Monograph

on

Avian Mycoses & Mycotoxicoses

A guide for postgraduate students

Section of "Geese of Meidum," a fragmentary wall painting from the mastaba of Nefermaat and Itet, now in the Egyptian Museum, Cairo, JE 34571/CG 1742. On the left, a bean goose (Anser fabalis) followed by two white-fronted geese (Anser albifrons) (photo by George B. Johnson)

By

Mohamed Kamal Refai

Amir Elbatrawi, Gamil Osman and Atef Hassan

2016

Refai, M.K. *et al*. (2016). Monograph on Avian Mycoses & Mycotoxicoses
A guide for postgraduate students,
https://www.academia.edu/21679188/
http://scholar.cu.edu.eg/?q=hanem/book/
https://www.researchgate.net/publication

Prof. Dr. Mohamed K Refai, Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Prof. Dr. Amir Elbatrawi, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Giza

Prof. Dr. Gamil Osman, Department of Poultry Diseases, Animal Health Research Institute, Dokki

Prof. Dr. Atef Hassan Department of Mycology and Mycotoxins, Animal Health Research Institute, Dokki

Preface

This monograph is dedicated to the eminent professors of poultry diseases, who introduced to the problems of fungal diseases of poultry, starting with ***Prof. Dr. Kamal Abbasi****, who invited me to visit a farm in Kafr El-Sheikh suffering from brooder pneumonia in 1966. Aspergillus fumigatus was isolated from the lungs of turkey poults and walls of the incubators. The results constituted the first paper I published after my return from Germany.* ***Prof. Dr Ahmed Bassiouni*** *was the first to honour me as a co-supervisor on the Ph.D. thesis of Amir Elbatrawi, who finished his thesis in 1980. Six years later, I was honoured by* ***Prof. Dt. Ibrahim Sokker*** *to be his co-supervisor of the Ph.D. thesis of Gamil Osman. The monograph is also dedicated to* ***Prof. Dr. Hamdy Abdelsalam****, who was the founder of the Department of Mycology in the Animal Health Research Institute and to* ***Prof. Dr. Salah Abdelhamid Youssef****, who joined me in seminars on mycotoxicosis in almost all governorates in Egypt.*

Prof. Kamal Abbasi Prof. Ibrahim SokkerPtof. Hamdu Abdelsalam

Prof. Ahmed Bassiouni Prof Salah Abedelhamid

Prof. Dr. Mohamed Kamal Refai, Cairo. 30.6.2016

Contents

Introduction *5*

1. **Avian Mycosis Caused by Dermatophytes** *25*
1.1. **Avian Favus** *25*

2. **Avian Mycosis Caused by Yeasts** *36*
2.1. **Avian Candidosis** *36*
2.2. **Avian Cryptococcosis** *61*
2.3. **Avian Macrorhabdiosis** *82*
2.4. **Avian Rhodotorulosis** *105*

3. **Avian Mycosis Caused by Moulds** *106*
3.1. **Avian aspergillosis** *106*
3.2. **Avian mucormycosis** *198*
3.3. **Avian dactylariosis (mycotic encephalitis)** *206*

4. **Avian Mycotoxicosis** *215*

4.1. **Aflatoxicosis (AF)** *215*
4.2. **Ochratoxicosis (OT)** *301*
4.3. **Sterigmatocystin (STC) mycotoxicosis** *370*
4.4. **Cyclopiazonic acid (CPA) mycotoxicosis** *386*
4.5. **Citrinin (CTN) mycotoxicosis** *407*
4.6. **Penicillic acid (PA) mycotoxicosis** *434*

4.7. **Avian fusariotoxicosis** *446*
4.7.1. **Deoxynivalenol (DON) mycotoxicosis** *459*
4.7.2. **Fumonisin (FB1) mycotoxicosis** *503*
4.7.3. **Moniliformin (M) mycotoxicosis** *549*
4.7.4. **T-2 mycotoxicosis** *573*
4.7.5. **Fusaric acid (FA) mycotoxicosis** *616*
4.7.6. **Zearalenone (ZEN) mycotoxicosis** *637*

4.8. **Oosporein Toxicosis (Avian gout)** *647*
4.9. **Avian ergotism** *653*

Introduction

It is doubtless that the Ancient Egyptians ate much better than people in any other ancient civilization of the world, even more so if the same timeline is considered for comparison. Much of the information about what the ancient Egyptians ate and drank comes from pictures on tomb walls, offering trays and foods left in the tombs, as well as a few scrolls of hieroglyphic writing that show further insight on the matter. Most common of art works are about growing, finding or making food. Many tomb walls also show pictures depicting people hunting, fishing and working in the fields.

Poultry foods in Ancient Egypt

The poultry foods were equally popular among both the rich and the peasant people who lived in the Ancient Egypt. The most commonly consumed poultry included the likes of *Offering of flowers, bred and pigeons (Berlin, Ägyptisches Museum)* Pigeons, Geese, Ducks and other tamed poultry were considered more popular among the richest of the Ancient Egyptians, and Crane, Swan, Wild Ostriches would end up being hard earned kills for the poor ones. Eggs from Ducks, Swans and Geese were also regularly consumed by people. Most of the times, the poultry kills were not eaten as soon as it was produced, but rather preserved with seasonings for a longer period of consumption.

Poultry products in Egypt www.ancienthistorylists.com

Chickens became a staple food in Egypt about 300 BC. Farmers in Egypt worked out ways to incubate chicken eggs in warm clay ovens, so that they didn't need to have hens sit on their eggs to hatch them, and instead the hens could lay more eggs. Did they get the idea from their beehives and honey farms? This factory system made chicken eggs cheaper, and more people began to eat them. After 300 BC, chickens

slowly made their way south and west across Africa, reaching South Africa either from Egypt or through the work of Indian traders along the coast of East Africa, probably around 500 AD.

According to Coltherd, in 1966, ‘there is no recorded mention of the domestic fowl in Ancient Egypt before the Middle Kingdom (2134-1786 BC). Evidence for its existence there before this time is completely negative. The hieroglyph which is found in the earliest inscriptions, and certain peculiarities in the mention of the indeterminate birds, led some early writers to believe that the fowl had already been introduced into Ancient Egypt at the dawn of history, by invaders from Mesopotamia.’

A century later Akhenaton (1364–47 B.C.), the so-called “heretic pharaoh” of Ancient Egypt, declares (if indeed he was really the author) in the Hymn to the Aton inscribed in the tomb of Ay:—

“When the chick crieth in the egg-shell,
Thou givest him breath therein, to preserve him alive,
When thou hast perfected him
that he may pierce the egg,
He cometh forth from the egg
To chirp with all his might;
”

(Breasted’s (1906) translation.)

Sentences describing hatching, : libraryblogs.is.ed.ac.uk

... which he had subdued, and from many beyond his reach, e.g. Assyria and Babylonia (Sngr). In the Royal Annals, carved in stone on the walls of the Great Temple of Karnak, in the section relating to tribute received after the campaign of Year 33, during which Egyptian troops crossed the Euphrates, occurs a sentence, part of which is missing, first discussed by Sethe (1916).

“4 birds of this country; they . . . every day.”

A former restoration of the missing characters had suggested that the bottom sign visible could only be restored as ms, meaning “to give birth”, so that the reading is therefore

A sentence on how four birds lay eggs every day: libraryblogs.is.ed.ac.uk

Historians have identified to which the first people began to breed poultry in incubators - this is ancient Egypt. Then be engaged in such activities had the right only to the priests. To do this, they used clay pots, which could hold tens of thousands of eggs. The pots are filled with a special liquid which on cooling became thicker. Thus, the internal temperature was determined to maintain it should have been with the fire, and watch the clock.

Geese, ducks, and other fowl were common in ancient Egypt. They were part of the daily diet and were used as offerings for deities and the deceased. Fishing and fowling were popular motifs in tomb and temple decoration. This colorful duck is unusual in its size and style, and it is possible that it was re-carved late

The Egyptian goose is believed to be most closely related to the shelducks (genus *Tadorna*) and their relatives, and is placed with them in the subfamily Tadorninae. It is the only extant member of the genus *Alopochen*, which also contains closely related prehistoric and recently extinct species. mtDNA cytochrome *b* sequence data suggest that the relationships of *Alopochen* to *Tadorna* need further investigation. Egyptian geese were considered sacred by the Ancient Egyptians, and appeared in much of their artwork. They have been raised for food and extensively bred in parts of Africa since they were domesticated by the ancient Egyptians. Because of their popularity chiefly as ornamental bird, escapes are common and small feral populations have become established in Western Europe

Egyptian goose. Alopochen-aegyptiacus.jpg, scotdir.com

The geese in the painting are commonly known as Egyptian geese (Alopochen aegyptiacus) which are members of the Tadorninae–the shelduck-sheldgoose subfamily (which means they are not exactly geese, taxonomically speaking). Egyptian Geese are 63–73 cm long (25-29 inches) and they range through most of sub-Saharan Africa and up the Nile valley. Domesticated by the ancient Egyptians in the depths of antiquity, the birds were also kept by the Greeks and Romans. There are feral populations in England and the United States (where Egyptophiles keep the fowl as ornamental birds!).

Detail of Geese in Frieze from Nefermaat's tomb (ca. 2600-2550 BC, An Ancient Masterpiece **ferrebeekeeper.wordpress.com**

The Nile valley was home to many kinds of ducks:

- Among these the pintail was most often kept.
- Many ducks remained wild and were a favourite prey for hunters in the Delta.
- They were often flushed out of their hiding places by dogs or civets. Throw sticks were used to bring them down.
- Netting was also popular.

mallard (*Anas platyrhynchos*, greyish **gadwall** (*Anas strepera*),), en.wikipedia.org

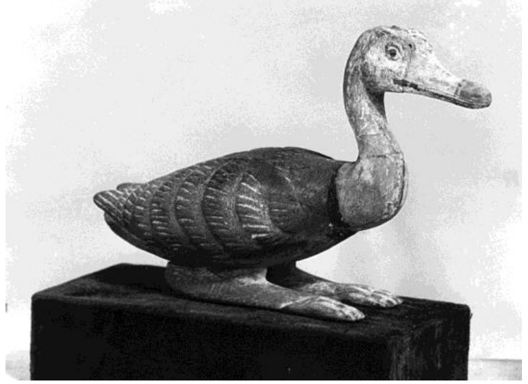
widgeon (*Anas penelope*), www.dreamstime.com the **teal** (*Anas crecca*), ibc.lynxeds.com

Tufted duck (*Aythya fuligula*),www.animalspot.net **porchard** (*Aythya ferina*), en.wikipedia.org

Ferruginous duck (*Aythya nyroca*) **pintail** (*Anas acuta*). commons.wikimedia.org

There's been a war going on — man against duck — since the beginning of time. Ancient Egyptian tombs were decorated with paintings of men in marshes, trying to catch ducks in traps. Native Americans made decoys out of cattail, bulrush and tule in an effort to lure the birds close enough to be bow-hunted, netted or snared.

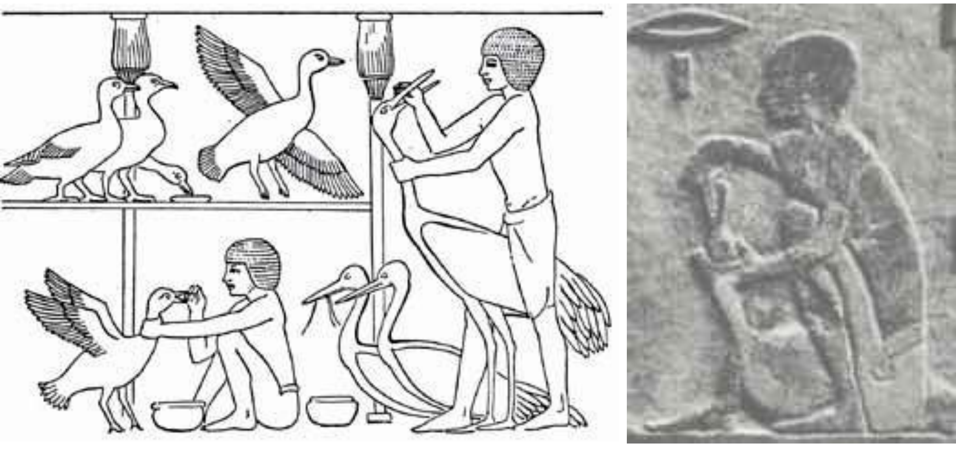
Ancient Egyptian Tomb Art detail, Nebamun hunting in the marshes, showing reeds, birds and a feral cat, painting from the tomb-chapel of Nebamun**,** www.pinterest.com



Duck Egyptian (Fayum?), Roman Period, ca. 1st-3rd century A.D.This duck figurine has wing feathers and eyes carved with a painted neck and head. There is a wood pin in the top of its head. commons.wikimedia.org

FORCED FEEDING ON AN ANCIENT EGYPTIAN POULTRY FARM

The Harris Papyrus mentions *fattening-houses containing fat geese*, and one wonders whether the ancient Egyptians knew how to prepare pâté de foie gras.

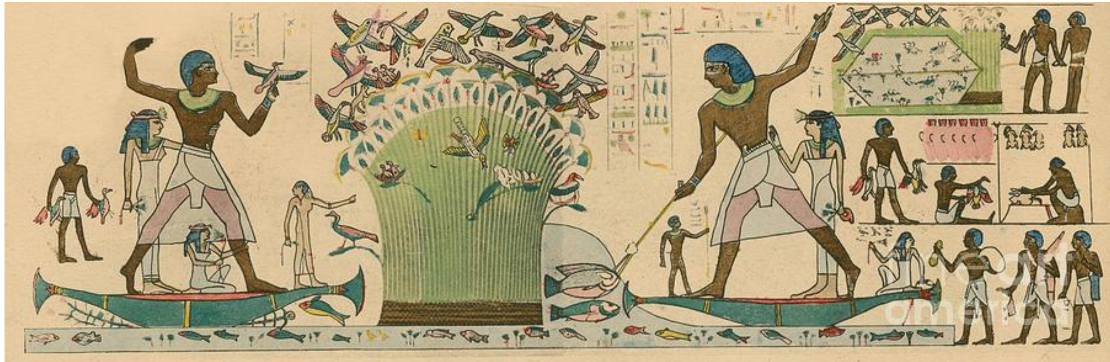


www.goldenageproject.org.uk

Force feeding a goose Saqqara, 1st Intermediate Period W.S.Smith, *Country Life in Ancient Egypt*, Museum of Fine Arts, BostonFrom *Everyday Life in Ancient Egypt* by Jon Manchip White www.earthmetropolis.com

Quails

Regarded as a delicacy, large numbers of migrating quail were caught when they landed exhausted after crossing the Mediterranean. Hunters spread nets and frightened the birds into rising. They got enmeshed in the nets and were easily picked off.



Ancient Egypt, Bird Hunting is a photograph by Science Source which was uploaded on March 14th, 2013. The photograph has colors ranging from desert sand to army green and incorporates ancient, egypt, and egyptian design themes. http://fineartamerica.com/featured/ancient-egypt-bird-hunting-science-source.html

Even the deceased enjoyed a feast of quail and death apparently did not spoil their appetite. In a letter to his dead mother Shepsi wrote:

This is an oral report concerning you saying to her son (i.e. the speaker): "You shall bring me poultry so that I can eat it," and when your son brought 7 quails (pAa.w.t) you ate them. Shall one act against me in your presence, so that my children are unhappy and your son is suffering? Who will pour water for you?

Pigeons

Pigeon houses were made of mud clay and their excrements were used as a natural fertilizer for agricultural purposes.

Ancient Egyptian pigeon houses 44 AD, www.pigeoncontrolresourcecentre.org

In 1100 BC, King Rameses III sacrificed 57,000 pigeons to the god Ammon at Thebes, confirming that the pigeon was well on the way to being domesticated not only for food but also for religious purposes. Mention of pigeon sacrifices can also be found in both the Old Testament and the New Testament

A BBQ at Amarna, palace of Akhenaton and Nefertiti. eating roasted pigeons (far left, and Nefertiti is handing a pigeon to her little girl) and brochettes. Their children are on chairs and the servants are standing. Source: Tombs of the Nobles, El Amarna dianabuja.wordpress.com

Offering of flowers, bred and pigeons (Berlin, Ägyptisches Museum)

Ostrich

The ostrich is the first species of bird for which we have pictorial evidence from Egypt. Its distinctive form can be recognized from the oldest series of rock drawings on cliffs of the Nile Valley and in the deserts of Upper Egypt, dating from predynastic times

- Ostriches are regularly represented in Egyptian art, and the ostrich feather, the sign of the deities Ma'at and Shu, was a common hieroglyphic
- The ostrich was an animal brought to ancient Egypt from southern Africa.
- The ancient Egyptians used the eggs of ostriches to make small containers for perfume, and the feathers to make fans.
- As symbols of their purity, the justified dead were pictured wearing ostrich feathers.
- The ostrich feather was the weight against which the heart of the deceased was weighed in the judgment of the dead.
- According to Horapollo: "The man rendering justice to all, was represented by the ostrich feather; because that bird, unlike others, has all its feathers equal."
- Priests drew an ostrich feather on their tongues with green dye, so that the words they spoke were truth.
- In a text from the New Kingdom, the ostrich is said to greet the dawn by "dancing" in the wadis in honor of the sun.
- In Predynastic times, the ostrich was connected with a mother-goddess cult.
- Ostrich feathers and eggs were often used for decoration.

Ostrich eggs and feathershttps://cowofgold.wikispaces.com/Ostrich

- Ostrich eggshells were sometimes used as vessels, and were among the earliest objects of any kind from ancient Egypt, as are small ornaments made from them.
- A large fan was discovered in the tomb of Tutankhamen, made of wood and sheathed in gold. The remains of thirty ostrich feathers, alternating white and brown, were found next to it, a few of the stumps still fixed in the holes on the outer edges of the fan.

https://cowofgold.wikispaces.com/Ostrich

- The feathers, according to an inscription on the handle, were obtained by "His Majesty when hunting in the desert east of Heliopolis." Embossed on each face of the fan are scenes of the young pharaoh hunting the birds for feathers for the fan. On one side is a dramatic hunting scene, showing Tutankhamen and his hound pursing the birds with a horse-drawn chariot, and the reverse shows the triumphant return of the hunt, with two attendants carrying the slain birds slung over their shoulders.

The front side of king Tutankhamun's large gilded wood fan: a dramatic ostrich huntin scene, https://cowofgold.wikispaces.com/Ostrich

The dead wearing ostrich feather, https://cowofgold.wikispaces.com/Ostrich

Packing Food for the Hereafter in Ancient Egypt (A. R. Williams)

When death came, as it inevitably did, the ancient Egyptian pharaohs and their relatives were ready for it. Each had spent years preparing a lavish tomb stocked with everything they might need or want in the afterlife, including food, preserved for eternity.

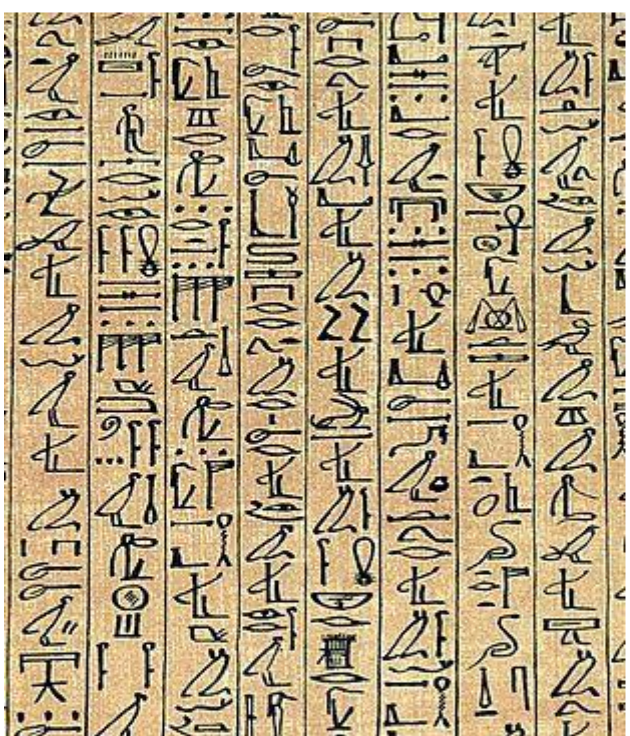
Even meat and poultry were on the menu. To keep these highly perishable foods tasty until the end of time, the Egyptians mummified them—slowly drying them with salt, bandaging them and covering the bundle with resins—much as they would a human body.

theplate.nationalgeographic.comPhotograph by Kenneth Garrett

Egyptian birds used in Hieroglyphics

HIEROGLYPHS are the word pictures that represent the sounds of the Ancient Egyptian language.There are two basic types of hieroglyphs:

- IDEOGRAMS are images that depict the object they represent. For example the image of a mouth can represent the word 'mouth'.
- PHONOGRAMS are images that represent the sounds of the Ancient Egyptian language, just like our alphabet represents the sound of our language. For example, the image of a mouth can also represent the sound 'R'.



A section of the *Papyrus of Ani* showing cursive hieroglyphs. 3500 BCE – 400 CE

The birds used as letters

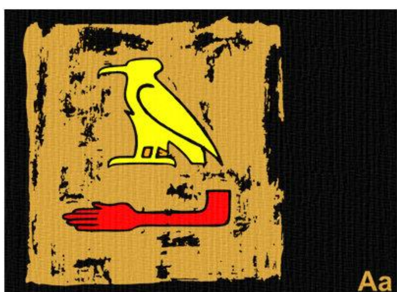
HIEROGLYPH: A

- There are two hieroglyphs for the letter "A". They represent the different sounds of the letter.
- **The vulture**, which is usually a sign for divine power, is used for the "ah" sound in words like around and about, and names like Adam.
- The arm is used for the "ay" sound in words like say, sail and sale, and names like Amy.

HIEROGLYPH: E

- There are two hieroglyphs for the letter "E". They represent the different sounds of the letter.

- The double reed is used for the 'ee' sound in words like need, piece and read, and names like Elaine.
- **The vulture is used for the 'E' sound** in words like get and learn, and names like Edward



HIEROGLYPH: M

The owl is used for the "M" sound in words like man and mask and names like Michael and Mary.

HIEROGLYPH: O

There are two hieroglyphs used for the letter "O". They represent the different sounds of the letter.

The quail chick is used for the long 'O', 'oa' and 'oo' sounds in words like overcoat and wood and names like Olivia

HIEROGLYPH: Q

- **The basket and quail chick combine for the "Q" sound in words like queen and names** like Quentin.

HIEROGLYPH: W

- **The quail chick is used for the "W**" sound in words like wise and why, and names like William and Wendy.

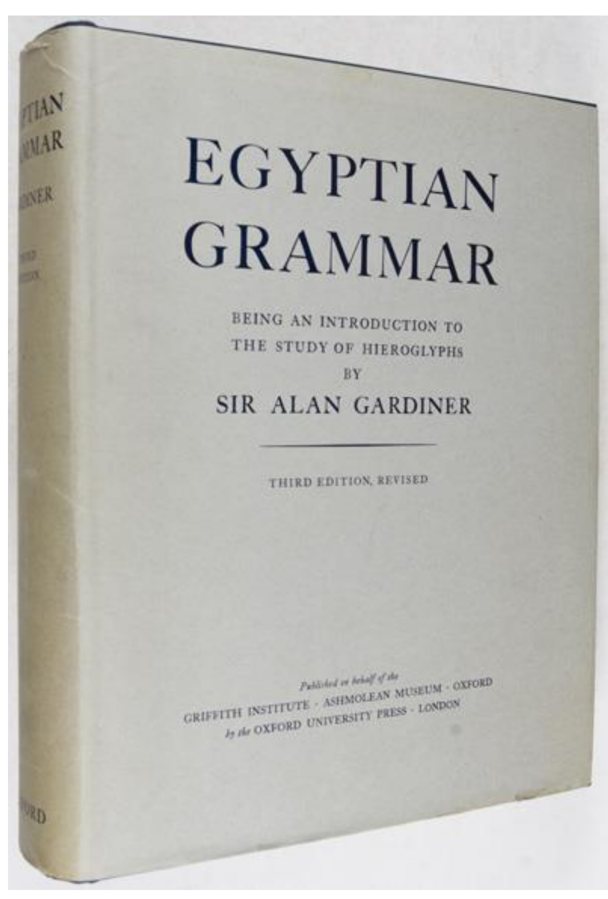
HIEROGLYPH: U

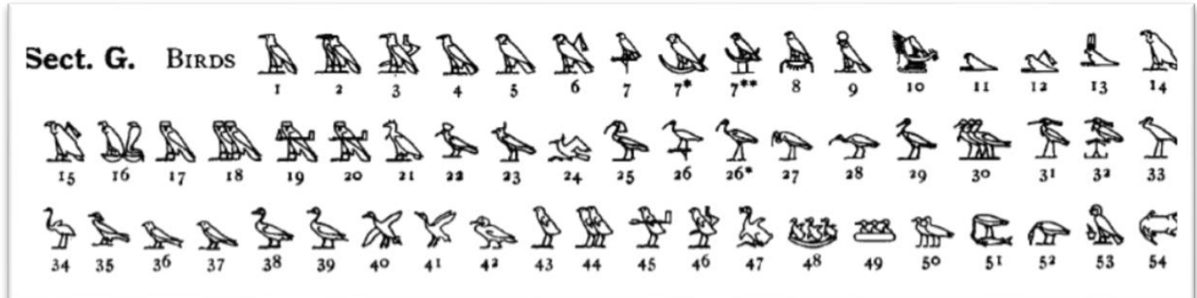
- There are two hieroglyphs for the letter "U". They represent the different sounds of the letter.
- **The quail chick** is used for the long "U" sound in words like rule, pull and duel, and names like Una.
- The reed and quail chick are combined for the short "U" sound in words like jump and up, and names like Ursula.

Signs of birds in the Ancient Egyptian language

Egyptian Grammar: Being an Introduction to the Study of Hieroglyphs was written by Alan Gardiner and first published in 1927 in London by the Clarendon Press. It has been reprinted several times since. The third edition published in 1957 is the most widely used version for the subject. Through a series of thirty-three lessons, the book gives a very thorough overview of the language andwriting system of Ancient Egypt. The focus of the book is the literary language of the Middle Kingdom. The creation of the book resulted in the development of an accurate and detailed hieroglyphic typeset, Gardiner's Sign List.

The birds in the Egyptian Grammer, A.Gardiner are found in section G. page 545. G1-G49





G. Birds

Examples G43-G49

GardinerNo	Hieroglyph	Description of Glyph	Details
G43		Quail chick, var. Z7	Phono. w.
G44		Two quail chicks	Phono. ww. Inpḥww “end.”
G45		Combination of G43 + D36	Phono wʿ.In wʿw“soldier.”
G46		Combination of G43 + U1	Phono. m3w.
G47		Duckling	Phono. ṯ3. In ṯ3y“male.”
G48		Three ducklings in nest	Ideo. in sš “nest.”
G49		Ducks’ heads protruding from pool	

Birds in the Ancient Egyptian relegion

Ancient Egyptians believed that we have different components to our being. The ba, our power to move, appears in iconography as a bird with a human head. When a person died, his or her ba lived on, leaving the tomb during the day and reuniting with the corpse in the netherworld at night. Mummies have masks in part, Bailleul-LeSuer explains, so the ba can recognize whom it should go back to. Statuettes like this one, found in Dendera, are seen in funerary assemblages and in tombs, perched atop the coffin, beginning around 1500 BC. Like most ba birds, this is a falcon, identifiable by its long wings that meet the tail.

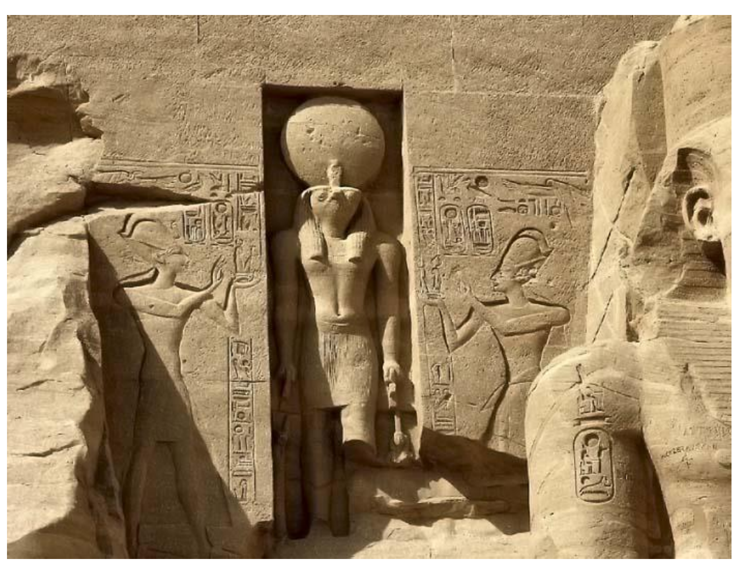
Key figures in the Egyptian pantheon were traditionally depicted as birds, notably the falcon-headed Horus and ibis-headed Thoth. Their worshippers mummified millions of the creatures as offerings, capturing and breeding thousands for the purpose each year, especially after the fall migration coinciding with the Nile flood. Birds' "ability to fly high in the sky led the ancients to believe that they could join the gods," Bailleul-LeSuer writes in the exhibition catalog, "and thus act as divine messengers, if not as receptacles of the divine themselves." Some funerary texts claim that they represent the souls of the dead coming back to Earth, "so it's actually a conquest of death when the birds come" each migration season.

Birds were food, communication – and the representatives of gods. Several gods had the forms of birds, and their shape-sake birds were respected for the likeness. Horus was falcon-headed, Thoth ibis-headed, Nekhbet a vulture.

Horus is one of the most significant deities in ancient Egyptian religion, who was worshipped from at least the late Predynastic periodthrough to Greco-Roman times. Different forms of Horus are recorded in history and these are treated as distinct gods by Egypt specialists. These various forms may possibly be different perceptions of the same multi-layered deity in which certain attributes orsyncretic relationships are emphasized, not necessarily in opposition but complementary to one another, consistent with how the Ancient Egyptians viewed the multiple facets of reality. He was most often depicted as a falcon, most likely a lanner or peregrine, or as a man with a **falcon head.**

Figure of a Horus Falcon, between circa 300 and circa 250 BC (Greco-Roman). [23] The Walters Art Museum. en.wikipedia.org

The ancient Egyptians revered Ra as the god who created everything. Also known as the Sun God, Ra was a powerful deity and a central god of the Egyptian pantheon. The ancient Egyptians worshiped Ra more than any other god and pharaohs often connected themselves with Ra in their efforts to be seen as the earthly embodiment of the Sun God.



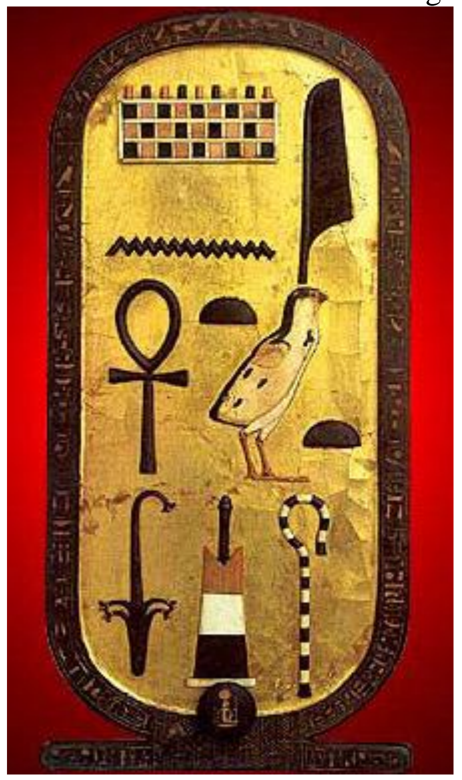
The Benu, according to ancient Egyptian mythology, was also believed to be the ba of Re, and by Egypt's Late Period, the hieroglyphic sign depicting the bird was used to write the name of this sun god. During the Middle Kingdom, it was said that the Benu of Re was the means by which Atum came into being in the Primeval water.

Like the sun god, the Benu's own birth is attributed to self generation. A mythological papyri of the 21st Dynasty provides a vignette of a heart-amulet and scarab beetle near to which stand the Benu, which is described as "the one who came into being by himself". It was believed to constantly rise renewed just like the sun, and was called the "lord of jubilees". The Benu Bird was said to each morning appear under the form of the rising sun, and was supposed to shine upon the world from the top of the famous persea tree in Heliopolis wherein he renewed himself.

The Ancient Egyptian Cartouche

In ancient Egypt, kings, and sometimes others, encircled their name hieroglyphs with a design that we now call a cartouche. While we may find it rarely used to enclose the name of non-kings, for the most part, the cartouche's presence identifies the name it encloses as the king of Egypt. A cartouche is an oval ring that is a hieroglyph representation of a length of rope folded and tied at one end. It symbolized everything that the sun encircled and is thus an indication of the king's rule of the cosmos. Later, in the demotic script, the cartouche was reduced to a pair of parentheses and a vertical line.

The term, "cartouche" is a relatively modern one coined by the soldiers of Napoleon's expedition in Egypt, who saw in the sign the likeness of the cartridges, or "cartouche" used in their own guns. The cartouche, known in ancient Egypt as the shenu, is derived from the Egyptian verb, Sheni, which means to encircle. It is very similar to the shen sign, a more circular form, and in fact the earliest use of the cartouche in which the king's name was written were circular and identical with that sign. So in order to understand the cartouche we must know something of the shen sign.



The circular shen sign, or ring evokes the concept of eternity through its form, having no beginning or end, and its solar aspect is symbolized by the sun disk often depicted in the center of the circle. It was also a symbol of protection, and as a hieroglyphic symbol in Egyptian art, it can have the meanings of both "eternity" and "protection". As a sign of "eternity", the shen is frequently associated with representations of Heh, the god of eternity, and often forms the base of the notched palm-branches

symbolizing "years," which is held by this deity. It is also mirrored in the shape of the ouroboros, the serpent which bites its own tail.

However, the sign is perhaps most commonly associated with the avian forms of the falcon god Horus and the various vulture goddesses. These divine birds are frequently depicted holding the shen in their claws, hovering above the king and guarding him beneath their outstretched wings. The shen signs represented with these avian deities may be regarded as symbols of eternity, and therefore life, but it is possible that the signs also carry the connotation of protection, and this double significance would certainly seem to be present in many of the small decorative items and amulets, and indeed the larger royal objects, which are adorned with this sign.

While the earliest use of a cartouche seems to have been identical in form to the shen sign, early in Egyptian history, the form of the shen ring was lengthened in order to hold the increased number of hieroglyphs resulting from longer royal names and fuller orthography. In this way, the shen continued to be used as a sign with its own meaning while the cartouche, or shenu, became the standard holder of the royal name. Occasionally, one may find the name of a god or goddess in a cartouche. This was

especially the case for Osiris-Onnophris and Isis in the temple inscriptions of the Greco-Roman Period.
many sarcophagi. Note also that in the tomb of Tuthmosis III, in the Valley of the Kings, the entire burial chamber, as well as the sarcophagus, was constructed in the form of a cartouche.

The ancient Egyptians depicted deities wearing headdresses, which often can be used to identify gods and goddesses. The headdress appears to have signified qualities or powers belonging to that specific deity. Vultures are among the most common symbols featured in Egyptian headdresses. A number of vulture species lived in ancient Egypt, so the bird was a recognizable image. In Egyptian mythology, vultures were not just scavenging birds, but symbols of femininity and maternal protection. When the goddess Nekhebet of Upper Egypt became associated with the vulture headdress, the bird evolved into a heraldic symbol for all of Upper Egypt.

BIRD MUMMIES

In collaboration with the University of Chicago Medicine and the Field Museum, Bailleul-LeSuer CT scanned ten bird mummies from the collection of the Oriental Institute, allowing her to identify the species and also to study the way they were mummified."The bird mummies are particularly fascinating," said Oriental Institute Chief Curator Jack Green. "They give us a glimpse into the religious beliefs of the ancient Egyptians and, with the help of modern technology, new insights into the birds themselves

Artfully Enrobed

Wrapped in linen, this so-called sacred ibis—a hatchling housed at Montreal's McGill University—provided some of the first evidence that ancient Egyptians sent animal mummies on their final journeys fully fed, a new study says. CT scans of the 2,500-year-old bird, one of four specimens used in the study, show that its body was packed with grains after death to sustain it in its afterlife mission as a messenger to the gods, according to findings published January 13 in the *Journal of Archaeological Science*. "The ancient Egyptians intended to send this ibis to eternity with a full belly," the study team writes.

1. Avian Mycosis Caused by Dermatophytes

1.1. Avian Favus (Avian ringworm, Avian dermatophytosis, white comb)

Definition

Favus is a mycotic infection found primary in gallinaceous birds. Favus is rare in commercial poultry today, but is occasionally reported in backyard flocks, especially exotic and game chickens. Characteristic lesions include white crusting on the comb and wattles-Favus; that can extend to the feathered portion of the skin to form scutula around the bases of feather follicles. *Microsporum gallinae* is the agent most often isolated, although *M. gypseum* and *Trichophyton simii* have also been isolated.

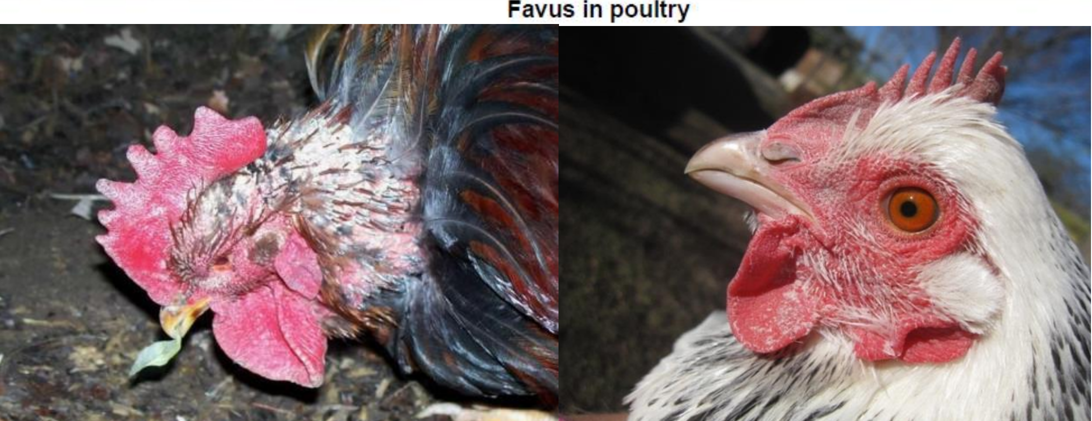
Clinical manifestations

- Lesions are observed on featherless skin areas like comb, wattle and shanks; initially appearing as few grayish/yellowish cup-like spots. They increase in

size and coalesce to make a wrinkled crust, which is mostly dry and scaly appearing like honeycomb about the size of a pea. Feathered skin may develop lesions of depression around follicles (favus cup), systemic signs are not observed

- Lesions are white on the wattles and combs of chickens. Lesions may spread to the head and neck.
- The feathers are normally not affected by the dermatophyte, although some feather loss can occur
- Roosters and chicks tend to be more susceptible to the infection, with fighting cocks having the highest rates of *M. gallinae* dermatophytosis
- Spread of infection occurs in birds by direct contact or via contaminated fomites
- Favus is not of much economical importance, and occurs sporadically.

Favus in poultry



Favus infection of the comb in a chicken.http://www.merckvetmanual.com. Wakenell, www.poultrycentral.co.nz

Aetiology

Favus is caused by :

1. *Microsporum gallinae* (Megnin) (*Trichophyton gallinae*),
2. *Trichophyton simii,*
3. *Microsporum gypseum*

1. *Microsporum gallinae* (Mégnin ex Guég.) Grigoraki, Ann. Dermatol. Syph.: 42

Synonyms:

≡Epidermophyton gallinae Mégnin, C.R. Soc. Biol.: 404 (1881)
≡Lophophyton gallinae (Mégnin) Matr. & Dassonv., Rev. Gén. Bot.: 429 (1899)
≡Epidermophyton gallinae Mégnin ex Guég.: tab. 8, fig. 9 (1907) [MB#153872]
≡Achorion gallinae (Mégnin) Sabour., Maladies du Cuir Chevelu 3: 553 (1910)
≡Sabouraudites gallinae (Mégnin ex Guég.) M. Ota & Langeron, Annales de Parasitologie Humaine Comparée 1: 327 (1923)
≡Closteroaleurosporia gallinae (Mégnin ex Guég.) Grigoraki, Annales des Sciences Naturelles Botanique 7: 412 (1925)
≡Trichophyton gallinae (Mégnin ex Guég.) Georg, Mycologia 44 (4): 486 (1952)
=Microsporum vanbreuseghemii Georg, Ajello, Friedman & S.A. Brinkm., Sabouraudia 1: 194 (1962)

Historical

- **Remak (1837)** detected fungal elements in the scutula in a case of favus
- **Schoenlein (1839**) described the nature of the fungus and recognized its aetiological role in favus.
- **Remak (1845)** named the fungus Achorion (the Greek name of scab or scurf) and coined the name of Schoenlein to the fungus as ***Achorion schoenleinii***
- **Megnin (1881)** described the cause of favus in poultry and named the fungus ***Achorion gallinae,***
- **Sabouraud (1910)** accepted the genus Achorion and classified it into human types including *A. schoenleinii* and animal types including ***Achorion gallinae***, *A. gypseum* and *A. quinckeanum* as causes of favus in poultry and animals
- **Grigorakis (1929)** classified the fungus in the genus Microsporum as ***Microsporum gallinae***
- Emmons (1934) deleted the clinically based genus Achorion and included the fungus in the genus Trichophyton as ***Trichophyton gallinae***
- **Langeron and Vanbreuseghem (1952)** replaced the genus Microsporum by the genus Sabourauditis.and included *Achorion gallinae* in this genus as ***Sabourauditis gallinae***

- **Conant *et al.*,1954**, adopted he classification of Emmons, where the fungus was included in the genus Trichophyton as ***Trichophyton gallinae (Megnin) Silva and Benham 1952*** with the *synonyms:Achorion gallinae- Epidermophyton gallinae- Microsporum tomentosum, M. umbonatum, M. velveticum- Sabourauditis audouinii- Trichophyton decalvans*
- Ajello (1962) accepted the fungus as ***Trichophyton gallinae***
- **Weitzam and Summerbell** (1995) accepted the fungus as ***Microsporum gallinae* (Megnin) Grigorakis 1929**
- **Simpanya (2000)** included the fungus in his classification as ***Microsporum gallinae (Megnin, 1881),***

Macroscopic morphology

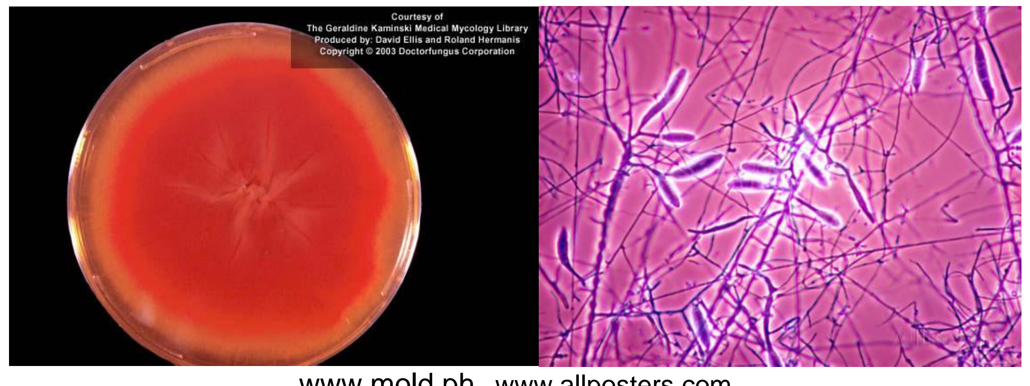
- Growth rate is moderately rapid and diameter of colonies ranges from 1 to 3 cm. incubated on Sabouraud dextrose agar at 25°C for 7 days;
- Colonies are moderately wrinkled and with velvety to wooly or cottony texture; and
- The surface colony color is white to gray turning pink to buff as it matures and the reverse is observed with diffusing deep strawberry – red pigment.

Microscopic morphology

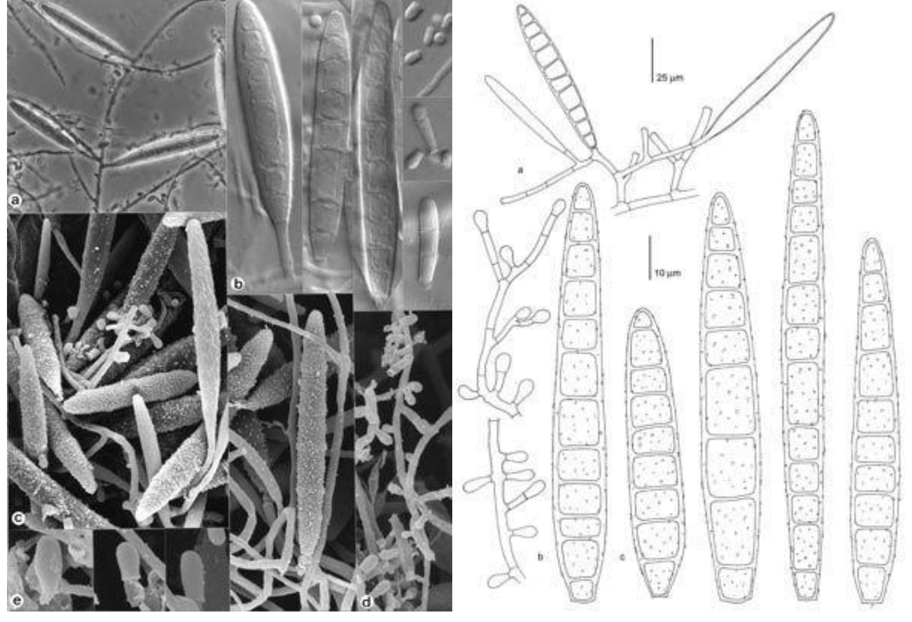
- Septate hyphae, macroconidia and macroconidia are present;
- Macroconidia are club – shaped, commonly curved or narrow at the tip, with a smooth or echinulate cell wall containing 2 to 10 cells, and may be rare or numerous; and
- Microconidia are ovoid to pyriform in shape, unicellular, and may be rare or numerous.

Colonies and macroconidia of *Microsporum gallinae*

Microsporum gallinae www.studyblue.com www.mycology.adelaide.edu.au



www.mold.ph www.allposters.com



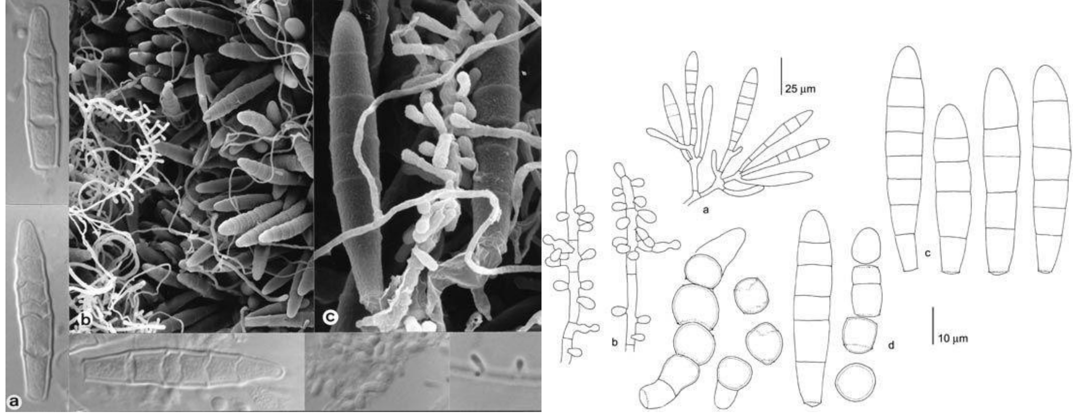
Mycobank

2. ***Trichophyton simii* (Pinoy) Stockdale, D.W.R. Mack. & Austwick, Sabouraudia 4 (2): 114 (1965)**
   Synonyms:

≡Epidermophyton simii Pinoy, C. R. Soc. Biol.: 59 (1912) [MB#416368]

≡Pinoyella simii (Pinoy) Castell. & Chalm., Manual of Tropical Medicine: 1023 (1919)

Colonies (SGA) spreading, evenly granular with fluffy margin, whitish to pale buff; reverse yellowish to salmon, becoming vinaceous.Microscopy. Macroconidia smooth-walled, fusiform, 30-85 x 6-11 ?m, 5-10-celled; individual cells often swelling and becoming liberated as chlamydospores



T. semi Mycobank

3. **Microsporum gypseum** (E. Bodin) Guiart & Grigoraki, Lyon Médical 141: 377 (1928)

Synonymy:

≡Trichophyton gypseum E. Bodin, Les champignons parasites de l'homme: 115 (1902)
≡Achorion gypseum (E. Bodin) E. Bodin, Annales de Dermatologie et Syphilis 8: 585 (1907)
≡Sabouraudites gypseus (E. Bodin) M. Ota & Langeron, Ann Parasitol 1: 328 (1923)
≡Closterosporia gypsea (E. Bodin) Grigoraki, Ann Sci Naturelles Botanique 7: 411 (1925)
≡Trichophyton mentagrophytes var. gypseum (E. Bodin) Kamyszek, Med. Wet146 (1945)
=Microsporum flavescens Horta, Memórias do Instituto Oswaldo Cruz 3 (2): 301-308 (1912)
=Microsporum scorteum Priestley, Ann. Trop. Med. Parasit.: 113 (1914)
=Microsporum xanthodes Fischer, Dermatol. Wochenschr.: 214-247 (1918)
=Favomicrosporon pinettii Benedek, Mycopathologia et Mycol Applicata 31 (2): 111 (1967

Colony characteristics.
Colonies (SGA) growing rapidly, powdery, cinnamon-tan; reverse yellowish-buff, sometimes with pinkish tinges.

Microscopy.
Macroconidia in large clusters, rather thin-walled, regularly verrucose, 3-6 (-8)-celled, fusiform, 25-60 x 8.5-15.0 ?m. Microconidia sessile or stalked, smooth- and thin-walled, clavate, 3.5-8.0 x 2-3 ?m.

Colonies and macroconidia of *Microsporum gypseum*

Diagnossis

Demonstration of the fungi in the smears:
Microscopic examination is performed with the skin scab examination on a glass slide with potassium hydroxide solution (20%) and heated until appearance of a few bubbles; subsequently it is examined for presence of fungi. Staining of the fungus can also be done with 10% Parker Superchrome 51 pen ink in sodium hydroxide which demonstrates the presence of fungus.

Isolation and identification
Transparent plastic tape 18 mm in width is cut into strips approximately 10 cm in length. Both 1-cm ends are folded for handling. The adhesive surface (approximately 6 cm in length) is placed on the comb and gently rubbed with both the thumb and index finger to collect scales. The tape is incubated at 42 °C for 4 h to remove the mites. Afterward, the tape is stamped on Sabouraud agar and cultured at 35 °C for up to 28 days.

White to slightly beige coloured, cottony or powdery colonies, which are characteristics of dermatophytes and/or related species, ae picked and transferred onto potato dextrose agar slants and incubated at 25 °C for further identification. In addition, the transparent tape can be placed on the first colonies to appear and observed them by light microscopy after staining with lactophenol cotton blue to detect round or pyriform conidia attached to the right angle of the hyaline septate hyphae.

Molecular Biological Identification

Sequences of the internal transcribed spacer (ITS) 1-5.8S-ITS 2 region of the rRNA gene (ITS rDNA)

- DNA is extracted with a kit
- 2.5 µL DNA extract are mixed with Ready-To-Go polymerase chain reaction (PCR) beads, 2.5 µL 10 pM primers ITS-5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′), and 17.5 µL distilled water.
- The reaction mixture is subjected to one denaturation cycle at 95 °C for 4 min; 30 amplification cycles at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; and a final extension cycle at 72 °C for 10 min in a PCR Thermal Cycler MP (TaKaRa).
- The PCR products are visualized by electrophoresis on 1.0 % agarose gels in 1× TBE buffer [0.04 M Tris-boric acid, and 0.001 M ethylenediaminetetraacetic acid (EDTA, pH 8.0)] followed by ethidium bromide staining. The PCR samples were purified using a PCR purification kit and labeled with BigDye Terminator. The labeled samples ae directly sequenced on an ABI PRISM 3100 sequencer using the primers ITS-5, ITS-4, ITS-2 (5′-GCTGCGTTCTTCATCGATGC-3′), and ITS3 (5′-GCATCGATGAAGAACGCAGC-3′). The DNA sequences ae aligned using GENETEX-MAC genetic information processing software. Sequences are analyzed by a basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi), and closely related sequences are obtained.

The confirmation of fungal species is based on >99 % identity to the known fungal species, and those of fungal genera was <98 %.

Prevention and treatment

Replacement of the birds with new stock need to be made with disease free birds (symptom/lesion free). Proper segregation isolation procedures need to be followed to avoid introducing the disease into a healthy flock and to have check on its spread amongst the birds. If necessary, birds should be culled and slaughtered. Dipping of the birds in 0.5% pentachlorophenol or 5-bromosalicyl-4-chloranilide.

- **Fonseca and Mendoza (1984)** treated favus in chickens in Costa Rica topically with tolnaftate and oraly with griseofulvin.
- Miconazole nitrate 2% was was used successfully in the treatment a flock of various Oriental breed (Shamo and Aseel) and crossbreed chickens infected with *Microsporum gallinae* (Bradley *et al*., 1995).
- Tinactin and Lotrimin are also used for favus. The disease can resolve naturally without treatment; however, the infection may persist for weeks prior to clearance.
- **Ferreira de Ferreira *et al.* (2015)** used ketoconazole. successfully

Zoonotic aspects

Microsporum gallinae has been isolated from the scalp, and smooth skin in human populations.[*Microsporum gallinae* infections are most commonly tinea capitis and tinea corporis. Very few human cases of *M. gallinae* infection have been reported, none of which were life-threatening. Of the reported cases, individuals ranged from 3–96 years old. They had cutaneous lesions on the glabrous skin or the scalp. These localized lesions are frequently accompanied by itching. The cutaneous manifestations are very similar to those of *Microsporum canis* therefore many cases of *Microsporum gallinae* could have been unreported.

.

Reports:

CARNAGHAN *et al* (1956) reported an outbreak of fowl favus (*Trichophyton gallinae*) in Berkshire that was diagnosed at the Veterinary Laboratory, Weybridge, in 1954. A total of 40 hens were affected following the introduction of twelve cockerels into the flock. Lesions consisted of small, multiple, white, powdery areas on the comb, wattles, and sides of the face. The isolate differed from the type description of *T. gallinae* in not forming pigment in the medium but its identity was confirmed by L. K. Georg. Experimental inoculations were successful in fowls and showed spontaneous recovery in six to seven weeks. Guinea-pigs and rabbits could not be infected. The outbreak was the first one confirmed out of 26 suspected ones investigated over a period of seven years.

Tewari (1969) reported *Trichophyton simii* infections in chickens, dogs and man in India

Singh and Singh (1972) described *Trichophyton simii* infection in poultry in India

Gugnani and Randhawa (1973) reported an epizootic of dermatophytosis caused by *Trichophyton simii* in poultry

Barsky ***et al.*** **(1978)** reported the first case of *Trichophyton simii* infection in the United States not traceable to India, The patient was a 40-year-old Nigerian male student who had not been out of the United States for more than three years and who had never been in India or had contact with animals or poultry.

Fonseca and Mendoza (1984) reported the first diagnosis of favus in chickens in Costa Rica in a 1-year-old fighting cock that had lesions surrounding the comb. The etiologic agent was isolated and identified as ***Microsporum gallinae***. The rooster recovered during topical treatment with tolnaftate and oral treatment with griseofulvin.

Droual ***et al.*** **(1991)** diagnosed avian ringworm in a backyard flock of game chickens from which ***Microsporum gallinae*** was isolated. Infected birds had white crusts on the comb and on the skin of the head and neck. Histopathological lesions included hyperkeratosis of the skin epithelium with invasion of the stratum corneum by fungal mycelia, acanthosis, acantholysis, and hydropic degeneration of cells in the stratum spinosum. The underlying dermis was infiltrated by mononuclear cells and contained lymphoid foci. Daily topical treatment with miconazole was applied in the field and in the laboratory, with apparent success.

Bradley ***et al.*** **(1993)** described chickens of various Oriental breeds (Shamo and Aseel) and crossbreeds in California's Central Valley to have an unusual skin condition and feather loss. The appearance of white plaques on the comb, face, and/or ear lobes was followed by feather loss starting at the caudal base of the comb and progressing down the neck. Although the cocks were affected first, the condition spread to the hens paired with those cocks. The birds showed no other signs of illness. The affected areas were scraped and biopsied. The samples were examined histologically and by culturing on Sabouraud's dextrose agar and dermatophyte test medium. ***Microsporum gallinae***, the causative agent of favus (avian dermatophytosis), was identified by the histological and mycological tests.

Bradley ***et al.*** **(1995)** tested miconazole nitrate 2% for its efficacy against ***Microsporum gallinae*** (the causative agent in favus) in a flock of various Oriental breed (Shamo and Aseel) and crossbreed chickens. Six adult males showing clinical signs of favus were randomized into control and experimental groups. The males were maintained on individual tiecords on the range, with no physical contact between birds. The experimental birds had the affected areas washed with soap and water and dried, and an ointment of miconazole nitrate 2% was applied. The experimental birds received the treatment twice a day for 34 days. Scrapings from the affected areas of all birds were cultured at the beginning and end of the test. At the end of the treatment period, the control birds were still positive for M. gallinae, but the organism could not be cultured from the treated birds.

Nweze (2001) carried out a survey of dermatophytoses amongst primary school children in Borno State, Nigeria, during February 1997 to January 1998. A total of

2,193 children aged 4-16 years were screened. Out of these, 154 (7.0%) were proved to be mycologically positive by microscopy, culture or both. Incidence was significantly higher (P <0.05) in young children aged 7-11 years (8.1%) and 4-6 years (6.9%) than in older children aged 12-16 years (3.6%). There was a significant difference in the incidence of dermatophytoses amongst children in urban and rural areas (P <0.05). Tinea capitis was the predominant clinical type followed by tinea corporis. Trichophyton schoenleinii was the most prevalent etiological agent (28.1%), followed by *T. verrucosum* (20.2%) and ***Microsporum gallinae*** (18.4%). Other species recovered included *T. mentagrophytes* (16.7%), T. tonsurans (10.5%), *T. yaoundei* (4.4%) and *M. gypseum* (1.8%).

Miyasato *et al.* (2011) reported a case of tinea corporis caused by *Microsporum gallinae* in a 96-year-old, otherwise healthy Japanese man. The patient had a long working history as a breeder of fighting cocks, and he suffered from two erythematous macules after being bitten by a cock. *M. gallinae* was identified as the infectious agent based on the morphology of isolates cultured on slides and analysis of DNA sequences of the internal transcribed spacers (ITS) from ribosomal DNA from cultured isolates. The patient was successfully treated with antifungal ointments. To our knowledge, this is the first case of M. gallinae infection in a human reported in Japan.

Murata *et al.* (2013) investigated 238 chickens and 71 fighting cocks in Okinawa and in the suburbs of Tokyo (Chiba, Tokyo, Ibaraki, and Sizuoka). One isolate of *M. gallinae* from a fighting cock in Chiba Prefecture in the Tokyo metropolitan area exhibited a different genotype, with a single base difference from the patient isolate based on the internal transcribed spacer 1-5.8s-ITS2 regions (ITS1-5.8S-ITS2) of the ribosomal RNA gene sequence. The isolation of *M. gallinae* from a fighting cock on the mainland of Japan is the first such finding in animals in our country.

Yamaguchi *et al.* (2014) investigated 793 bird combs [645 chickens and 148 fighting cocks (Shamo)] to determine the prevalence of dermatophytes and their related fungal species. The targeted fungal species were recovered from 195 of the 793 examined birds (24.6 %). Prevalence ratios were compared in temperate (the mainland) and subtropical (Nansei Islands) areas, genders, strains, breeding scale (individual and farm), and housing system (in cage and free ranging). The frequency of the fungal species in the mainland, males, fighting cocks, breeding scale by individual nursing, and free-range housing system exhibited significantly higher positive ratios than that in the other groups. A total of 224 dermatophytes and related species were isolated, including 101 Arthroderma (Ar.) multifidum, 83 Aphanoascus (Ap.) terreus, five Uncinocarpus queenslandicus, two U. reesii, two Ap. pinarensis, one Amauroascus kuehnii, one Ar. simii, one Gymnoascus petalosporus, one Microsporum gallinae, and 28 Chrysosporium-like (Chrysosporium spp.) isolates, which were identified using internal transcribed spacer regions of ribosomal RNA gene sequences. The predominant fungal species in the mainland was Ap. terreus and that in the Nansei Islands was Ar. multifidum. Pathogenic fungal species to humans and animals were limited to ***M. gallinae and Ar. simii***, which corresponded to 0.025 % of the isolates in this study.

Ferreira de Ferreira *et al.* (2015) reported a dermatophytosis case by Microsporum gallinae in chicken (Gallus gallus domesticus), in the Pelotas city, Brazil, with subsequent recovery after treatment with ketoconazole.

References:

1. Barsky S, Knapp D, McMillen S. Trichophyton simii infection in the United States not traceable to India. Arch Dermatol. 1978 Jan;114(1):118
2. Bradley FA, Bickford AA, Walker RL. Diagnosis of favus (avian dermatophytosis) in Oriental breed chickens. Avian Dis. 1993 Oct-Dec;37(4):1147-50.
3. Bradley FA, Bickford AA, Walker RL. Efficacy of miconazole nitrate against favus in oriental breed chickens. Avian Dis. 1995 Oct-Dec;39(4):900-901.
4. CARNAGHAN, R. B. A. ; GITTER, M. ; BLAXLAND, J. D. Favus in Poultry: an outbreak of Trichophyton gallinae infection, Veterinary Record 1956 Vol. 68 pp. 600-601
5. Droual R, Bickford AA, Walker RL, Channing SE, McFadden C. Favus in a backyard flock of game chickens. Avian Dis. 1991 Jul-Sep;35(3):625-30.
6. Fonseca E, Mendoza L. Favus in a fighting cock caused by Microsporum gallinae. Avian Dis. 1984 Jul-Sep;28(3):737-41.
7. Gracialda Ferreira de Ferreira , Carolina Lambrecht Gonçalves, Josiara Furtado Mendes, Ana Paula Neuschrank Albano , Mário Carlos Araújo Meireles , Patrícia da Silva Nascente. Dermatophytosis (ringworm) in Microsporum gallinae in Gallus gallus domesticus: Case report, Acta Veterinaria Brasilica, v.9, n.2, p.180-184, 2015
8. Gugnani HC, Randhawa HS. An epizootic of dermatophytosis caused by Trichophyton simii in poultry. Sabouraudia. 1973 Mar;11(1):1-3.
9. Miyasato H, Yamaguchi S, Taira K, Hosokawa A, Kayo S, Sano A, Uezato H, Takahashi K. Tinea corporis caused by Microsporum gallinae: first clinical case in Japan. J Dermatol. 2011 May;38(5):473-8.
10. Murata M, Takahashi H, Takahashi S, Takahashi Y, Chibana H, Murata Y, Sugiyama K, Kaneshima T, Yamaguchi S, Miyasato H, Murakami M, Kano R,Hasegawa A, Uezato H, Hosokawa A, Sano A. Isolation of Microsporum gallinae from a fighting cock (Gallus gallus domesticus) in Japan. Med Mycol. 2013 Feb;51(2):144-9.
11. Nweze EI. Etiology of dermatophytoses amongst children in northeastern Nigeria. Med Mycol. 2001 Apr;39(2):181-4.
12. Singh MP, Singh CM. Trichophyton simii infection in poultry. Vet Rec. 1972 Feb 19;90(8):218.
13. Tewari RP. Trichophyton simii infections in chickens, dogs and man in India. Mycopathol Mycol Appl. 1969 Dec 29;39(3):293-8.
14. Yamaguchi S, Sano A, Hiruma M, Murata M, Kaneshima T, Murata Y, Takahashi H, Takahashi S, Takahashi Y, Chibana H, Touyama H, Ha NT, Nakazato Y,Uehara Y, Hirakawa M, Imura Y, Terashima Y, Kawamoto Y, Takahashi K, Sugiyama K, Hiruma M, Murakami M, Hosokawa A, Uezato H. Isolation of dermatophytes and related species from domestic fowl (Gallus gallus domesticus). Mycopathologia. 2014 Aug;178(1-2):135-43.

2. Avian Mycosis Caused by Yeasts

2.1. Avian Candidosis

Syn. thrush, moniliasis, sour crop, soar, crop mycosis, mycosis of the digestive tract, stomatitis oidica, oidiomycosis, muguet**, levurosis,**

Candidosis in poultry is a disease of the mucocutaneous areas of the body and gastrointestinal mucosa, particularly of the oropharynx, crop and esophagus the mucocutaneous areas of the body and gastrointestinal mucosa, particularly of the oropharynx, crop and esophagus is reported in a variety of avian species, such as, chickens, turkeys, pigeons, game birds, waterfowl, and geese. The disease is caused mainly by yeasts of the genus *Candida* , where *C. albicans* is the most common and significant species. Other Candida species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. lusitaniae* have also been implicated as causative agents. Candidiasis affects.

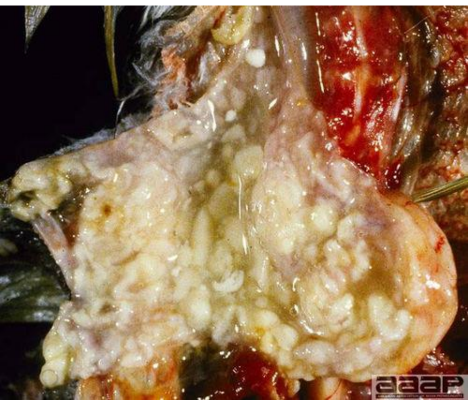
The yeasts of the genus *Candida* belong to the normal digestive flora of birds. In the case of confinement structures densely stocked with birds, the environmental contamination by dropping is massive

Clinical signs

- There are no unique signs of the disease. Affected poultry show
  - anorexia,
  - retarded/slow growth,
  - stunted appearance,
  - listlessness,
  - ruffled feathers,
  - dejection,
  - poor appetite,
  - slow growth and
  - diarrhoea.
- Clinical signs are present only in severely affected individual birds with superficial oral or crop infections may fail to gain weight and become dehydrated.
- Localized infections may occur in the beak or oral cavity and white, caseous plaques in the oral cavity may be the only noticeable clinical sign.
- Systemic invasion and signs of neurological, renal or intestinal disease may be present.

The characteristic *Candida* lesion:

- A catarrhal to mucoid exudate consisting of raised white mucosal plaques and white to clear mucus that may be associated with a foul odor. Lesions are generally confined to the upper areas of the digestive tract.
- With inhibition of competing microflora or immunosuppression yeasts proliferate on the surface and hyphae or pseudohyphae invade superficial epithelial layers, that stimulates epithelial hyperplasia and pseudomembrane or diptheritic membrane formation in the form of multifocal to confluent cheesy material in the crop and less frequently in the esophagus and pharynx.
- The membranous mass that appears adheres to the surface of the crop and cannot be easily removed.
- Other areas of the upper digestive tract develop false membranes that resemble those which develop during diphtheria, areas of dead tissue and contain considerable tissue debris.
- In chronic cases the mucosa of crop gives "turkish towel" like appearance produced by multiple tag-like plaques of mucosa and inflammatory cells or it may produce round raised ulcers



Sour crop www.backyardchickens.com , Edited by casportpony - 2/10/13

Candida. White cheesy lesions in crop and gizzard, www.nadis.org.uk

Transmission

- In most cases, the infection is endogenous in origin, occurring secondarily to stress, immunosuppression, inadequate nutrition, poor sanitation, debilitation or in birds that have been extensively treated with antibiotics.
- The organisms can be transmitted from the parent bird to chicks during regurgitative feeding.
- The infection also may be spread throughout the nursery population by the use of contaminated fomites and feeding utensils.
- Fecal contamination of feed undoubtedly accounts for the spread of candidiasis.
- Ingestion through food or water is the usual means for the transmission of the disease, and Candida probably becomes part of the resident flora of the mouth, esophagus and crop.
- Contaminated environments such as litter from poultry and game bird rearing facilities refuse disposal areas, discharge sites for poultry operations, and areas contaminated with human waste have all been suggested as sources for *Candida* exposure for bird.

Predisposing factors

- Most common disease risk factor is the prolonged use of antibiotic administration., which suppresses normal bacterial flora and competition for nutrients thus allowing *Candida* to proliferate.
- Young animals with immature immune systems are particularly susceptible.
- Inbreeding and line breeding techniques for unusual color patterns may create less genetically hardy birds and probably contributes to a decline in resistance to disease particularly in the smaller pet bird species.
- Poor hygiene and contamination of feeds and feeding utensils
- Dietary deficiencies, particularly vitamin A and D deficiencies, are another common predisposing factors.
- immunosuppression.
- Over crowding.
- Environmental stress.
- Nutritional diseases.
- Gizzard erosions, intestinal coccidiosis

Pathology

- *C. albicans* possesses a number of putative virulent factors like adhesins having an affinity for the fibronectin on the cell surfaces.
- The yeast forms are responsible for tissue damage and inhibition of yeast cell division resulting in hyphal elements that invade tissues.
- Phospholipase concentrated in hyphal tips, may enhance invasiveness. and penetration of the fungus into tissues.
- Haematogenous spread may occur following vascular invasion by hyphae or pseudohyphae, producing systemic lesions.

- Neuraminidase and proteases may play a role in virulence.
- Cell wall glycoprotein has an endotoxin like activity.
- Other virulence factors are chitin, mannoprotein and lipids.
- Phenotypic switching in *C. albicans*may facilitate evasion of host defense mechanisms.Inflammatory responses are predominantly neutrophils and granulomatous lesions are rare.

Aetiology:

C. albicans is the most abundant and significant species. Other Candida species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* , C rugose and *C. lusitaniae*

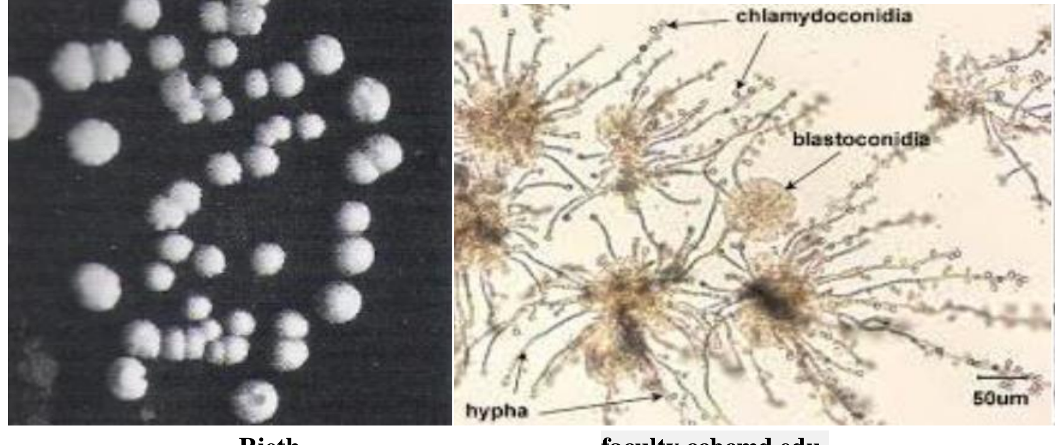
1. *Candida albicans* (Robin) Berkhout 1923

Synonyms

1. =Blastomyces albicans Brownlie: 425-431 (1920) [MB#456196]
2. =Candida biliaria Bat. & J.S. Silveira, Hospital Rio de Janeiro 56 (2): 295 (1959)
3. =Candida claussenii Lodder & Kreger, The Yeasts: a taxonomic study: 578 (1952)
4. =Candida desidiosa Cif. & Redaelli, Archiv für Mikrobiologie 6: 65 (1935) [MB#263052]
5. =Candida genitalis Bat. & J.S. Silveira, Public Instit Micol Unive do Recife 170: 11 (1962)
6. =Candida intestinalis Bat. & J.S. Silveira, Hospital Rio de Janeiro 56 (2): 293 (1959)
7. =Candida langeronii Dietrichson, Annales de Parasitol Humaine Comparée 29: 479 (1954)
8. =Candida mycotoruloidea Redaelli & Cif., Archiv für Mikrobiologie 6: 50 (1935)
9. =Candida nouvelii Saëz, Bulletin de la Société Mycologique de France 89 (1): 82 (1973)
10. =Candida truncata Vanbreus., Archives Belge de Derm et Syphil 4: 307-313 (1948)
11. =Endomyces albicans Okabe, Cblatt Bakteriol, Parasit Infek, Erste Abt: 181-187 (1929)
12. =Monilia alba Castell. & Chalm., Manual of Tropical Medicine: 1089 (1919) [MB#481761]
13. =Monilia albicans Plaut (1919) [MB#479429]

Morphology

On Sabouraud's dextrose agar colonies are white to cream coloured, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to subspherical budding yeast-like cells or blastoconidia, 2.0-7.0 x 3.0-8.5 um in size.



Rieth **faculty.ccbcmd.edu**

Physiological Tests:

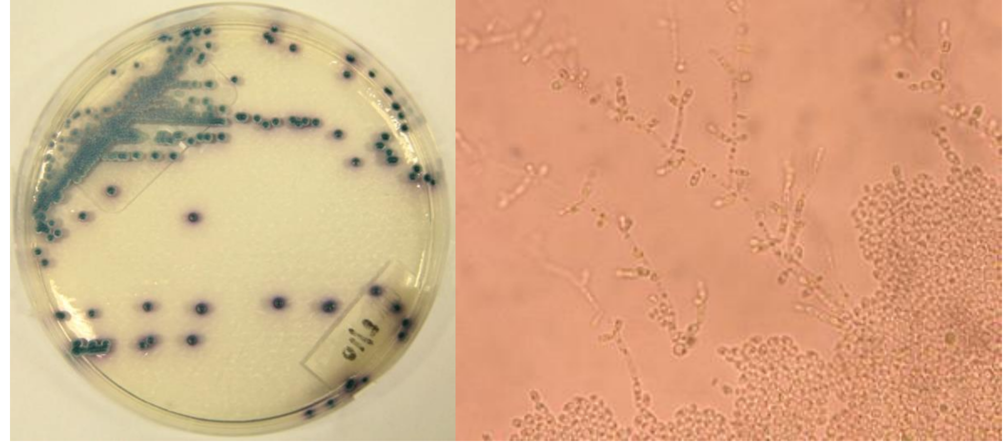
Germ Tube test + within 3 hours. Hydrolysis of Urea +,Growth on Cycloheximide medium +. Growth at 37C +, fermentation: Glucose +; Maltose +, Galactose +/-; Trehalose+/-, Sucrose (some strains +); Lactose -. **Assimilation:** Glucose +; Maltose +; Galactose +; Trehalose +; Sucrose (some negative);D-Xylose +; Soluble Starch +; D-Mannitol +; D-Glucitol (Delayed), Melezitose +/-; Glycerol +/-; Succinic acid +/-; L-Arabinose +/-; L-Sorbose +/-; D-Ribose (some positive); Citric acid +/-; DL-Lactic acid +/-. Potassium nitrate -; Lactose -; Ribito-

2. ***Candida tropicalis*** (Castell.) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923)

1. Atelosaccharomyces tropicalis (Castell.) Mello, Arquivos de Higiene e Patologia Exóticas 6: 263 (1918)
2. Candida albicans var. tropicalis (Castell.) Cif., Manuale de Micologica Medica 2: 252 (1960)
3. Candida tropicalis var. tropicalis
4. Castellania tropicalis (Castell.) C.W. Dodge, Medical mycology. Fungous diseases of men and other mammals: 258 (1935)
5. Endomyces tropicalis (Castell.) Castell., Centbl. Bakt. ParasitKde, Abt. 1: 236 (1911)
6. Monilia tropicalis (Castell.) Castell. & Chalm., Manual of Tropical Medicine: 1086 (1919)
7. Myceloblastanon tropicale (Castell.) M. Ota, Jap. J. Dermatol. Urol.: 178 (1927)
8. Mycotorula tropicalis (Castell.) Cif. & Redaelli, Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia 3 (1): 48 (1943)
9. Oidium tropicale Castell., Philippine Journal of Science Section B Medical Science 5 (2): 202 (1910)
10. Procandida tropicalis (Castell.) E.K. Novák & Zsolt, Acta Botanica Academiae Scientiarum Hungarica 7: 133 (1961)

Colonies (YPGA) cream-coloured, off-white, soft, smooth and creamy or wrinkled near the margin. Microscopy. Budding cells (RA) ellipsoidal. Pseudomycelium abundant, consisting of long, poorly branched elements, often narrowed towards a sterile apex; conidia arranged in small groups around the middle of each cellular element.

Differential diagnosis. Species signature: fermentation of maltose, +, and assimilation: galactose +, lactose, raffinose, l-rhamnose, meso-erythritol, myo-inositol, d-tryptophan (N), w/o biotin, growth at 40°C



CHROMAgar™ image of Candida tropicaliswww.life-worldwide.org

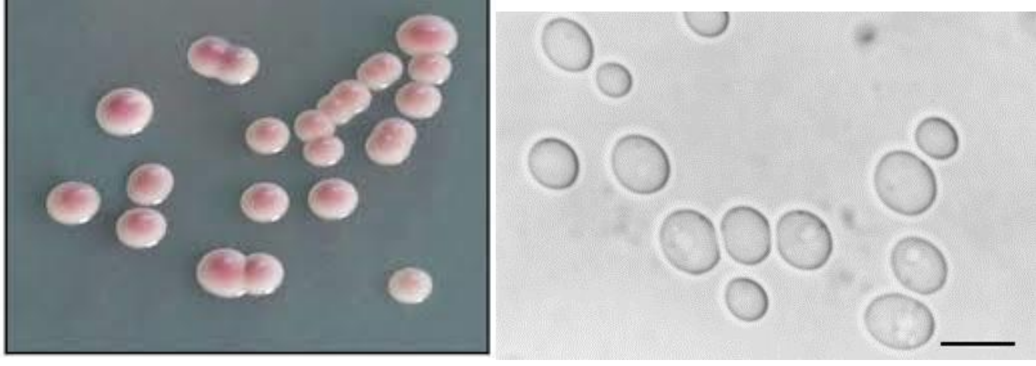
3. ***Candida glabrata*** (H.W. Anderson) S.A. Mey. & Yarrow, International Journal of Systematic Bacteriology 28: 612 (1978)

≡Cryptococcus glabratus H.W. Anderson, Journal of Infectious Diseases 21: 379 (1917)
≡Torulopsis glabrata (H.W. Anderson) Lodder & N.F. de Vries, Mycopathologia 1 (2): 102 (1938)
=Torulopsis stercoralis Uden

Colonies on Glucose Peptone Agar at 25°C: after 3 days cream-coloured, smooth, dull, regular in shape, spherical, domed.Yeast-like cells are generally ovoid, single or budding 2·0-4·0 x 3·0-5·5 µm. Cultures on Corn Meal Agar: ovoid, budding cells only. No pseudomycelium (chains of elongated yeast-likecells) produced.

Germ Tube Test: negative.

Fermentation of Carbohydrates: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose.
Assimilation of Organic Compounds: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose +Cellobiose - Inositol - Melezitose - Melibiose - Mannitol - L-Sorbose - D-Xylose - L-Arabinose - D-Arabinose - D-Ribose - L-Rhamnose - Glycerol v Erythritol - Ribitol - Galactitol - D-Glucitol - Salicin - DL-Lactic Acid - Succinic Acid - CitricAcid - Soluble Starch -.
Assimilation of Inorganic Compounds: Nitrate -.
Ability to split urea: -.



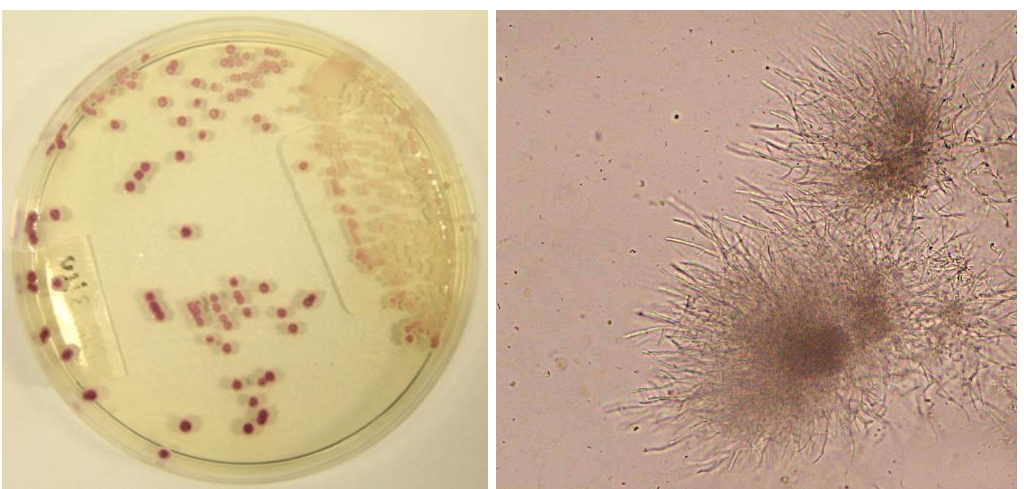
C. glabrata on HardyCHROM Candida www.hardydiagnostics.com

4. ***Candida parapsilosis*** (Ashford) Langeron & Talice, Annales de Parasitologie Humaine Comparée 10: 54 (1932)

1. Candida parapsilosis var. parapsilosis , Annales de Parasitologie Humaine Comparée 10: 1 (1932) [MB#426111]
2. Monilia parapsilosis Ashford, American Journal of Tropical Medicine 8: 518 (1928) [MB#253820]
3. Mycocandida parapsilosis (Ashford) C.W. Dodge, Medical mycology. Fungous diseases of men and other mammals: 294 (1935) [MB#253821]
4. Mycotorula parapsilopsis (Ashford) Cif. & Redaelli (1943) [MB#288681]
5. Mycotorula parapsilosis (Ashford) Cif. & Redaelli, Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia 3 (1): 47 (1943) [MB#535232]

Colonies (YPGA) cream-coloured to yellowish, glistening and soft, mostly smooth or partly or entirely wrinkled. Pseudomycelium (RA) present, mostly abundant, consisting of branched chains of elongate cells in more or less christmastree-like arrangement, lateral branches gradually becoming shorter towards the hyphal apex.

Differential diagnosis. Species signature: fermentation of glucose +, and assimilation: cellobiose, raffinose, melebiose, melezitose +, soluble starch, d-xylose +, salicin, arbutin, 5-keto-d-gluconate (but may be slowly positive), nitrate, growth at 37À¸C +, d-tryptophan (N), w/o thiamine +. Physiologically



Candida parapsilosis on CHROMAgar™ www.life-worldwide.org , www.medical-labs.net

Candida krusei (Castell.) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923)

Synonyms:
1. Candida krusei var. krusei
2. Endomyces krusei (Castell.) Castell., British Medical Journal 2: 1210 (1912)
3. Geotrichoides krusei (Castell.) Langeron & Talice, Annales de Parasitologie Humaine Comparée 10: 67 (1932)
4. *Monilia krusei (Castell.) Castell. & Chalm., Manual of Tropical Medicine: 826 (1913)*
5. *Myceloblastanon krusei (Castell.) M. Ota, Jap. J. Dermatol. Urol.: 178 (1928) =*
6. Mycotoruloides krusei (Castell.) Langeron & Guerra, Annales de Parasitologie Humaine Comparée 10 (1932)
7. Saccharomyces krusei Castell., Journal of Tropical Medicine and Hygiene 11 (1908)
8. Trichosporon krusei (Castell.) Cif. & Redaelli, Archiv für Mikrobiologie 6: 19 (1935)

Colonies on Glucose Peptone Agar incubated at 25°C: after 3 days cream-coloured, smooth, dull with 'ground glass'appearance. After 7 days the colonies are flat-topped with a broad mycelial edge (the entire colony is 'hat-shaped'). Yeast-like cells oval to elongate to long rectangular 2,0-5,5 x 4,0-15,0 µm, single, budding and in short chains. After 7 days branched chains of elongated cells (15-25 µm long) are produced.
Dalmau Plate Cultures on Corn Meal Agar: extensive long, branched chains of elongated cells are produced after 3 days.Globose to ovoid, thin-walled spores are produced singly, in pairs or clusters mainly at the junctions of the elongated cells. Germ Tube Test: negative.
Fermentation of Carbohydrates: Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose -Assimilation of Organic Compounds Glucose + Sucrose - Maltose - Lactose - Galactose - Raffinose - Trehalose -Cellobiose - Inositol - Melezitose - Melibiose - Mannitol - L-Sorbose v D-Xylose - L-Arabinose - D-Arabinose - D-Ribose - L-Rhamnose - Glycerol + Erythritol - Ribitol - Galactitol - D-Glucitol - Salicin - DL-Lactic Acid + Succinic Acid + CitricAcid v Soluble Starch -.

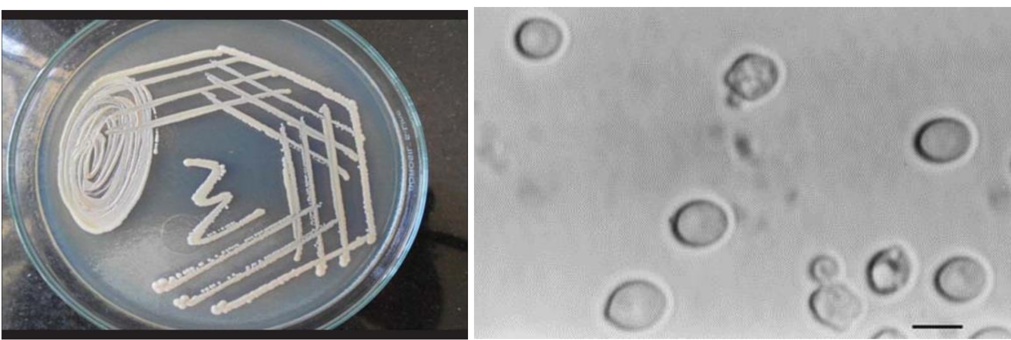
Assimilation of Inorganic Compounds: Nitrate -.
Ability to split urea: variable.

Candida krusei catalog.hardydiagnostics.com, s3.amazonaws.com

5. ***Candida rugosa*** (H.W. Anderson) Diddens & Lodder, Die anaskosporogenen Hefen, II Hälfte: 280 (1942)

≡Mycoderma rugosum H.W. Anderson, Journal of Infectious Diseases 21: 341-385 (1917)
≡Candida rugosa var. rugosa , Die anaskosporogenen Hefen, II Hälfte: 280 (1942)
≡Candida rugosa var. elegans Dietrichson, Annales de Parasitol Humaine Comparée 29: 485 (1954)
≡Azymocandida rugosa E.K. Novák & Zsolt, Acta Bot Acad Sci Hungarica 7: 134 (1961)
=Endomyces rugosus Castell., British Medical Journal 2: 1209 (1912)
=Torula rugosa Saito, Journal of Japanese Botany 1: 49 (1922)
=Trichosporon rugosum (Castell.) M. Ota (1926)
=Candida rugosa var. elegans Dietrichson, Annales de Parasitol Humaine Comparée 29: 485 (1954)

Growth in glucose-yeast extract-peptone broth: After 3 days at 25°C, the cells are ovoid, ellipsoidal to cylindrical, (2.4-6.4) × (3.2-8.0) µm, single, in pairs, and cluster, multilateral budding. Growth on glucose-yeast extract-peptone agar: Aerobic growth is white to cream, butyrous, colonies. Dalmau plate culture on corn meal agar: After 7 days at 25°C, primary pseudohyphae are found. Formation of ascospores: Ascospores are not formed.



C. rugosa on CHROM agar www.jdrntruhs.org , www.bcrc.firdi.org.tw

6. ***Candida lusitaniae*** Uden & Carmo Souza, Portugaliae Acta Biologica, Série B: 251 (1959) [MB#294031]

=Candida parapsilosis var. obtusa Dietrichson, Annales de Parasitologie Humaine Comparée 29: 483 (1954)
=Candida obtusa var. obtusa (1970)

On Sabouraud's dextrose agar colonies are white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows numerous subglobose, ovoid, or elliptical budding yeast-like cells or blastoconidia, 1.5-6.0 x 2.5-10.0 um in size. **India Ink Preparation: Negative - no capsules present. Dalmau Plate Culture on Cornmeal and Tween 80 Agar: Abundant pseudohyphae with short chains of** blastoconidia.

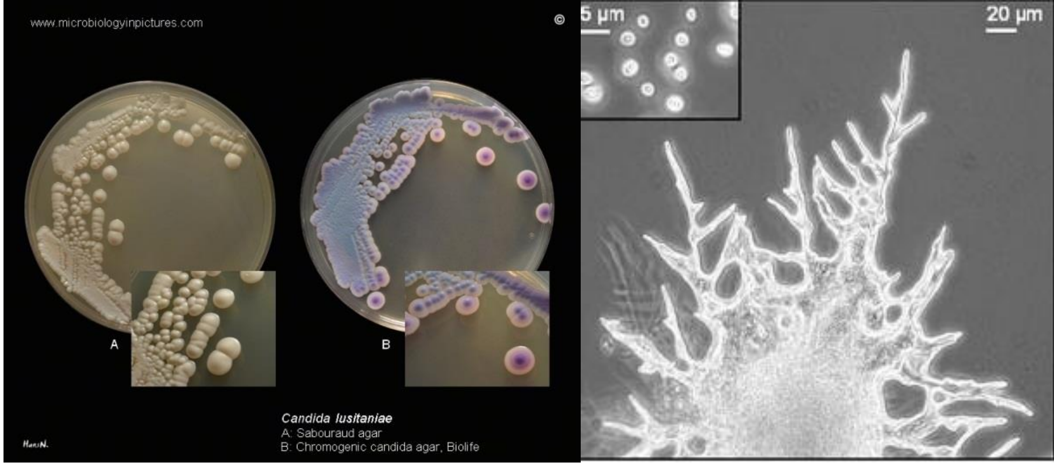
Physiological Tests:

Germ Tube test is Negative Hydrolysis of Urea is Negative Growth on Cycloheximide medium is Negative Growth at 37C is Positive

Fermentation Reactions: Where fermentation means the production of gas and is independent of pH changes. Positive: Glucose; Sucrose (delayed); Trehalose (delayed). Variable: Galactose; Maltose. Negative: Lactose.

Assimilation Tests:

Positive: Glucose; Maltose; Sucrose; Trehalose; D-Xylose; Glycerol; Cellobiose; L-Rhamnose; D-Ribose (delayed); D-Mannitol; Ribitol; D-Glucitol; Salicin; DL-Lactic acid; Succinic acid. Variable: Galactose; Melezitose; D-Arabinose; L-Arabinose; L-Sorbose; Citric acid. Negative: Potassium nitrate; Lactose; Raffinose; Melibiose; Galactitol; Erythritol; Inositol; Soluble Starch.



Candida lusitaniae www.microbiologyinpictures.com ww.researchgate.net

Diagnosis

- clinical signs and P.M.
  - visualization of lesions,
- histopathology
  - microscopic examination of smears for the hyphal forms of the yeast in the tissue isolated either by cytology or on culture.
- Isolation
  - *C. albicans* can be isolated from clinical samples viz. faeces, crops, gizzards, lungs and livers from fowls.
  - Colonies of this fungus appear as white to ivory colour, smooth and with a yeasty smell. The yeast form of organism is small, (3 to 6 pm in diameter) and similar in size to the nucleus of an avian red blood cell.
  - Gram staining although provides a rapid determination of the presence of yeast within a lesion cannot act as confirmative test because the organism is common inhabitant of the gut.
  - Air dried smears may be stained with a hematology stain such as Diff-Quick, Wrights, Geimsa , or new methylene blue.
- Serological tests
  - might be useful in diagnosis of systemic infections that are not shedding yeast from the gastrointestinal or respiratory tract.
  - Tests described include
    - slide latex agglutination (LA),
    - immunodiffusion (ID),
    - counterimmunoelectrophoresis (CEP), and
    - enzyme immunohistochemistry.
- Diagnosis by embryo inoculation test involves administration of isolated fungi into chorioallantoic membrane (CAM) of the chicken embryo. *Suspension of Candida* organisms in 0.1 ml distilled water produce lesions within 48 hr and 50% of the embryos may die between 48 and 72 hr.
- Differential diagnoses for oral and upper gastrointestinal candidiasis include hypovitaminosis A, trichomoniasis, avian poxvirus, bacterial infection, etc.

Treatment

- Correction of the diet and husbandry are necessary for successful treatment of candidiasis.
- **Nystatin** is the first drug of choice for yeast infections confined to the alimentary tract.
  - It is not absorbed from the digestive tract and is effective for oral or topical use only.
  - Nystatin h is fungistatic in action and must come in contact with the organism to be effective.
  - Oral lesions may not respond if the drug is administered by gavage tube beyond this site of infection.
  - The drug also can be applied directly to lesions of the mucous membranes in the oropharynx.

- The recommended dose of 290,000 units/kg PO q8-12h is safe and effective for use in psittacine neonates.
- For flock treatment, nystatin h can be added to the drinking water at 100,000 IU/L.102
- Severe yeast infections may be refractory to nystatin h therapy. If the organism is resistant to nystatin h or is in the hyphal stage, having penetrated the wall of the digestive tract,

- **Fluconazole**
  - is one of the most effective antifungal agents for the treatment of tissue-based yeast infections.
  - A dose of 5 to 15 mg/kg PO q12h is recommended for most avian species.
  - It also is effective against alimentary tract yeast when added to the drinking water at 50 mg/L.
- **Ketoconazole**
  - can be used to treat systemic yeast infections at 10 to 30 mg/kg PO q12h.
  - It can be added to the drinking water at 200 mg/L for flock treatment of pigeons.

- **Itraconazole**
  - has been used in the successful treatment of candidal tracheitis in a blue and gold macaw (Ara ararauna) and candidal infection of the uropygial gland in a king penguin (Aptenodytes patagonicus).
  - Some Candida spp. are, however, extremely resistant to itraconazolec
  - The drug is unlikely to achieve therapeutic concentrations at 5 mg/kg and should be used at the higher dose of 10 mg/kg PO q24h.
- **Oral chlorhexidine**
  - Can be used at 10 to 20 ml per gallon drinking water for 3 weeks
  - can be used for flock control of Candida infections but generally will not eliminate them.
  - Mild cases of candidiasis may respond to acidification

Prevention and Control

- Cages, equipments and other materials in contact with infected birds should be disinfected
- Cleanliness and proper managemental care,
- Adequate vitamin A supplement are essential for prevention.
- Avoid excessive use of antibiotics and other stressors.
- Ensure good hygiene, propionic acid, sodium or calcium propionate at 1 kg/tonne continuously. $CuSO_4$ at 200 g/tonne upto 14-16 weeks in replacement pullets

- Administration of 71-125 mg Nystatin/ kg feed helps in prevention. 200g Mycostatin/ ton of feed completely destroy the contaminating fungi in the crop. Gentian violet @ 8mg/ kg feed also helps in prevention.
- Gentian violet, 8mg/ kg feed also helps in prevention.
- Garlic at 2-5% in feed has been shown to protect chicks from experimental *C. albican* infection.
- Control of *Candida* through drinking water is sometimes practiced with chlorination (e.g. chlorax, sodium hypochlorite) at 5 ppm.
- Addition of vinegar to the drinking water will acidify the gastrointestinal contents, making the environment less favorable for fungal growth, and may resolve some cases.
- The addition of chlorhexidine in the drinking water can help in preventing overgrowth in some flocks or nurseries but may have contraindication of the immune suppression associated with the overuse of disinfectants.
- Dipping the egg in an iodine solution before incubation may be effective measure of disease control.

Reports:

Schlegel (1912) isolated ***Candida albicans*** from fowls in Germany**.**

Gierke (1932) reported the first major epidemic of avian candidosis in the USA., that caused 8-20% mortality in young turkeys.

Jungherr (1933), in the USA, reported the loss of 10,000 chicks in a commercial hatchery due to ***Candida albicans*** infection.

Hart (1947) recorded outbreaks among turkeys and fowls caused by Candida albicans in New South Wales

Blaxland and Fincham (1950) surveyed mycosis of the crop (moniliasis) in poultry raised in Great Britain. They reported serious mortality occurring in young turkeys and no beneficial effects from copper sulfate therapy.

Underwood (1955) used a panendoscope for detection of crop mycosis (moniliasis) in chickens and turkey poults

Underwood *et al.* (1956) reported that copper sulfate administered in the feed or water was ineffective for preventing and treating the disease in chicks and poults. In two of five trials the use of copper sulfate apparently resulted in a more favorable environment in the crop for establishment of Candida albicans infection. In an experiment conducted at The Squibb Institute, copper sulfate at a dilution of 1 to 2000 in the drinking water was ineffective in preventing moniliasis in turkey poults.

Yacowitz *et al.* (1957) used Mycostatin to retard yeast growth on chicken meat. Preliminary studies in chicks showed that Mycostatin in the ration was effective in preventing the spread of moniliasis from infected to control birds.

KUPROWSKI (1960) recognized the 3 forms of infection by *Candida albicans*-thrush, blackbead, and toxicosis produced by inoculation experiments on turkeys, the

best results being secured by the use of birds a few days old and material from the organs of diseased rabbits. Gram + vegetative forms of the fungus were unmistakably revealed in slides stained by a modification of the Kuhne-Weigert method. The ovoid structures in the blood-vessels of the large parenchyma, hitherto described as blastospores, proved to be erythrocytic nuclei without a plasmatic margin. Although microscopic examination disclosed no developing fungal elements in slides from the liver, kidneys, spleen, and lungs, their presence therein was confirmed by cultures. *C. albicans* caused β-haemolysis in glucose-blood agar at a temp, range of 38.5-44° C., the production of haemolysins being dependent on mycelial growth. The absence of marked post-mortem changes and the simultaneous presence of mycelia in the crop mucosa is regarded as evidence that the pathogenesis of the toxic form of moniliasis is determined primarily by the haemolytic properties of the fungus.

Wind *et al.* (1960) reported that nystatin dispersed in water with sodium lauryl sulfate was effective in the treatment of established crop mycosis of turkeys when used continuously at levels of 62.5 to 250 mg. per litre (p.p.m.) of drinking water for 5 days. It was well tolerated and did not reduce water consumption.

Mayeda (1961) presented data from 167 cases examined during 1957-60 at Livestock and Poultry Path. Lab., Calif. Dept Agric., Sacramento. Incidence of moniliasis was highest in the dry season, July being the peak month; in turkeys 80% of infections occurred in the 1st 3 months of life, in chickens ş were in the 7-10 months age group. It was commonly associated with insanitary watering systems and extensive antibacterial antibiotic therapy, and was basically an upper alimentary tract disease. Nystatin, quaternary ammonium compounds, and copper sulfate administered in the drinking water or feed controlled the outbreaks.

Tripathy *et al.* (1965) reported aortic changes associated with candidiasis of turkeys

Balish and Phikips (1966) investigated bacterial protection against intestinal infection by *Candida albicans* in chicks with a monoflora of either *Escherichia coli* or *Streptococcus faecalis*, by orally inoculating germ-free chicks (3 days old) with pure cultures of bacteria. Each bacterial species was established in large numbers in the gut of separate groups of animals within 24 hr of inoculation; these numbers were similar in chicks examined 34 days later, at which time all birds were killed. The numbers of bacteria from contents of the crop, small intestine, and ceca were similar in chicks with the *E. coli* monoflora. Comparable results were obtained in chicks with the *S. faecalis*monoflora, except for decreased numbers in the duodenum and jejunum. Some of the monoflora chicks (7 days old) were transferred into separate isolators, orally inoculated with *C. albicans*, and observed for 34 days. All chicks grew well and appeared healthy. However, examinations at autopsy revealed severe crop infections in chicks with a diflora containing *S. faecalis*. Preferential growth of hyphae (*C. albicans*) occurred in the lesions and throughout the gut. The numbers of *S. faecalis* in the gut were comparable to those found in unchallenged animals. Agglutinins against *C. albicans* were not detected in test or control chicks. Chicks with a diflora containing *E. coli* and *C. albicans* had a few microscopic crop lesions containing small numbers of hyphae. *C. albicans* was well established in the gut of

these animals, largely as the yeast form. The numbers of *E. coli* in the gut were similar to those in control chicks. Thus, it was concluded that *E. coli*provided protection against crop infection by *C. albicans*. In crop contents from unchallenged animals, chicks with *S. faecalis* monoflora were about *p*H 5, whereas birds with *E. coli* monoflora were about *p*H 7. The challenge did not greatly change the former value, and the latter was slightly decreased. In the crop of unchallenged birds, negative E_h values were found in chicks with *S. faecalis* and positive E_h values in those with *E. coli*. Challenge did not greatly change these values. These data on *p*H and E_h were related to conditions for morphogenesis of *C. albicans* and virulence. No major difference in the concentrations of serum proteins was seen in chicks with *E. coli* or *S. faecalis* after challenge with *C. albicans*. Possible mechanisms of the protective effect of *E. coli* are discussed.

Kuttin *et al.* (1975).described chicken dermatitis and loss of feathers from *Candida albicans.*

Wyatt and Hamilton (1975) mentioned that a mycological survey of the crops of approximately 100 healthy birds from each of 6 grow-out operations revealed that the incidence of Candida in the crops ranged from 17.4 to 51.5% with a mean value of 32.3%. The population of Candida in the crops of birds found positive was of low magnitude in the majority of the chickens examined. Of the 573 birds examined in this study less than 1% exhibited visible lesions attributable to Candida. C. albicans comprised 95% of the isolates while C. ravautii, C. salmonicola, C. gulliermondi, C. papapsilosis, C. catenulata and C. brumptii comprised the remainder. The incidence and number of Candida in the crop was related apparently to management practices on the farm. The crops from four field outbreaks of crop mycosis were also studied. Three of the four cases of crop mycosis were characterized by multiple strains of C. albicans in the crop. In one case, C. parapsilosis also was isolated from the crop.

Kuttin (1976) described an epidemic of dermatitis affecting the skin of the back and thighs of chickens in Israel. The causal organism was shown to be Candida albicans.

Crispin and Barnett (1978). described cases of ocular candidosis in ornamental ducks.

Panigrahy *et al.* (1979) described death of cockatiel nestlings caused by ***Candida albicans***,. Lesions in the cockatiels were pseudomembranes and ulcers in the mouth, esophagus, and crop.The adult cockatiels were emaciated. At necropsy, no gross pathologic lesions were observed. However, the 3 nestlings had pseudomembranes in the mouth, esophagus, and crop. Smears made from the necrotic epithelial surfaces revealed large numbers of oval and budding yeast cells. The owner was asked to clean up the premise and treat the nursing adults for 4 to 5 days with copper sulfate at 1:2,000 in drinking water.The nestlings continued to die, and 2 months later the owner submitted a live 3-week-old cockatiel. At necropsy, ulcerlike patches were noticed in the oral cavity, esophagus, and crop. Wet mounts from the necrotic epithelium of crop and upper intestine revealed numerous oval and budding yeast cells. Treatment for 10 days with nystatin at a 1 g per 20 lb of feed was recommended.

Bacteriologic cultures on blood agar were made from liver, heart, crop, and intestine. Forty-eight hours after incubation at 37C, pure cultures of nonhemolytic yellowish colonies not unlike those of Staphylococcus aureus were obtained on all blood-agar plates. Examination of Gram-stained preparations revealed Gram positive yeast cells. The organism was grown in 1 ml of inactivated fetal calf serum at 37 C (3). Microscopic examination of the growth after 5 hours of incubation revealed germ tubes characteristic of Candida albicans. Growth in Sabouraud's broth and fermentation of dextrose, maltose, lactose, and sucrose were tested. In Saubouraud's broth, growth occurred at the bottom, and fermentation with acid production occurred in dextrose, maltose, sucrose, but not in lactose. The characteristic gross lesions, demonstration of germ tubes in serum medium, and fermentation reactions suggested that C. albicans was the cause of the deaths of the cockatiel nestlings.

The nursing adult cockatiels were probably the source of infection in the nestlings, although the disease could not be positively diagnosed in these birds. The young cockatiels were presumably more susceptible to the disease than the adults. Candidiasis in several young cockatiels from an aviary was diagnosed in this laboratory on 14 October 1976. The clinical features were persistent death losses, ulcerlike lesions in the esophagus and crop, and erosion of the gizzard lining.

Schmidt *et al.* (1985) described an outbreak of ocular candidiasis in ornamental ducks kept privately in Norfolk. Ocular signs included small lesions on the nictitating membrane, keratitis and intraocular infection. A method of successful treatment is given and attention is drawn to the importance of an abnormal environment in the pathogenesis of this condition.

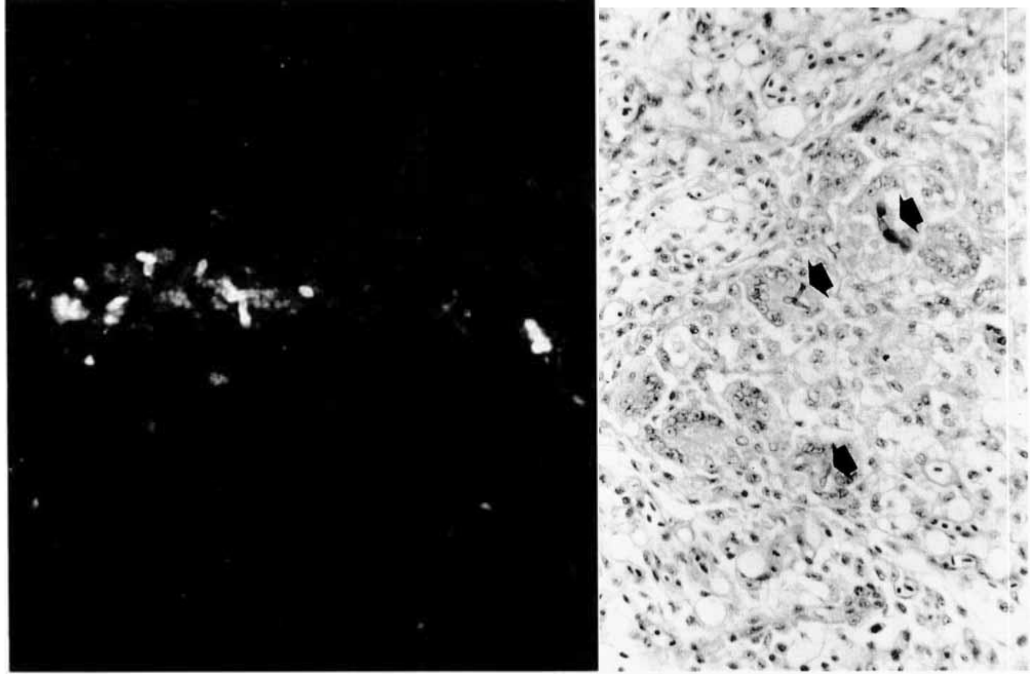
TSAI *et al.* (1992) encountered high incidences of candidiasis (15.4%) in 241 psittacines and passeriformes which died within 2 weeks of quarantine after being imported into Japan. Candidiasis was observed in 35 psittacines and two finches. The prevalence of this infection was as follows; rosellas from Holland (50%)>Amazons from Argentina (31.6%)>lovebirds from Taiwan (25.0%)>parakeets from India (22.4%)>parakeets from Philippine (12.8%) > lories from Indonesia and finches from Taiwan (ll.l%)>cockatiels from Taiwan (5.8%). The disease involved various organs including the respiratory system (24 cases; 64.9%), digestive tract (20 cases; 54.1%) and skin (2 cases; 5.4%). Out of them, eight birds had candidial lesions in both respiratory and digestive organs.

The affected organs of respiratory candidiasis were as follows; nasal cavity (22 cases; 91.7%)>larynx and lung (2 cases; 8.3%)>trachea (one case; 4.2%). Nasal cavity seemed to be the target site for candidial infection, and especially the vestibular region was involved in all infected cases. Candida spp. often invaded into this region leading to hyperkeratosis and desquamation of the superficial layer. All cases had numerous blastospores in the desquamated keratin. Six birds showed pseudohyphae invading into the lamina propria where there was induced a mild to severe cellular reaction, mainly consisting of heterophils and macrophages. Both blastospores and pseudohyphae sometimes aggregated on the mucosal surface of respiratory region without inducing apparent cellular reaction. Candida spp. frequently attacked the

upper digestive tract. The involved organs were as follows; crop (17 cases; 85.0%)>oesophagus (12 cases; 60.0%)>proventriculus and gizzard (6 cases; 30%)>small intestine (2 cases; 10%). The histological changes of the digestive tract were similar to those described in the respiratory tract.

Asrani *et al.* (1993) produced candidiasis experimentally in young Japanese quail by oral administration of Candida albicans cells. Lesions were confined to upper digestive tract with most characteristic changes occurring on the mucosa of crop. No lesions were observed in other tissues of the body. The initial changes in the crop were characterized by thickening and yellowish-white necrotic plaques on the mucosa. From 10th day onwards, there was marked thickening and corr.ugations of the crop mucosa giving it a typical 'turkish towel' appearance. Varying degree of mucosal swelling was also observed in the oesophagus and proventriculus. Two of the infected birds also revealed yellowish-white necrotic plaques on the tongue at 7th and 10th day post-infection. The prominent microscopic lesions in the crop and tongue consisted of hyperkeratosis and parakeratosis with congestion of the subepithelial tissues. Varying degree of parakeratosis and epithelial hyperplasia coupled with subepithelial oedema and hypertrophy of glands was observed in the oesophagus. The proventriculus and small intestine revealed congestion, oedema, mild to marked goblet cell hyperplasia and focal epithelial sloughing. Fungal elements could be demonstrated in the sections of tongue upto 10 days while in crop upto 14 days postinfection. Reisolation of the fungus was consistently achieved from the crop of infected birds throughout the duration of the experiment.

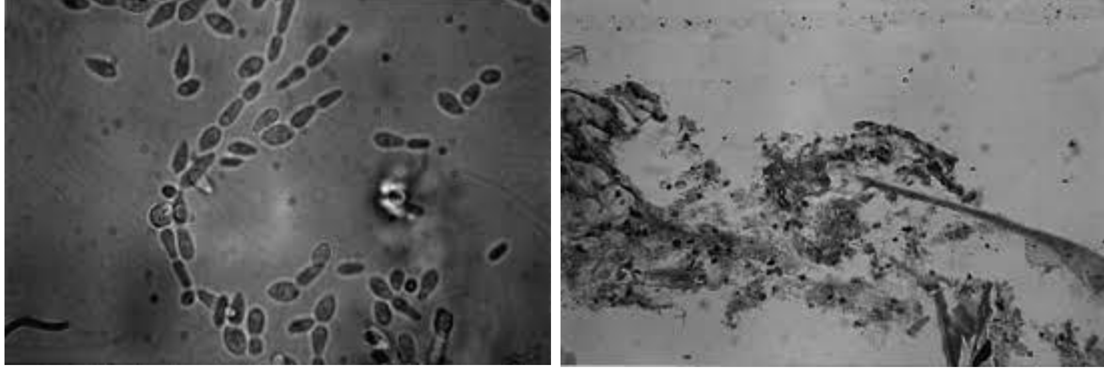
Carrasco *et al.* (1993) diagnosed systemic candidosis immunohistochemically in two Amazon parakeets (Amazona aestiua). In the bird with systemic candidosis, subacute necrotic lesions were present in the lung and the gastrointestinal tract, whereas chronic giant cell-containing granulomas were located in the liver, heart, spleen and on the serosal lining of the small intestine. Although the lesions in the liver, heart and spleen most likely developed as a result of haematogenous spread, the granulomas on the serosal surface may have developed after a local transmural intestinal invasion.



Intestinal candidosis in an Amazon parakeet (bird A). Within an area of local necrosis of the epithelial lining of the small intestine fungal elements are strongly stained by a specific polyclonal anti-Cundidu antibody. Indirect immunofluorescence, x 480. Peritoneal granulomatous candidosis in an Amazon parakeet (bird A). Within some of the giant cells pseudohyphae arc seen
(arrows). PAS, x 480.

Moretti *et al.* (2000) described the isolation of Candida rugosa from 6 weeks old turkeys that died 10 days after the end of a therapeutic treatment for an outbreak of coccidosis. Macroscopic examination of the birds showed caseous material and small pseudo-caseous nodules in the mucosa of glandular stomach, crop and abdominal air sacks, *C. rugosa* was isolated from all the birds examined. In 12 of these birds, *C. rugosa* was isolated in association with *C. albicans* and *C. tropicalis*, *C. albicans* and *C. guilliermondii*, *C. albicans* and *Trichosporon pullulans* and *C. albicans* and *C. famata*. Histological examination of samples from lung, liver and kidney did not show important lesions. Areas of inflammation were seen in the mucosal epithelium of glandular stomach and crop in four of the eight birds positive for *C. rugosa* alone, and in five of the 12 birds positive for yeast associations. Lesions were characterized by infiltration of mononuclear cells, including macrophages, lymphocytes and plasma cells, with localized granulomatous reaction and necrosis of enterocytes. In the contest of the mucosa of the glandular stomach a large number of yeast cells was seen in association with degeneration of enterocytes.

Turkey crop showing caseous material and white nodules (arrows) in the mucosa. White nodules (arrows) in abdominal air sacks



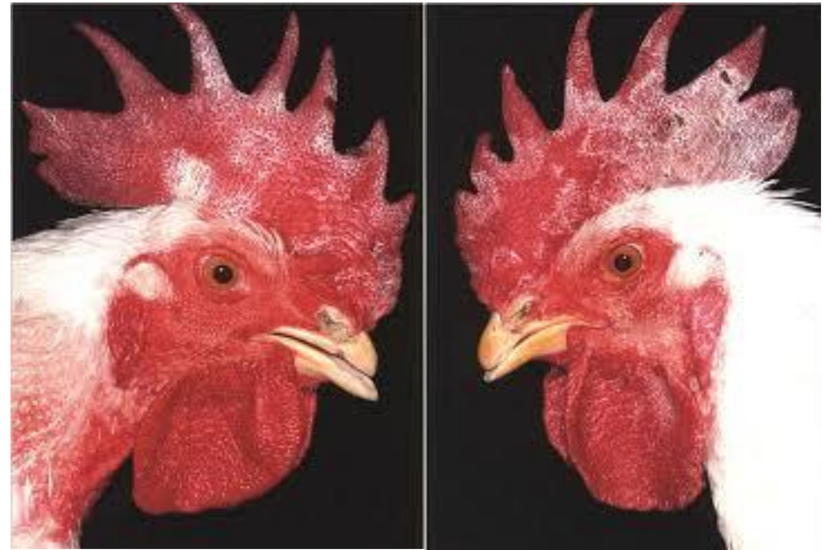
Unstained smear of C. rugosa cells. Epithelial mucosa of glandular stomach. Presence of numerous C. rugosa cells and degeneration of the mucosal cells (PAS, × 32).

Velasko (2000) mentioned that candidiasis and cryptococcosis are the 2 most common yeast infections of birds. Although most commonly thought to be opportunistic fungi, primary disease may be caused by either yeast. The biology, epidemiology, diagnosis, and treatments for these two disease conditions were discussed.

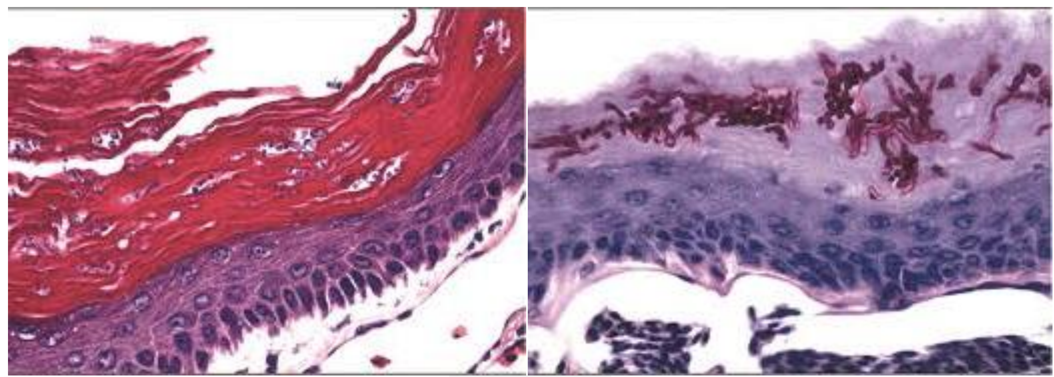
Sato *et al.* (2001) reported 2 cage birds, a two-month-old Fisher's lovebird (Agapornis fischeri) and a one-year-old budgerigar (Melopsittacus undulatus), that manifested clinical symptoms with general weakness, loss of appetite and ruffled feathers, then died. Pathological findings revealed a large quantity of yellowish-white pseudomembrane on the mucosal membrane of the esophagus and crop in these twobirds. Histopathologically, blastospores (5.5 mum long x 3.4 mum wide) and pseudohyphae were detected in the lesions of conspicuous parakeratosis and moderate acanthosis in the stratified squamous epithelium. These two birds were diagnosed as having had candidiasis.

Fulleringer *et al.* (2006) detected *C. albicans* at wk 4 from litter samples and at wk 7 from poultry feed. Densities of *C. albicans* remained very high in litter samples (63.2 cfu/g) even after new litter was added at wk 10. *C. albicans* was also isolated from many environmental samples (68.7%), including air samples from wk 4 to the end of the study.

Osorio *et al.* (2007) described a cutaneous mycosis caused by Candida albicans that involved the combs and less frequently the wattles, facial skin, ear lobes, and neck of male broiler breeders is described. Roosters were 35 wk old and housed with hens in two conventional broiler breeder houses on a farm in western North Carolina. Morbidity was approximately 10% in one house and less than 2% in the other house. Mortality and flock fertility were not affected. Three birds from the most affected house were examined. All birds had white adherent material on their combs that presented as crusty patches or lighter diffuse areas. Often, lesions were roughly circular or had a defined margin. Small black scabs were present in a few lesions. Similar but less extensive lesions were located on the wattles, facial skin, ear lobes, and rictus. In one bird, lesions extended down the neck, and they were accompanied by hyperemia and feather loss. Hyperkeratosis with little to no inflammation and intralesional fungi occurring as yeast and pseudohyphae were seen microscopically. High numbers of C. albicans were isolated and identified from the lesions.



Comb Candidiasis Affecting Roosters in a Broiler Breeder Flock ...www.jstor.org



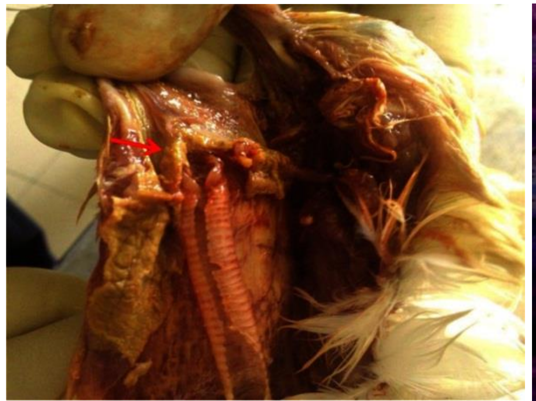
www.jstor.org

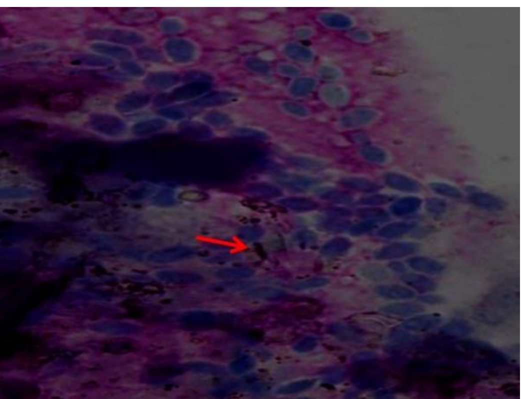
Nouri and Kamyab (2010) described a young Fisher's lovebird (Agapornis fischeri), with general weakness, diarrhoea, ruffled feathers and unilateral extrarhinoectasia and died finally. Gross necropsy revealed marked edema, congestion and hemorrhage on the distal part of the gizzard and proximal part of the duodenum. On microscopic examination, a large number of oval budding yeast-like fungi were observed in a stained smear sample from the gizzard and duodenum by the Giemsa method. Histopathologically, there was epithetlial necrosis in the proventriculus and gizzard and inflammation of the proximal intestine. The pseudohyphae and budding yeast-like organisms were most numerous on the surface and extended deep into the submucosa and muscularis layer. Severe hemorrhage, vasculitis with invasion of fungal organisms into the vessels' wall associated with infiltration of inflammatory cells were observed. The disease was diagnosed as candidiasis in the middle part of digestive tract

Tiwari *et al.* (2012) mentioned that yeast like fungi as the causative agents of intestinal tract infections were recognized in humans during early 1800s. These mycotic infections were frequently associated with poor hygiene. A fungal organism then named *Monilia albicans* was reported by Lagenbeck to be associated with most cases of thrush in chickens and turkeys. The 3rd International Microbiological Congress in 1839, decided to replace the older generic name *Monilia* with the term *Candida.* Candidiasis has emerged as a very commonly diagnosed disease in

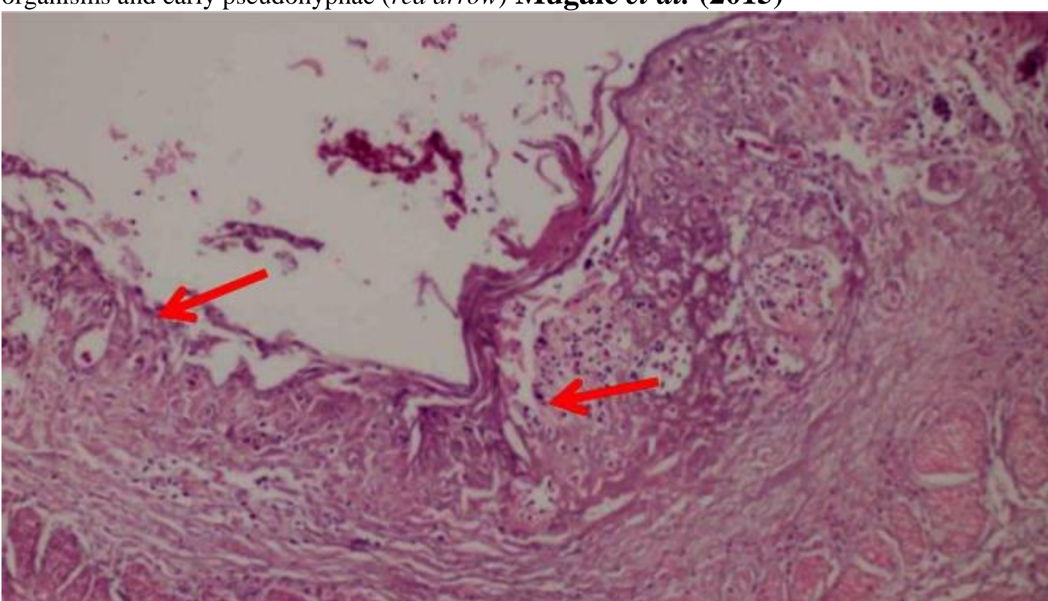
most of the avian species like chicken, turkeys, guinea fowl, quails etc. *Candida albicans,* the causative agent, is capable of causing both superficial and deep infections. Although commonly thought to be a secondary invader, it has been documented as the causative organism in primary disease of many avian species. The disease is usually associated with unhygienic surroundings and secondary debilitating conditions.

Mugale *et al.* (2015) reported a hundred pigeons that were unable to feed properly and regurgitate feed. Birds lost body condition gradually, and three among these died. Both alive and dead pigeons were cachectic with wasting of breast muscles. On necropsy, no significant gross lesions were recorded in most of the visceral organs, except mottling of the liver. However, in the oral cavity, gray Turkish towel-like lesions were seen at the opening of the pharynx which continued into the larynx and proximal esophagus. Microscopic examination of material scrapped from lesions revealed a large number of budding yeast-like organisms and pseudohyphae, suggestive of *Candida* spp. Histologically, marked necrosis and sloughing of oral and esophageal mucosal epithelium with the presence of pyogranulomatous inflammation containing a large number of *Candida* organism were observed..

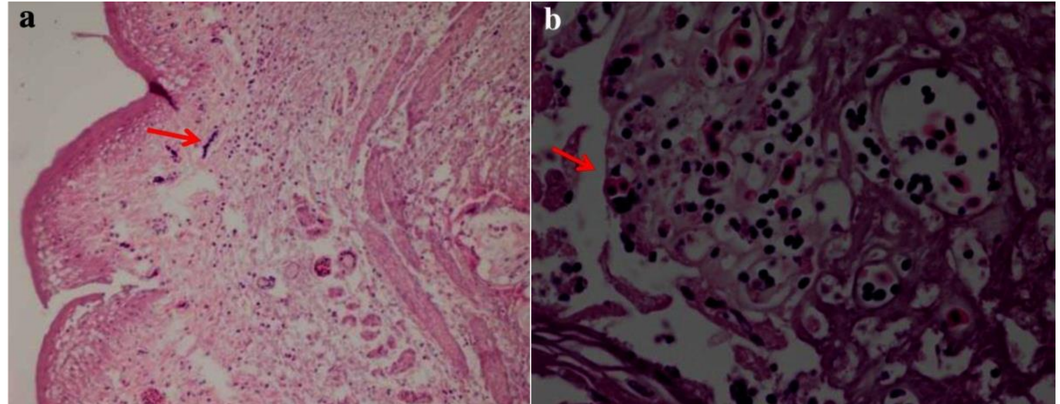




Pseudomembrane on esophagus, pharynx, and larynx (*red arrow*), Large number of yeast-like organisms and early pseudohyphae (*red arrow*) **Mugale *et al.* (2015)**



Section of esophagus showing marked sloughing of epithelium and inflammation (*red arrow*)



a Section of esophagus showing hyperkeratosis and candida organism (*red arrow*, ×10). **b** Higher magnification of **a** showing division of and candida organism (*red arrow*, H & E, ×40) **Mugale *et al.* (2015)**

Reports on Candida species and other yeasts isolated in association with poultry

Mancianti *et al.* (2002) cultured 325 droppings from parrots raised in the premises of 4 breeders and in several private households for yeasts. One-hundred sixty droppings (49.2%) resulted positive. From these specimens 212 isolates belonging to 27 different species were obtained. Mainly Candida species such as C. albicans, C. catenulata, C. curvata, C. famata, C. glabrata, C. guilliermondi, C. holmii, C. intermedia, C. krusei, C. lambica, C. lusitaniae, C. membranaefaciens, C. parapsilosis, C. pelliculosa, C. sake and C. valida were isolated. Debarvomyces marama, D. polymorphus, Geotrichum sp., Pichia etchelsii, P. ohmeri, Rhodotorula glutinis, R. rubra, Rhodotorula sp., Saccharomyces cerevisiae, S. kluyiveri and Zygosaccharomyces sp. were also obtained. Dark colonies on Staib medium were never observed. The psittacine birds apparently serve as carriers for several Candida species or their perfect states and to a lesser extent for other opportunistic yeasts such as Rhodotorula, Trichosporon and Saccharomyces spp., which are considered part of the transient microbiota of the gastrointestinal tract. The most striking finding was the absence of Cryptococcus spp. among the isolates. The present survey confirms the role of pet birds in carrying potential zoonotic yeasts.

Grundet *et al.* (2005) examined 500 **combs** of adult chickens from two different locations in Germany (Hessen and Schleswig-Holstein) clinically and mycologically. The chickens came from three battery cages (n = 79), one voliere system (n=32), six flocks maintained on deep litter (n = 69) and 12 flocks kept on free outdoor range (n=320). Twenty-two of the 500 chicken combs (4.4%) were found to have clinical signs: only non-specific lesions neither typical of mycosis nor of avian pox such as desquamation with crust formation, yellow to brown or black dyschromic changes, alopecia in the surrounding area and moist inflammation. Only seven of the 22 clinically altered combs showed a positive mycological result; the non-pathogenic and geophilic Trichophyton terrestre in one case and non-pathogenic yeast in six cases. The following fungi were seen in the different housing systems: 13 dermatophytes (2.6% of 500 samples): 12 x T. terrestre, 1 x Trichophyton mentagrophytes, 11 isolates of Chrysosporium georgiae (2.2% of 500 samples) and 149 isolates of yeasts (29.8%): Malassezia sympodialis: n = 52, Kloeckera apiculata: n = 33, Trichosporon

capitatum (syn. Geotrichum capitatum): n = 23, Trichosporon cutaneum/Trichosporon mucoides: n = 12, Trichosporon inkin (syn. Sarcinosporon inkin): n = 8 and Candida spp.: n = 21, including pathogenic or possibly pathogenic species: Candida albicans: n = 3, Candida famata: n = 4, Candida guilliermondii: n = 3, Candida lipolytica: n = 3, Candida dattila: n = 2 and one isolate each of Candida glabrata, Candida parapsilosis, Candida aaseri, Candida catenulata sive brumpti, Candida fructus and Candida kefyr sive pseudotropicalis. There is no stringent correlation between the clinical symptoms diagnosed on the chicken combs and the species of yeasts isolated. The causative agent of favus in chickens, Trichophyton gallinae, and the saprophytic yeast in pigeons, Cr. neoformans were not isolated. The most frequently isolated yeasts M. sympodialis and Kloeckera apiculata are suggested to be classified as members of the resident flora of the chicken comb.

Carfachia *et al*. (2006) studied the yeasts present in the cloacae of 421 wild birds (24.39% out of 1726 birds caught in Romania, Hungary and Bulgaria). Samples were collected directly from the cloacae and cultured, and colonies were identified in each positive sample. Yeasts were isolated from 15.7% of the birds sampled, with the highest percentage found in coots (Fulica atra -58.8%) and the lowest in quails (Coturnix coturnix -1.7%). A total of 131 isolates belonging to 15 species of yeast were identified. Rhodotorula rubra was the yeast with the highest number of isolates (28.2%), followed by Cryptococcus albidus (18.4%), Candida albicans (9.2%), Trichosporon cutaneum (8.4%), Candida guilliermondii (6.1%), Candida tropicalis (6.1%) and other species. The present study represents the first survey on the occurrence of yeasts in the cloacae of migratory birds. The prevalence and species of yeasts isolated is discussed on the basis of the ecology, diet, and habitat of the birds.

Musgrove *et al.* (2008) collected washed and unwashed eggs (treatments) aseptically on three separate days (replications) from a commercial processing facility and stored for 10 weeks at 4 degrees C. Ten eggs from each treatment were sampled weekly (110 eggs per treatment per replication). Yeasts and moulds were enumerated from external shell rinses by plating onto acidified potato dextrose agar. Yeast colonies were picked randomly and stored for subsequent identification by gas chromatographic analysis of fatty acid methyl esters using the MIDI Microbial Identification System. Of 688 isolates analyzed, 380 were identified to genus or species. Genera identified by this method included Candida, Cryptococcus, Hansenula, Hyphopichia, Metschnikowia, Rhodotorula, Sporobolomyces, and Torulaspora. Candida spp. accounted for 84.5% (321 of 380) of the isolate identifications. Candida famata was the most prevalent species (n = 120), followed by Candida lusitaniae (n = 38). A group of 20 isolates was subjected to molecular or biochemical analyses for comparison with the MIDI results. Biochemical tests were performed using automatic and mini systems. Results of biochemical tests and ribosomal DNA sequencing were in agreement for 11 of the isolates, but only 7 of the 20 MIDI-identified isolates were in agreement with the sequencing results. C. famata, an anamorph of Debaryomyces hansenii var. hansenii, was the most commonly identified isolate by all methods. These data indicate that there was limited correlation between results obtained with the MIDI system and the information obtained from molecular databases. However, both systems were able to correctly identify C. famata, the species most often isolated throughout egg storage.

Costa *et al.* (2010) carried out a study to investigate pigeons as a potential source of pathogenic yeast species, 47 samples of pigeon droppings and 322 samples from pigeon cloacae were evaluated. The samples were also collected from trees located near the pigeon habitats, in the city of Fortaleza, Ceará, Northeast Brazil. In addition, we evaluated the in vitro antifungal susceptibility of these environmental Cryptococcus strains to amphotericin B, azoles and caspofungin. C. neoformans var. neoformans (n = 10), C. laurentii (n = 3), Candida spp. (n = 14), Rhodotorula mucilaginosa (n = 6) and Trichosporon sp. (n = 3) were isolated from pigeon droppings. In contrast, only Candida spp. (n = 4), Trichosporon sp. (n = 3) and R. mucilaginosa (n = 2) were recovered from cloacae specimens. Only Candida glabrata (n = 1) was recovered from plant samples. Azole resistance was detected in only one environmental strain of Cryptococcus, which was resistant to itraconazole (MIC = 1 microg/ml). As expected, all Cryptococcus strains were resistant to caspofungin. In summary, the present study confirms that urban pigeons are a potential source of Cryptococcus spp. and other pathogenic yeasts. Additionally, antifungal resistance was observed in one environmental strain of Cryptococcus neoformans var. neoformans in Northeast Brazil.

AL-Shimmery (2011) recovered 58 yeast isolates belonging to 3 genera and 6 species from the intestinal tracts of 35 out of 50 birds. The occurrences of individual yeast species were Saccharomyces (31.03 %), Candida glabrata (20.69 %), C. tropicalis (15.51 %), C . albicans (15.51 %), C . fmata and Creptococcus neoformans (8.62%) .
Kemoi (2012) carried out a study to isolate and characterize pathogenic yeasts from domestic Chicken (Gallus gallus) droppings. The droppings were collected from Kabigeriet Villages, Olenguruone Division, Kuresoi District and Nakuru County. The samples were collected from cages, houses and roosting sites. The samples (droppings and soil) were collected by swabbing or scooping fresh dropping from Chicken houses, grass, soil and trees using sterile plastic spoons, labeled and inserted in a zip lock safety bag. A total of 84 samples (dropping and soil enriched with chicken droppings) were sampled during the study. The droppings were tested for Cryptococcus by direct plating on Niger seed while Candida and Saccharomyces species by direct plating on Typan blue agar. Candida and Saccharomyces species were sub cultured on CHROM agar and Corn meal agar for presumptive identification of various Candida species. Cryptococcus neoformans were sub cultured onto Christensen"s urease agar. Geotrichum species were presumptively identified by lactophenol cotton blue. Analytical profile index test (API 20C AUX) was used for confirmation. Four types of yeasts were isolates; 35(57.4%) Candida species (9 Candida lusitanie, 7 Candida glabrata, 5 Candida albicans, 5 Candida tropicalis, 3 Candida parapsilosis, 2 Candida lipolytica and 2 Candida krusei), 23(37.7%) Geotrichum candidum, 2(3.3%) Cryptococcus species (Cryptococcus neoformans and Cryptococcus laurenti) and 1(1.6%) Saccharomyces cerevisiae were isolated from Chickens dropping sampled.

Rad (2013) isolated Candida unigattulatus 4 cases (5.72%), Candida laurentii 3 cases (4.28%), Candida albidus 2 cases (2.86%) and Candida humicola 1 cases (1.43from the excreta of 50 ppigeons collected in Qazvin

Soltani *et al.* (2013) examined 120 samples of pigeon droppings for *Candida* species. The identification was based on the presence of a capsule on India ink preparation, urease production on urea agar medium and RapID yeast plus system. The

identification of *candida* species was based on micro-morphological analysis on corn meal-Tween 80 agar, RapID yeast plus system and growth in CHROMagar *candida*. The frequency rate of *Candida albicans* was 6.6%

Mendes *et al.* (2014) investigated the presence of potentially pathogenic fungi in the feces of wild birds collected in Screening Centers. Samples were collected from the feces of 50 cages with different species of birds. The samples were processed according to the modified method STAIB and the plates incubated at 32 °C for up to ten days with daily observation for detection of fungal growth. The isolation of the following species was observed: Malassezia pachydermatis, Candida albicans, C. famata, C. guilliermondii, C. sphaerica, C. globosa, C. catenulata, C. ciferri, C. intermedia, Cryptococcus laurentii, Trichosporon asahii, Geotrichum klebahnii, Aspergillus spp., A. niger and Penicillium spp. Knowing the character of some opportunistic fungi is important in identifying them, facilitating the adoption of preventive measures, such as proper cleaning of cages, since the accumulation of excreta may indicate a risk for both health professionals and centers for screening public health.

References:

1. Asrani , R.K, R.K. Paul Gupta, J.R. Sadana & Ajay Pandita. Experimental candidiasis in Japanese quail: Pathological changes. Mycopathologia 121: 83-89, 1993
2. BALISH, EDWARD AND A. W. PHILLIPS. Growth and virulence of *Candida albicans* after oral inoculation in the chick with a monoflora of either *Escherichia coli* or *Streptococcus faecalis*. J. Bacteriol. **91:**1744–1749. 1966.
3. BLAXLAND, J.D. & FINCHAM, I.H. (1950). Mycosis of the crop (moniliasis) in poultry, with particular reference to serious mortality occurring in young turkeys. *British Veterinary Journal,* 106, 221-231.
4. Carfachia, C., A. Camarda, D. Romito, M. Campolo, N.C. Quaglia and D. Tullio, 2006. Occurrence of yeasts in cloacae of migratory birds. Mycopathologia, 161: 229-234.
5. Carrasco, L., Gomez-Villamandos, J.C. and Jensen, H.E. (1998) Systemic candidosis and concomitant aspergillosis and zygomycosis in two Amazon parakeets (Amazona aestiva). Mycoses 41, 297-301
6. Costa AK, Sidrim JJ, Cordeiro RA, Brilhante RS, Monteiro AJ, Rocha MF. Urban pigeons (Columba livia) as a potential source of pathogenic yeasts: a focus on antifungal susceptibility of Cryptococcus strains in Northeast Brazil. Mycopathologia. 2010 Mar;169(3):207-13.
7. CRISPIN, S.M. & BARNETT, K.C. (1978). Ocular candidiasis in ornamental ducks. *Avian Pathology,* 7, 49-59.
8. Fulleringer ,S.L, D. Seguin, S. Warin, A. Bezille, C. Desterque, P. Arné, R. Chermette, S. Bretagne,and J. Guillot. Evolution of the Environmental Contamination by Thermophilic Fungi in a Turkey Confinement House in France Poultry Science (2006) 85 (11): 1875-1880
9. Grundet S., Mayser P., Redmann T., Kaleta E.F., (2005). Mycological examination on the fungal flora of the Chicken comb. Mycoses, 48:114-119.
10. Jungherr, E.L., 1933. Observations on a severe outbreak of mycosis in chicks. J. Agric., 2: 169-178.
11. Kemoi EK, Okemo P, Bii CC. PRESENCE OF CRYPTOCOCCUS SPECIES IN DOMESTIC CHICKEN (GALLUS GALLUS) DROPPINGS AND THE POSSIBLE

RISK IT POSED TO HUMANS IN KABIGERIET VILLAGE, NAKURU COUNTY, KENYA. East Afr Med J. 2013 Jun;90(6):202-6.

12. KUPROWSKI, MARIAN. On the pathogenesis and morphology of moniliasis in Poultry. Deutsche Tierarztliche Wochenschrift 1960 Vol. 67 No. 7 pp. 185-189
13. Kuttin, E.S., BEEMER, A.M. & MEROZ, M. (1975). Chicken dermatitis and loss of feathers from *Candida albicans. Avian Diseases,* 20, 216-218.
14. Kuttin ES, Beemer AM, Meroz M. Chicken dermatitis and loss of feathers from Candida albicans. Avian Dis. 1976 Jan-Mar;20(1):216-8.
15. Mancianti F, Nardoni S, Ceccherelli R. Occurrence of yeasts in psittacines droppings from captive birds in Italy. Mycopathologia. 2002;153(3):121-4.
16. Mayeda, B. (1961) Candidiasis in turkeys and chickens in the Sacramento Valley of California. Avian Disease 5, 232-243.
17. Mendes JF, Albano AP, Coimbra MA, Ferreira GF, Gonçalves CL, Nascente Pda S, de Mello JR. Fungi isolated from the excreta of wild birds in screening centers in Pelotas, RS, Brazil. Rev Inst Med Trop Sao Paulo. 2014 Nov-Dec;56(6):525-8.
18. Mohsen Nouri and Zahra Kamyabi, Occurrence of Ventricular Candidiasis in a Lovebird (Agapornis fischeri). Iranian Journal of Veterinary Science and Technology Vol. 2, No. 1, 2010, 51-56
19. Moretti A, Piergili Fioretti D, Boncio L, Pasquali P, Del Rossi E. Isolation of Candida rugosa from turkeys. J Vet Med B Infect Dis Vet Public Health. 2000 Aug;47(6):433-9.
20. Mugale, M., Bhat, A. A., Gavhane, D. S., & Bhat, S. A. (2015). Outbreaks of thrush in pigeons in Punjab State of India. *Comparative Clinical Pathology*,*24*(3), 635–638. http://doi.org/10.1007/s00580-014-1958-y
21. Musgrove MT, Jones DR, Hinton A Jr, Ingram KD, Northcutt JK. Identification of yeasts isolated from commercial shell eggs stored at refrigerated temperatures. J Food Prot. 2008 Jun;71(6):1258-61.
22. Nash, H. (2010) Candidiasis in birds: Signs, Treatment, and Prevention of Yeast Infections in Birds. http://www.peteducation.com/article.cfm.
23. Osorio, Claudia, Oscar Fletcher, Michael J. Dykstra, Karen Post and H. John Barnes, Comb Candidiasis Affecting Roosters in a Broiler Breeder Flock (Candidiasis de la cresta afectando machos en una parvada de reproductores de engorde). *Avian Diseases*. 51, 2 (Jun., 2007), 618-622
24. Panigrahy, B., Naqi, S.A., Grumbles, L.C. and Hall, C.F. (1979) Candidiasis in Cockatiel Nestlings and Mucormycosis in a Pigeon. Avian Disease 23, 757-760.
25. Rad, Samiee F. Isolation of Cryptococcus neoformans from pigeon excreta in Qazvin. Life Sci J 2013;10(1):214-219] (ISSN:1097-8135). http://www.lifesciencesite.com. 32
26. Sato, Y., Aoyagi, T., Kobayashi, T. and Inoue, J. (2001) Occurrences of candidiasis in a Fischer´s love bird and a Budgerigar. Journal of Veterinary Medical Science 63, 939-941.
27. Schmidt, R.E., Eisenbrandt, D., L., Witt, W.M. and Fletcher, K.C. (1985). Ocular candidiasis in ornamental ducks. Avian Pathology 7, 49-59. Hubbard, G.B.,
28. Soltani M, Bayat M, Hashemi SJ, Zia M, Pestechian N. Isolation of*Cryptococcus neoformans* and other opportunistic fungi from pigeon droppings.*Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences*. 2013;18(1):56-60.
29. Tiwari. R., M. Yakoob Wani and K. Dhama. Candidiasis (Moniliasis, Thrush or Sour crop) in Poultry : An Overview (2012), http://poultrytechnology.com/about-us/5.html
30. Tripathy SB, Mathey WJ, Kenzy SG. Study of aortic changes associated with candidiasis of turkeys. Avian Dis. 1965 Nov;9(4):520-30.

31. TSAI, S. S., J. H. PARK , K. HIRAI & C. ITAKURA Aspergillosis and candidiasis in psittacine and passeriforme birds with particular reference to nasal lesions. Avian Pathology (1992) 21: 699-709
32. UNDERWOOD PC. Detection of crop mycosis (moniliasis) in chickens and turkey poults with a panendoscope. J Am Vet Med Assoc. 1955 Sep;127(942):229-31.
33. Velasko, M.C., 2000. Candidiasis and Cryptococcosis in birds. Semin. Avian Exotic Pet Med., 9: 75-81.
34. Wind, S.; Yacowitz, H. Use of Mycostatin in the drinking water for the treatment of crop mycosis in turkeys. Poultry Science 1960 Vol. 39 pp. 904-905
35. Wyatt RD, Hamilton PB. Candida species and crop mycosis in broiler chickens. Poult Sci. 1975 Sep;54(5):1663-6.
36. Yacowitz, H.., S. Wind, W. P. Jambor, N. P. Willett, J. F. Pagano. Use of Mycostatin(R) for the Prevention of Moniliasis (Crop Mycosis) in Chicks and Turkeys. Poultry Science (1959) 38 (3):653-660

2.2. Avian Cryptococcosis

Introduction

Cryptococcosis, also known as Torulosis, Yeast meningitis, Busse-Buschke’s disease and European blastomycosis), is caused by *Cryptococcus neoformans* that affects animals including poultry and humans (**Singh and Dash, 2008; Dhama *et al.*, 2011**).

Infections in birds are rare. *C. neoformans* has been isolated from the faeces of canaries (26%), carrier pigeons (18%), budgerigars (2%) and psittacine birds (1%), apart from domestic poultry (**Saremi *et al.*, 2004; Singh and Dash, 2008**).

C. neoformans is known to inhabit natural environments such as soil and grows in bird excreta, especially that of pigeons (Ajello, **1958**; Denton and DiSalvo, **1968**; Yamamoto *et al.*, **1995a**, **1995b**).

Cryptococcus species isolated from avian droppings

1. ***Cryptococcus neoformans***
   (Abou-Gabal and Atia, 1978, Yilmaz *et al.*, 1989, Yamamoto *et al.*, 1995, Khosravi, 1997, Kuroki *et al.*, 2004, Faria *et al.*, 2010, ZARRIN *et al.*, 2010, AL-Shimmery, 2011, Ferreira-Paim *et al.*, 2011, Kemoi, 2012, Kemoi *et al.*, 2013, Soltani *et al.*, 2013, Takahara *et al.*, 2013, Tangwattanachuleeporn *et al.* , 2013, Teodoro *et al.*, 2013, Xavier *et al.*, 2013, Ior *et al.*, 2015)
2. ***Cryptococcus neoformans var neoformans***

(Khosravi, 1997, Filiu *et al.*, 2002, Baroni *et al.*, 2006, Costa *et al.*, 2010, Kuchak *et al.*, 2012, Kangogo *et al.*, 2014)

3. ***Cryptococcus neoformans var. grubii***
   (Granados *et al.*, 2005, Chee and Lee , 2005, Carvalho, *et al.*, 2007, Kuchak *et al.*, 2012)
4. ***Cryptococcus laurentii***
   (Costa *et al.*, 2010, Ferreira-Paim *et al.*, 2011, Kemoi (2012, Teodoro *et al.*, 2013, Mendes *et al.*, 2014)
5. ***Cryptococcus gattii***
   (Abegy *et al.* (2006**,**Teodoro *et al.*, 2013, Kangogo *et al.*, 2014)
6. ***Cryptococcus luteolus***
   (Teodoro *et al.*, 2013)
7. ***Cryptococcus ater***
   (Teodoro *et al.*, 2013)
8. **Cryptococcus species**
   Hamasha *et al*. (2004)

- Cryptococcosis seems to be very rare in pigeons. Racing pigeon may develop localized subcutaneous swelling or even disseminated lesions.
- Fatal C. gattii infections have been reported in captive kiwis. Extensive granulomatous pneumonia was found in two of these birds at necropsy, while the third had disseminated disease involving the heart, kidneys and proventriculus.
- Some psittacine birds with cryptococcosis have signs of an upper respiratory tract obstruction. These birds often have proliferative lesions, which may resemble neoplasia, around the beak or nares.
  - The infection can progress to involve structures close to the nasal cavity, such as the rhamphotheca, nasopharynx, palate and sinuses.
  - Severe invasive or disseminated disease affecting the lung, air sacs, CNS or other internal organs has been reported in a few psittacines.

Cryptococcus species reported from infected birds

- *Cryptococcus neoformans* was reported to be associated with the trachea of fowls, isolated from broilers of a poultry-processing plant **Laubscher *et al.* (2000**).
- *Cryptococcus* **laurentii** was reported to be associated with feather loss in a glossy starling (Lamprotornis chalybaeus). The bird exhibited patchy feather loss, especially around the back and beak area, and greyish crusts sticking quite firmly to the underlying skin. The feathers had a greasy appearance and disseminated a musty odour. Treatment was installed with fluconazole in the drinking water. One month following the onset of treatment, the condition of the plumage had markedly improved **Decostere *et al.* (2003).**.

- ***Cryptococcus. gattii*** produced localized invasive disease of the upper respiratory tract of captive parrots living in Australia. This resulted in signs referable to mycotic rhinitis or to involvement of structures contiguous with the nasal cavity, such as the beak, sinuses, choana, retrobulbar space and palate. Cryptococcus appeared to behave as a primary pathogen of immunocompetent hosts (**Malik *et al.* (2003)**.
- ***C. neoformans var. grubii*** was isolated from a tissue specimen from an Australian racing pigeon with minimally invasive subcutaneous disease; and was demonstrated immunohistology in the subcutis tissues (**Malik *et al.* (2003)**..
- Two similar cases had been reported in pigeons domiciled in America. Data for parrots, one pigeon and other birds studied principally in America and Europe (and likely infected with ***C. neoformans***) suggested a different pattern of disease, more suggestive of opportunistic infection of immunodeficient hosts. Infections typically penetrated the lower respiratory tract or disseminated widely to a variety of internal organs (**Malik *et al.* (2003)**...
- Three captive North Island brown kiwis, one residing in Australia, the other two in New Zealand, died as a result of severe diffuse **cryptococcal pneumonia** (two cases) or widely disseminated disease (one case). ***Cryptococcus. gattii*** strains were isolated from all three cases, as reported previously for another kiwi with disseminated disease in New Zealand (**Malik *et al.* (2003)**..
- **Cryptococcus gattii** was reported in a 14-yr-old female Pesquet's parrot (Psittrichas fulgidus) with lethargy and decreased ability to fly. Physical exam was unremarkable. Blood work showed an elevated white blood cell count and a strong positive Aspergillus galactomannan titer. Empirical Aspergillus treatment was initiated with compounded generic itraconazole. Radiographs revealed an irregular osteolytic lesion isolated to the distal right humerus. Bone biopsy of the lesion, cytology, and histopathology were diagnostic for osteomyelitis with intralesional yeasts confirmed to be on fungal culture. After 2 mo of compounded itraconazole treatment, the bird developed dyspnea and dysphagia due to new Cryptococcus lesions in the proximal trachea and glottis. **(Molter *et al.* (2014)**

Description of main Cryptococcus species reported in association with birds

1. Cryptococcus neoformans (San Felice) Vuillemin, 1901

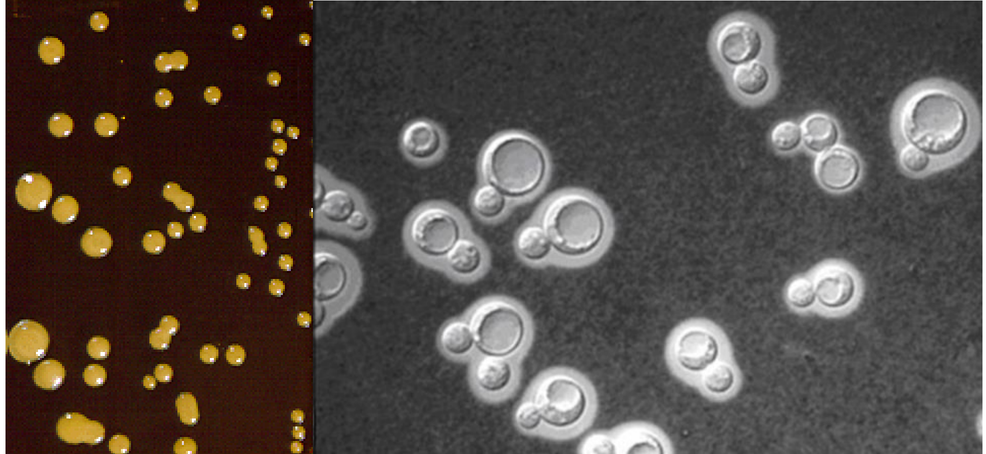
Synonyms:

1. *Saccharomyces neoformans San Felice, Annali Ig. Sperim.: 241 (1895)*
2. *Torula neoformans* (San Felice) J.D. Weis, Journal of Medical Research 7 (1902)
3. *Blastomyces neoformans* (Vuill.) Arzt, Archiv Dermatolo und Syphilis 145: 311 (1924)
4. *Debaryomyces neoformans* (San Felice) Redaelli, Cif. & Giordano, Boll. Sez. Ital. Soc. Int. Microbiol.: 24 (1937)
5. *Lipomyces neoformans* (San Felice) Cif., Manuale de Micologica Medica 2: 214 (1960)
6. *Torulopsis neoformans var. sheppei* A. Giord.
7. *Saccharomyces hominis* Costantin, Bulle. Soc. Mycol. de France 17: 145-148 (1901)

8. *Cryptococcus guilliermondii* Beauverie & Lesieur, Journal de Physiologie et Pathologie Général 14 (1912)
9. *Torula histolytica* J.L. Stoddart & Cutler, Studies from the Rockefeller Institute for Medical Research (1916)
10. *Torulopsis neoformans var. neoformans* (1931)
11. *Cryptococcus neoformans var. grubii* Franzot et al., J. Clin. Microbiol. 37: 839 (1999)

Morphology

Colonies of *Cryptococcus neoformans* are fast growing, soft, glistening to dull, smooth, usually mucoid, and cream to slightly pink or yellowish brown in color. The growth rate is somewhat slower than *Candida* and usually takes 48 to 72 h. It grows well at 25°C as well as 37°C. Ability to grow at 37°C is one of the features that differentiates *Cryptococcus neoformans* from other *Cryptococcus* spp. However, temperature-sensitive mutants that fail to grow at 37°C in vitro may also be observed . At 39-40°C, the growth of *Cryptococcus neoformans* starts to slow down.



Cryptococcus neoformans colonies and capsules

Micromorphology

On cornmeal tween 80 agar, *Cryptococcus neoformans* produces round, budding yeast cells. No true hyphae are visible. Pseudohyphae are usually absent or rudimentary. The capsule is best visible in India ink preparations. The thickness of the capsule is both strain-related and varies depending on the environmental conditions. Upon growth in 1% peptone solution, production of capsule is enhanced

Physiological data

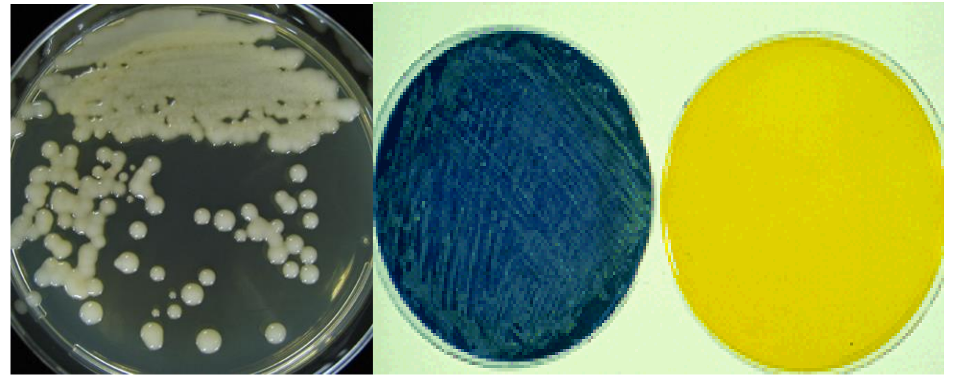
C1 D-Glucose+	C20 Melezitose+	C39 Succinated
C2 D-Galactose+	C21 Inulind	C40 Citrate-
C3 L-Sorbose+	C22 Starch+	C43 Propane 1,2 diold
C4 D-Glucosamine+	C23 Glycerol-	C44 Butane 2,3 diol-
C5 D-Ribose+	C24 Erythritol+	C45 Quinic acid-
C6 D-Xylose+	C25 Ribitol+	C46 D-glucarate+
C7 L-Arabinose+	C26 Xylitol+	C47 D-Galactonated
C8 D-Arabinose+	C27 L-Arabinitol+	N1 Nitrate-
C9 L-Rhamnose+	C28 D-Glucitol+	N2 Nitrite-
C10 Sucrose+	O3 Acetic acid 1%-	N3 Ethylamine+
C11 Maltose+	C29 D-Mannitol+	N4 L-Lysine+
C12 a,a-Trehalose+	C30 Galactitol+	N5 Cadaverine-
C13 Me a-D-Glucoside+	C31 myo-Inositol+	N6 Creatine-

C14 Cellobiose+	C32 D-Glucono-1,5-lactone+	N7 Creatinine+
C15 Salicin+	C33 2-Keto-D-Gluconate+	N8 Glucosamine-
C16 Arbutin+	C35 D-Gluconate-	N9 Imidazole-
C17 Melibiose-	C36 D-Glucuronate+	N10 D-Tryptophan-
C18 Lactose-	C37 D-Galacturonate+	V1 w/o vitamins-
C19 Raffinose+	C38 DL-Lactate	O1 Cycloheximide 0.01%-

2. *Cryptococcus gattii* (Vanbreusghem & Takashio) Kwon-Chung & Boekhout, Taxon 51 (4): 806 (2002)

Synonyms:

- *Cryptococcus neoformans var. gattii* Vanbreuseghem& Takashio, Annal. de la Soci. Belge de Méd.Trop. 50 (6): 701 (1970)
- *Cryptococcus neoformans var. gattii* Vanbreuseghem & Takashio ex De Vroey & Gatti, Mycoses 32 (12): 675 (1989)
- *Cryptococcus bacillisporus* Kwon-Chung & J.E. Benn., Intern.J.Syste. Bacteriol.28: 618 (1978)
    - *Cryptococcus neoformans var. shanghaiensis* W.Q. Liao et al., Chinese Med. J.: 287 (1983)



Cryptococcus gattii colonies. YPGA*, 25°C, 5 days www.pf.chiba-u.ac.jp, CGB agar turns blue for *Cryptococcus gattii*, gattii www.mycology.adelaide.edu.au

Physiological data

C1 D-Glucose+	C22 Starch+	C44 Butane 2,3 diol-
C2 D-Galactose+	C23 Glycerold	C45 Quinic acid-
C3 L-Sorbose+	C24 Erythritold	C46 D-glucarate+
C4 D-Glucosamined	C25 Ribitol+	C47 D-Galactonated
C5 D-Ribose+	C26 Xylitol+	N1 Nitrate-
C6 D-Xylose+	C27 L-Arabinitol+	N2 Nitrite-
C7 L-Arabinose+	C28 D-Glucitol+	N3 Ethylamine+
C8 D-Arabinose+	C29 D-Mannitol+	N4 L-Lysine-, d, w
C9 L-Rhamnose+	C30 Galactitol+	N5 Cadaverine-
C10 Sucrose+	C31 myo-Inositol+	N6 Creatine-
C11 Maltose+	C32 D-Glucono-1,5-lactone+	N7 Creatinine+
C12 a,a-Trehalose+		N8 Glucosamine-
C13 Me a-D-Glucoside+	C33 2-Keto-D-Gluconate+	N9 Imidazole-

C14 Cellobiose+	C35 D-Gluconate+	N10 D-Tryptophan-
C15 Salicind	C36 D-Glucuronate+	V1 w/o vitamins-
C16 Arbutin+	C37 D-Galacturonate+	V2 w/o myo-Inositol+
C17 Melibiose-	C38 DL-Lactate-	V3 w/o Pantothenate+
C18 Lactose-	C39 Succinate+	V4 w/o Biotin+
C19 Raffinose+	C40 Citrate+	V5 w/o Thiamin-
C20 Melezitose+	C43 Propane 1,2 diol-	V6 w/o Biotin & Thiamin
C21 Inulind		

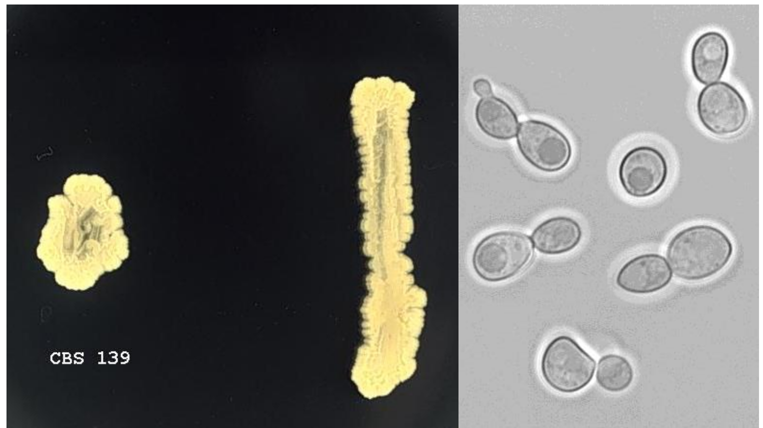
3. *Cryptococcus laurentii* (Kuff.) C.E. Skinner, The American Midland Naturalist 43: 249 (1950)

Synonyms:

- *Torula laurentii* Kuff., Bulletin de la Societé Royale des Sciences Medicales et Naturelles de Bruxelles 1: 1-31 (1920)
- *Torulopsis laurentii* (Kuff.) Lodder, Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde 32: 160 (1934) [MB#269102]
- Cryptococcus laurentii var. laurentii (1952) [MB#429219]
- *Rhodotorula laurentii* (Kuff.) T. Haseg., Banno & Yamauchi, J Gen Appl Microbiol Tokyo 6 (3): 212 (1960)
- Rhodotorula nitens Mackenzie & Auret, Journal of General Microbiology 31 (2): 171 (1963)

Morphology

Colonies are yellowish to orange, sometimes pink in colour. Colony texture is smooth. Budding cells are round, oval or somewhat cylindrical.



Physiological data :

C1 D-Glucose+	C24 Erythritol-, +	N2 Nitrite-, +
C2 D-Galactose+	C25 Ribitol-, +	N3 Ethylamine-, +
C3 L-Sorbose-, +	C26 Xylitol+, d, w	N4 L-Lysine-, +
C4 D-Glucosamine-, +	C27 L-Arabinitol+, d, w	N5 Cadaverine-, +
C5 D-Ribose+, d, w	C28 D-Glucitol+, d, w	N6 Creatine-, +
C6 D-Xylose+	C29 D-Mannitol+, d, w	N7 Creatinine-, +
C7 L-Arabinose-, +	C30 Galactitol-, +	N8 Glucosamine-, +
C8 D-Arabinose-, +	C31 myo-Inositol+, d, w	N9 Imidazole-
C9 L-Rhamnose+, d, w	C32 D-Glucono-1,5-	N10 D-Tryptophan-, +
C10 Sucrose+	lactone-, +	V1 w/o vitamins-, +
C11 Maltose+	C33 2-Keto-D-	V2 w/o myo-Inositol+

C12 a,a-Trehalose+, d, w
C13 Me a-D-Glucoside+, d,w
C14 Cellobiose+
C15 Salicin+, d, w
C16 Arbutin+, d, w
C17 Melibiose+
C18 Lactose+
C19 Raffinose-, +
C20 Melezitose+
C21 Inulin-, d, w
C22 Starch-, +
C23 Glycerol-, +

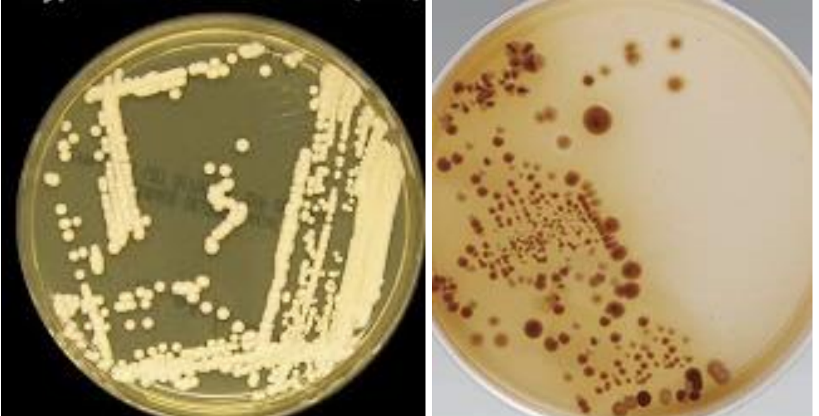
Gluconate+
C34 5-Keto-D-Gluconate+
C35 D-Gluconate+
C36 D-Glucuronate+
C37 D-Galacturonate-, +
C38 DL-Lactate-, +
C39 Succinate+, d, w
C40 Citrate+, d, w
C43 Propane 1,2 diol-, +
C44 Butane 2,3 diol-
C45 Quinic acid-, +
C46 D-glucarate-, +
C47 D-Galactonate-, +
N1 Nitrate-

V3 w/o Pantothenate+
V4 w/o Biotin+
V5 w/o Thiamin-, d, w
V6 w/o Biotin & Thiamin-, d, w
V7 w/o Pyridoxine+
V8 w/o Pyridoxine & Thiamin-, d, w
V9 w/o Niacin+
V10 w/o PABA+
O1 Cycloheximide 0.01%-, +
O2 Cycloheximide 0.1%-, +
O3 Acetic acid 1%-
O6 10% NaCl-, +
O7 16% NaCl-

Diagnosis

Isolation and identification

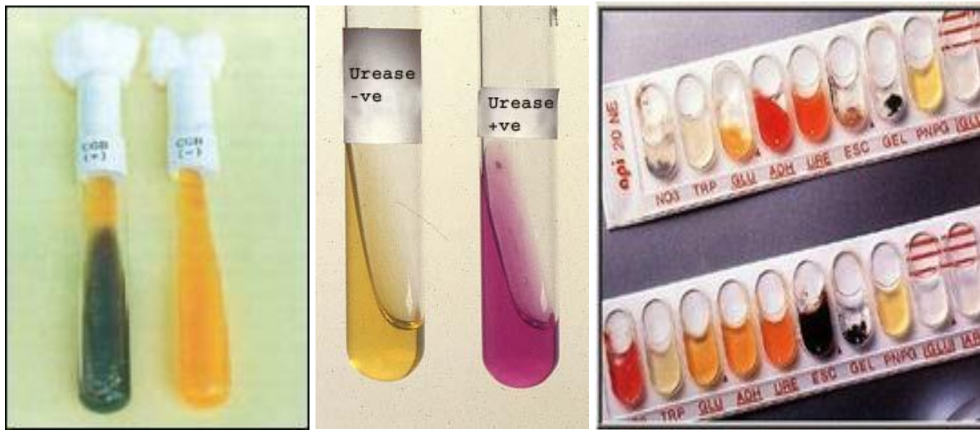
Avian droppings are suspended 1:10 in saline solution and then cultured on Sabouraud agar. Cryptococcus colonies are brown on bird seed agar, modified tobacco and Eucalyptus leave extract agar as well as on Pal's medium. Other yeasts develop white to creamy colonies.



Cryptococcus colonies on Sabroud's dextrose agar, Colonies on bird seed

Differentiation of *C. gattii* and *C. neoformans* on canavanine glycine bromothymol blue (CGB)

On canavanine gjycin bromthymol blue (CGB) medium, *Cryptococcus neoformans* develop non-coloured coloniesand , while *C. gattii* develops blue colonies.



Blue colonies of *C. gattii* (left) and non-coloured colonies of *C. neoformans* (right)
Urease and sugar fermentation tests

Biochemical identification
Cryptococcus neoformans and *C. gattii* do not ferment sugars, but assimilate several sugars such as glucose, galactose, sucrose, maltose and inositol, but not lactose or nitrate and hydrolyses urea.

Serotyping of *Cryptococcus neoformans* and *C. gattii*

Method: To determine the antigenic formulas of *Cryptococcus* species, equal volumes of factor serum and heat-killed cell suspension are mixed on a glass slide and rotated for 5 min, and then the results of agglutination are observed. The formation of aggregates within 5 min is considered positive. Smaller clumps are recorded as weakly positive. PSS is used for a negative control.

Molecular typing
Numerous molecular techniques have been applied to subtype *C. neoformans* and *C. gattii* strains, only three methods were proved to produce comparable results: PCR Fingerprinting, AFLP, and MLST. M13 PCR Fingerprinting and *URA5* RFLP:

Reports:

Abou-Gabal and Atia (1978) recovered Cryptococcus neoformans from droppings collected within the first 24 h from pigeons experimentally fed with a dose of 5 X 10(6) cells. The fungus proved to multiply well though differently in the sterilized pigeon and chicken excreta seeded with the organism. In both unsterile types of droppings no viable cells of C. neoformans were detected after 4 weeks incubation. Isolated bacterial flora from the intestinal contents of apparently healthy pigeons showed a complete inhibitory effect on the growth of C. neoforms in vitro. It has been concluded that pigeons do not favor multiplication of the fungus in their gut and consequently they do not seem to play an active biological role in dissemination of C. neoformans in nature.

Yilmaz *et al.* (1989) investigated C neoformans in faecal droppings obtained from pigeon coops that were placed in several suburbs of Bursa City. In sixteen of the 115 samples (13.9%), C neoformans strains has been isolated and studied their morphological and biochemical properties. But no isolation has been obtained from 8 soil samples and 14 samples of chicken dung.

Yamamoto *et al.* (1995) isolated C. neoformans from pigeon excreta in hospitals, private houses, parks in Nagasaki from October to December in 1994. C. neoformans was isolated from 4 of 8 samples (50%) of pigeon excreta and the isolation rate increased to 80% (4/5) if they were weathered excreta. Two patients with pulmonary cryptococcosis occurred in the last two years in the area where C. neoformans was isolated during that period. Epidemiological studies of clinical isolates and environmental isolates are important to determine its origin of infection and the route of transmission.

Kielstein (1996) showed that Cryptococcus neoformans in bird droppings originated from different ornamental birds and chickens had less chance to survive in non-sterile or bacteria-free droppings of large parakeets and chickens in comparison with droppings of small parakeets. Survival rates of Cr. neoformans in buffer solutions with pH-values ranging from 8.5-9.5 allowed to conclude that this species is not alkali-sensitive. Therefore, the increase of pH is not regarded responsible for the survival of Cr. neoformans in bird droppings. Possibly fungistatic substances present in droppings are involved.

Khosravi (1997) examined 983 specimens of pigeon droppings, collected in different regions of northern Iran, for the occurrence of Cryptococcus neoformans. Of these samples, 175 (17.8%) were positive for Cryptococcus neoformans. All isolates obtained were C. neoformans var. neoformans. Most of these isolates of C. neoformans were from pigeon shelters. There were significant differences in isolation frequency between pigeon shelters and the other collection sites.

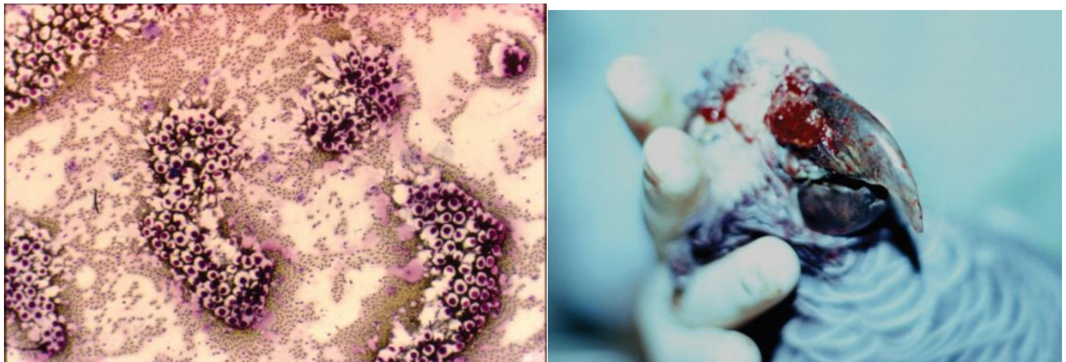
Laubscher *et al.* (2000) studied the predominant yeasts associated with the trachea of fowls, isolated from broilers of a poultry-processing plant. 38 representative yeast isolates were obtained and identified. Species belonging *Candida*, *Cryptococcus*, *Debaryomyces*, *Rhodotorula*, *Torulaspora*, *Trichosporon*, were isolated at various stages of the broiler program.

Isfahani *et al.* (2001) isolated C. neoformans from 11 of 136 samples (8.1 percent). All of them were identified as C. neoformans var. neoformans. However, five of them (45.5 percent) caused disease in mice. There was no statistically significant relation between the pH of the pigeon dropping and the precence of C. .neoformans. According to the results, it was concluded that the actual prevalance of cryptococcosis should be more than the reported cases.

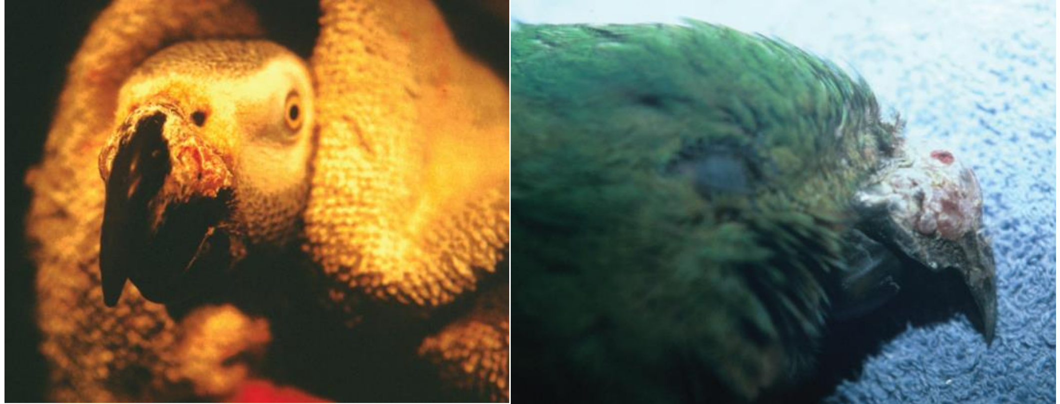
Decostere *et al.* (2003) reported the first isolation of C. laurentii associated with feather loss in a glossy starling (Lamprotornis chalybaeus). The bird exhibited patchy feather loss, especially around the back and beak area, and greyish crusts sticking quite firmly to the underlying skin. The feathers had a greasy appearance and disseminated a musty odour. Treatment was installed with fluconazole in the drinking water. One month following the onset of treatment, the condition of the plumage had markedly improved.

Filiu *et al.* (2002) studied the saprophytic sources of *C. neoformans* in the city of Campo Grande on 20 samples of avian droppings collected from distinct environments within the city. The samples were suspended in sterile saline and then smeared on niger seed agar medium. Five days later smooth dark-brown colonies were subcultivated for identification by morphophysiologic tests. The variety and serotype was determined. *C. neoformans* var. *neoformans* serotype A was isolated from 10 (50%) of the samples collected. Consequently, the saprophytic presence of *C. neoformans* is related to avian habitats.

Malik *et al*. (2003 analyzed clinical and laboratory findings in 15 unreported cases of avian cryptococcosis from Australia contrasted with 11 cases recorded in the literature. Cryptococcus species produced localized invasive disease of the upper respiratory tract of captive parrots living in Australia. This resulted in signs referable to mycotic rhinitis or to involvement of structures contiguous with the nasal cavity, such as the beak, sinuses, choana, retrobulbar space and palate. Parrots of widely differing ages were affected and of the seven birds for which sex was determinable, six were male. Cryptococcus bacillisporus (formerly C. neoformans var. gattii) accounted for four of five infections in which the species or variety was determinable, suggesting that exposure to eucalyptus material may be a predisposing factor. In these cases, Cryptococcus appeared to behave as a primary pathogen of immunocompetent hosts. One tissue specimen was available from an Australian racing pigeon with minimally invasive subcutaneous disease; immunohistology demonstrated a C. neoformans var. grubii (formerly C. neoformans var. neoformans serotype A) infection, presumably subsequent to traumatic inoculation of yeast cells into the subcutis. Two similar cases had been reported previously in pigeons domiciled in America. Data for parrots, one pigeon and other birds studied principally in America and Europe (and likely infected with C. neoformans) suggested a different pattern of disease, more suggestive of opportunistic infection of immunodeficient hosts. In this cohort of patients, the organism was not restricted to cool superficial sites such as the upper respiratory tract or subcutis. Instead, infections typically penetrated the lower respiratory tract or disseminated widely to a variety of internal organs. Finally, three captive North Island brown kiwis, one residing in Australia, the other two in New Zealand, died as a result of severe diffuse cryptococcal pneumonia (two cases) or widely disseminated disease (one case). C. bacillisporus strains were isolated from all three cases, as reported previously for another kiwi with disseminated disease in New Zealand.



Smear made from a tissue specimen from the beak of a long-billed corella with nasal cryptococcosis. Note the numerous clusters of spherical capsulate yeasts with a background of nucleated erythrocytes. C. bacillisporus was cultured from this material. African grey parrot with nasal cryptococcosis. Note thefleshy lesions on either side of the beak. The nasopharynx, choana and infraorbital sinus were involved also. **Malik *et al*. (2003**



An African grey parrot with localized cryptococcosis involving the nasal cavity and nearby structures. A king parrot with localized nasal cryptococcosis. Note thedistortion of the cere. **Malik *et al.* (2003**

Localized cutaneous cryptococcosis in a stud racing pigeon also viewed in profile. Eclectus parrot with severe localized nasal cryptococcosis **Malik *et al.* (2003**

Haag-Wackernagel and Moch (2004) performed a comprehensive literature search of epidemiological studies and reports of transmissions of disease from feral pigeons to humans. There were 176 documented transmissions of illness from feral pigeons to humans reported between 1941 and 2003. Feral pigeons harbored 60 different human pathogenic organisms, but only seven were transmitted to humans. Aerosol transmission accounted for 99.4% of incidents. The most commonly transmitted pathogens continue to be Chlamydophila psittaci and Cryptococcus neoformans. Although feral pigeons pose sporadic health risks to humans, the risk is very low, even for humans involved in occupations that bring them into close contact with nesting sites. In sharp contrast, the immunocompromised patient may have a nearly 1000-fold greater risk of acquiring mycotic disease from feral pigeons and their excreta than does the general population.

Granados *et al.* (2005) examined 89 avian droppings samples collected from different places., where 7.9% yielded C. neoformans strains, all of them were C. neoformans var. grubii, serotype A. The yeast was obtained more frequently from dry droppings than from moist ones, but neither the sunlight exposure nor the site of collection of samples was correlated with this occurrence. Population density was significantly higher in droppings than in tree samples. Under laboratory conditions, isolates of different serotype showed similar capsular sizes. Water content and pH

ranges were wide and did not show any significant difference between positive and negative samples.

Hamasha *et al.* (2004) performed a study to determine the environmental occurrence of both varieties of Cryptococcus neoformans 509 samples of pigeon droppings collected from cities of Amman, Irbid, Jerash, and Ajlun. Also, were collected from After inoculating the samples onto modified Staib agar medium in Petri dishes, a total of 336 melanoid yeast colonies were picked up during screening process. At the end of serial mycological studies, none of these isolates was identified as Cryptococcus neoformans, but all were Cryptococcus species other than C. neoformans.

Kuroki *et al.* (2004) isolated Cryptococcus neoformans from chicken faeces in suburban areas of Thailand. C. neoformans was isolated from 36/150 houses (24.0%) in the dry season and 6/150 (4.0%) in the rainy season. All environmental isolates were of serotype A. The high isolation rate of 24% from chicken faeces has never been reported previously.

Chee and Lee (2005) examined 72 pigeon dropping samples collected from 26 different localities in Seoul and investigated them for the occurrence of Cryptococcus neoformans. Seventeen samples from 8 different localities were found to be positive for C. neoformans. All isolates were obtained from withered pigeon droppings. Identification and serotyping of the isolates were determined by means of serological testing and DNA fingerprinting. All isolates belonged to C. neoformans var. grubbi (serotype A).

Abegy *et al.* (2006) analyzed fecal samples from 59 species of captive birds kept in cages at a local Zoological Garden, belonging to 12 different orders. Thirty-eight environmental isolates of C. neoformans were obtained only from Psittaciformes (Psittacidae, Cacatuidae and Psittacula). Their variety and serotype were determined, and the genetic structure of the isolates was analyzed by use of the simple repetitive microsatellite specific primer M13 and the minisatellite specific primer (GACA)4 as single primers in the PCR. The varieties were confirmed by pulsed-field gel electrophoresis (PFGE). Thirty-three isolates (87%) were from the var. grubii, serotype A, molecular type VNI and five (13%) were Cryptococcus gattii, serotype B, molecular type VGI. All the isolates were mating type a. Isolates were screened for some potential virulence factors. Quantitative urease production by the environmental isolates belonging to the C. gattii was similar to the values usually obtained for clinical ones.

Baroni *et al.* (2006) demonstrated that C. neoformans has been present in every church selected in Rio de Janeiro city and was present in 37.8% of 219 pigeon dropping samples. As well as, the yeast was isolated from soil, insects, eggs, pigeon nests and feathers. Fifteen air samples (4.9%) were positive. The growth on C.G.B. medium showed that all strains belonged to C. neoformans var. neoformans, with 98.8% of the strains belonging to serotype A.

Carvalho, *et al.* (2007) carried out a study to verify the presence of the yeast in pigeon droppings, and to identify the isolates obtained in serotypes and mating types (MAT). Ten samples of pigeon droppings were collected in the rural area of the city of Alfenas, Brazil. Samples were inoculated in agar Niger medium for fungal isolation and 22 isolates with characteristics of C. neoformans were obtained. The serotypes and MAT were determined by multiplex PCR using specific primers. Serotypes were

also determined by using the Kit Crypto Check. Among the 22 samples evaluated, eight were identified as C. neoformans by classic identification tests. These samples were characterized as serotype A by the Kit Crypto check and as serotype A MAT alpha by the multiplex PCR. The present study reinforces the evidence that pigeon droppings are a reservoir for C. neoformans and confirms the prevalence of C. neoformans var. grubii (A alpha) among environmental isolates.

Lugarini *et al.* (2008a) performed a study) to verify the existence of C. neoformans and C. gattii in crop and cloaca of wildlife and captivity birds hypothesizing about a possible primary source of this yeast in the excreta, and to determine the fungal invasive capability in avian species through latex agglutination. For that purpose, 172 cloacal and 77 crop samples of domestic pigeon, Passerine, and Psittacine birds were collected. None of these samples was positive, suggesting that the yeast is not saprobiotic in the digestive tract of these birds. Only one out of 82 serum samples collected from pigeons and Psittacine birds was positive (titre 1:2) showing that Cryptococcus sp. probably has a low invasive capability in birds, and is thus considered only a dry excreta colonizer.

Lugarini *et al.* (2008b) emphasized that the isolation of *C. neoformans* from bird excreta collected in the environment in which they live, in nature or in captivity, does not mean that a particular bird species has as specific role as a reservoir or that this fungus is part of the natural microbiota of these vertebrates. Furthermore, there is no scientific evidence that the fungus has the ability to grow or survive in the excreta of a specific bird.

Rosario and Colom (2008) stated that in the last 25 years, the cases of human and animal cryptococcosis have increased significantly. This is mostly due to the improvement in the survival of immunocompromised patients. The disease is frequently related to the exposure of this type of patients to avian droppings. Among birds, pigeon, Columba livia, is undoubtedly the most important reservoir for the Cryptococcus species. Nevertheless, the study of a large number of bird's species demonstrated that pigeons are not the only Cryptococcus spp. carriers. The suspicion of the birds being the source for the infection is now becoming a demonstrable fact thanks to the use of molecular typing methods. These methods allow the comparison between strains from birds to patients living around them, with high level of discrimination.

Costa *et al.* (2010) carried out a study to investigate pigeons as a potential source of pathogenic yeast species, 47 samples of pigeon droppings and 322 samples from pigeon cloacae were evaluated. C. neoformans var. neoformans (n = 10), C. laurentii (n = 3. The present study confirms that urban pigeons are a potential source of Cryptococcus spp. and other pathogenic yeasts. Additionally, antifungal resistance was observed in one environmental strain of Cryptococcus neoformans var. neoformans in Northeast Brazil.

Faria *et al.* (2010) surveyed 70 different environments in the city of Pelotas, Rio Grande do Sul, for the purpose of investigating Cryptococcus neoformans occurrences in pigeon excreta. The environments included buildings, public squares and outdoor locations in the city. After collection, chloramphenicol saline solution was added to the excreta, which were then homogenized and seeded onto Sabouraud agar with

chloramphenicol and onto Niger agar, and incubated at 32 degrees C. Identification was performed by direct examination and by means of the phenoloxidase and urease tests, carbohydrate assimilation and culturing in CGB medium. Out of the sites investigated (n = 70), 26 (37.1%) of them contained pigeon excreta. These included historical buildings (n = 8), a church tower (n = 1), rice mills and warehouses (n = 7), a public square (n = 1) and outdoor locations (n = 9). Cryptococcus neoformans was isolated from 26.9% (n = 7/26) of these locations. This study drew attention to isolation of this fungus in urban areas that presented large accumulations of pigeon excrement. This represents a risk to public health, especially for immunocompromised individuals.

Zarrin *et al.* (2010) carried out a study to evaluate the presence of Cryptococcus neoformans in Ahwaz, Iran. Sixty-five samples of pigeon droppings were collected from 10 different regions in Ahwaz. Each sample was suspended 1:10 in saline solution and then cultured in Sabouraud's dextrose agar medium including chloramphenicol. Identification of C. neoformans was performed on the basis of melanin synthesis on bird seed agar, presence of a capsule on India ink preparation, urease production on urea agar medium, and ability to grow at 37 °C. An assimilation test was also used to confirm C. neoformans. Results: Of the 65 samples, 22 (34%) were positive for C. neoformans. The highest frequency was observed in droppings from site 7 (86%). The lowest frequency was obtained on samples from sites 2, 3, and 4 (17%). They confirmed the presence of C. neoformans in urban environmental sources at places with a large population in Ahwaz.

AL-Shimmery (2011) recovered 58 yeast isolates belonging to 3 genera and 6 species from the intestinal tracts of 35 out of 50 birds. The occurrences of individual yeast species were Saccharomyces (31.03 %), Candida glabrata (20.69 %), C. tropicalis (15.51 %), C . albicans (15.51 %), C . fmata and Creptococcus neoformans (8.62%) .

Ferreira-Paim *et al.* (2011) carried out a study to evaluate Cryptococcus spp. molecular types isolated from captive birds' droppings, an epidemiological survey was carried out in Uberaba, Minas Gerais, Brazil, from December 2006 to September 2008. A total of 253 samples of bird excreta (120 fresh and 133 dry) were collected from pet shop cages and houses in different neighbourhoods. Cryptococcus neoformans was isolated in 19 (14.28%) dry samples and one fresh sample (0.84%). Cryptococcus laurentii was recovered from seven (5.26%) dry samples, but not in the fresh samples. The canavanine-glycine-bromothymol blue test was positive in all but one of the C. laurentii isolates. Cryptococcus neoformans molecular typing was performed using URA5-RFLP and the mating type locus using mating type specific PCR. Nineteen (95.0%) presented genotype VNI and one VNII (5.0%). In addition, all isolates presented mating type α. Thus, the genotype of the environmental C. neoformans isolates observed in this study is in accordance with others already reported around the world and adds information about its distribution in Brazil. Cryptococcus laurentii strains were typed using URA5-RFLP and M13 fingerprinting, which showed similar profiles among them. Thus, despite the low number of C. laurentii isolates analysed, their molecular profile is different from another already reported.

Ferreira and Raso (2012) investigated the presence of antigens of C. neoformans in the blood serum of urban pigeons (Columba livia) in São Paulo and Tatuí cities,

Brazil. During a year 240 birds had their serum evaluated, with a latex agglutination test, for the presence of cryptococcal antigens. All the birds showed negative results.

Kemoi (2012) carried out a study to isolate and characterize pathogenic yeasts from domestic chicken (Gallus gallus) droppings. The droppings were collected from Kabigeriet Villages, Olenguruone Division, Kuresoi District and Nakuru County. The samples were collected from cages, houses and roosting sites. The samples (droppings and soil) were collected by swabbing or scooping fresh dropping from Chicken houses, grass, soil and trees using sterile plastic spoons, labeled and inserted in a zip lock safety bag. A total of 84 samples (dropping and soil enriched with chicken droppings) were sampled during the study. The droppings were tested for Cryptococcus by direct plating on Niger seed Cryptococcus neoformans were sub-cultured onto Christensen"s urease agar. 2 (3.3%) Cryptococcus species (Cryptococcus neoformans and Cryptococcus laurenti) and 1(1.6%) Saccharomyces cerevisiae were isolated from Chickens dropping sampled.

Kuchak *et al.* (2012) performed a study to verify the presence of Cryptococcus neoformans in pigeon excreta in Mazandaran province, Iran, and to identify the varieties of the C. neoformans isolates using D1/D2 and IGS sequencing, and determining the presence of the two mating types: α and a. Four hundred pigeon droppings samples were collected from 15 different cities in Mazandaran province over a period of 1 year (February 2010-March 2011). Identification of C. neoformans was determined based on growing brown colonies on Niger seed agar (NSA) and biochemical characteristics. We used MATα and MATa specific primers for determining mating type and sequence analysis of the D1/D2 and intergenic spacer regions were done. Out of 400 samples, 20 samples (5%) were positive for C. neoformans and all of these isolates were α mating types. Sequence analysis of polymerase chain reaction (PCR) amplicons of D1/D2 regions revealed that all of the isolates were C. neoformans var. grubii except two isolates that were C. neoformans var. neoformans. These results reinforced that the pigeon excreta is a favorable environment rich in nitrogen and supports the growth of C. neoformans and the pigeon could play an important role in spread of this organism.

Kemoi *et al.* (2013) performed a cross sectional study to isolate and identify Cryptococcus from domestic chicken droppings in Kabigeriet village, Olenguorone Division, Nakuru county, Kenya. Sixty four domestic chicken droppings were sampled in thirty two homesteads after obtaining the farmers consent. Two species of Cryptococcus were isolated. It was concluded that domestic chicken (Gallus gallus) harbor pathogenic Cryptococcus in their dropping and their close proximity to human habitation poses a risk of AIDS to immunocompromised persons

Soltani *et al.* (2013) examined 120 samples of pigeon droppings for *C. neoformans* . The identification was based on the presence of a capsule on India ink preparation, urease production on urea agar medium and RapID yeast plus system. The identification of *candida* species was based on micro-morphological analysis on corn meal-Tween 80 agar, RapID yeast plus system and growth in CHROMagar *candida*. The frequency rate of *C. neoformans* isolation was 2.5%

Takahara *et al.* (2013) evaluated the occurrence of C. neoformans in 122 samples of dried pigeon excreta collected in 49 locations in the City of Cuiabá, State of Mato Grosso, Brazil, including public squares (n = 5), churches (n = 4), educational

institutions (n = 3), health units (n = 8), open areas covered with asbestos (n = 4), residences (n = 23), factory (n = 1) and a prison (n = 1). Samples collected from July to December of 2010 were seeded on Niger seed agar (NSA). Dark brown colonies were identified by urease test, carbon source assimilation tests and canavanine-glycine-bromothymol blue medium. Polymerase chain reaction primer pairs specific for C. neoformans were also used for identification. Cryptococcus neoformans associated to pigeon excreta was isolated from eight (6.6%) samples corresponding to six (12.2%) locations. Cryptococcus neoformans was isolated from urban areas, predominantly in residences, constituting a risk of acquiring the disease by immunocompromised and immunocompetent individuals.

Tangwattanachuleeporn *et al.* (2013) carried out a study to characterize the prevalence of C. neoformans, its serotypes and antifungal drug susceptibilities in environmental isolates from Chon Buri, Eastern Thailand. C. neoformans was isolated from 10% of fifty pigeon excreta examined from this province. All C. neoformans isolates were of serotype A and although the isolates displayed slightly decreased susceptibility towards fluconazole, all tested sensitive to amphotericin B, fluconazole and itraconazole. This study is the first report of the occurrence of C. neoformans in pigeon excreta in eastern Thailand.

Teodoro *et al.* (2013) performed a study to determine the occurrence of pathogenic Cryptococcus in pigeon excrement in the City of Araraquara, Samples were collected from nine environments, including state and municipal schools, abandoned buildings, parks, and a hospital. The isolates were identified using classical tests, and susceptibility testing for the antifungal drugs (fluconazole, itraconazole, voriconazole, and amphotericin B) independently was also performed. After collection, the excrement samples were plated on Niger agar and incubated at room temperature. A total of 87 bird dropping samples were collected, and 66.6% were positive for Cryptococcus neoformans (17.2%), Cryptococcus gattii (5.2%), Cryptococcus ater (3.5%), Cryptococcus laurentti (1.7%), Cryptococcus luteolus (1.7%) and70.7% of the isolates were not identified to the species level and are referred to as Cryptococcus spp. throughout the manuscript.

Xavier *et al.* (2013) performed a study to evaluate the presence of Cryptococcus neoformans in pigeon droppings. Thirty three samples of pigeon droppings were collected from 10 different regions in Tiruchirappalli district. Of the thirty three samples, 20 (60.6%) were positive for Cryptococcus neoformans. The highest frequency was observed in droppings from site 1(100%), 3(80%), 5(80%). The lowest frequency was observed in 2(57%), 4(60%), 8(50%), 9(50%), 10(50%). The samples from the sites 6, 7 did not show any contamination to Cryptococcus neoformans.

Kangogo *et al.* (2014) performed a study to establish the environmental reservoirs of Cryptococcus neoformans and Cryptococcus gattii in Nairobi, Kenya. A total of 400 environmental samples from different sites were analysed including; avian droppings, tree swabs, soil contaminated with avian droppings and swabs from garbage damping sites. Samples were subjected to various phenotypic tests including microscopic morphology, physiological and biochemical tests, pigmentation on bird seed agar and reaction on Canavanine-Glycine-Bromothymol Blue agar. Cryptococcus neoformans was isolated from 23/200 (11.5%) dropping samples and Cryptococcus gattii in 5/200 (2.5%) of the same samples. Cryptococcus gattii was isolated from 7/60 (11.7%) tree swabs and Cryptococcus neoformans in 5/60 (8.5%) of the same samples. From other sites there was no Cryptococcus gattii recovered

with (5/50: 10%), (6/60: 10%), (2/30: 6.7%) Cryptococcus neoformans recovered from chicken cage, garbage damping site and soil respectively. Findings clearly showed a high presence of Cryptococcus neoformans and Cryptococcus gattii from several environmental sites in Nairobi, Kenya. This could probably explain the high incidence of cryptococcal meningitis in HIV/AIDS patients in Kenya.

Mendes *et al.* (2014) investigated the presence of potentially pathogenic fungi in the feces of wild birds collected in Screening Centers. Samples were collected from the feces of 50 cages with different species of birds. The samples were processed according to the modified method STAIB and the plates incubated at 32 °C for up to ten days with daily observation for detection of fungal growth. The isolation of Cryptococcus laurentii was reported,

Molter *et al.* (2014) presented a 14-yr-old female Pesquet's parrot (Psittrichas fulgidus) with lethargy and decreased ability to fly. Physical exam was unremarkable. Blood work showed an elevated white blood cell count and a strong positive Aspergillus galactomannan titer. Empirical Aspergillus treatment was initiated with compounded generic itraconazole. Radiographs revealed an irregular osteolytic lesion isolated to the distal right humerus. Bone biopsy of the lesion, cytology, and histopathology were diagnostic for osteomyelitis with intralesional yeasts confirmed to be Cryptococcus gattii on fungal culture. After 2 mo of compounded itraconazole treatment, the bird developed dyspnea and dysphagia due to new Cryptococcus lesions in the proximal trachea and glottis. Plasma itraconazole levels were measured and found to be undetectable; therefore, treatment was changed to fluconazole. Twenty-four months after initial presentation, clinical signs improved, but radiographic and histopathology lesions were static.

Ior *et al.* (2015) conducted a study to evaluate the presence of Cryptococcus neoformans in four environmental sources; water, soil poultry droppings and pigeon in Nigeria. Two hundred samples, fifty samples each of water, soil, poultry droppings and pigeon guano were collected from five different settlements in Jos. Each sample was suspended 1:10 in saline solution and then cultured in Sabouraud's dextrose agar medium including chloramphenicol. Identification of C. neoformans was performed on the basis of melanin synthesis on bird seed agar, presence of a capsule on India ink preparation, urease production on urea agar medium, and ability to grow at 37 °C. An assimilation test was also used to confirm C. neoformans. Of the 200 samples, 17(8.5%) were positive for C. neoformans. The highest frequency was observed in pigeon guano 8(16.0%) followed by soil 6(12.0%) and poultry droppings 3(6.0%) no isolate was made from water. The study showed the presence of C. neoformans in environmental sources especially in domestic birds.

References:

1. Abegy M. A., Cella F.L., Faganello J., Valente P., Schrank A., Vainstein M.H. (2006). Cryptococcus neoformans e Cryptococcus gattii isolated from excreta of psittaciformes in a Southern Brazilian Zoological garden. Mycopathologia. 161 (2):83-91.
2. Abou-Gabal M, Atia M. Study of the role of pigeons in the dissemination of Cryptococcus neoformans in nature. Sabouraudia. 1978 Mar;16(1):63-8.
3. Baroni, F.A., C.R. Paula, E.G. Silva, F.C. Viani, I.N.G. Rivera, M.T.B. Oliveira and W. Gambale, 2006. Cryptococcus neoformans strains isolated from church towers in Rio de Janeiro city, RJ. Brazil. Rev. Inst. Med. Trop. S. Paulo, 48: 71-75

4. Böhm KH, Trautwein G, Weiland E, Abdallah IS. Experimental infection of pigeons and chickens with Cryptococcus neoformans and further epidemiologic studies. Mycopathol Mycol Appl. 1974 Nov 29;54(3):317-28..
5. Carfachia, C., A. Camarda, D. Romito, M. Campolo, N.C. Quaglia and D. Tullio, 2006. Occurrence of yeasts in cloacae of migratory birds. Mycopathologia, 161: 229-234.
6. Carvalho, V.G., M.S. Terceti, A.L.T. Dias, C.R. Paula, J.P. Lyon, A.M. Siqueira and M.C. Franco, 2007. Serotype and Mating type characterization of Cryptococcus neoformans by multiplex PCR. Rev. Inst. Med. Trop. S. Paulo, 49: 207-210.
7. Chee HY, Lee KB. Isolation of Cryptococcus neoformans var. grubii (serotype A) from pigeon droppings in Seoul, Korea. J Microbiol. 2005 Oct;43(5):469-72.
8. Costa AK, Sidrim JJ, Cordeiro RA, Brilhante RS, Monteiro AJ, Rocha MF. Urban pigeons (Columba livia) as a potential source of pathogenic yeasts: a focus on antifungal susceptibility of Cryptococcus strains in Northeast Brazil. Mycopathologia. 2010 Mar;169(3):207-13.
9. Decostere, A. K. Hermans1 , T. De Baere2 , F. Pasmans1 and F. Haesebrouck. First report on Cryptococcus laurentii associated with feather loss in a glossy starling (Lamprotornis chalybaeus), Avian Pathology (June 2003) 32(3), 309/311
10. Fadhil.A.AL-Shimmery. Investigation On The Occurrence of Yeast Species in the Digestive Tracts of Broiler. RESEARCHES of THE FIRST INTERNATIONAL CONFERENCE (BABYLON AND RAZI UNIVERSITIES) (2011), 37-42
11. Faria RO, Nascente Pda S, Meinerz AR, Cleff MB, Antunes Tde A, Silveira Eda S, Nobre Mde O, Meireles MC, Mello JR. Occurrence of Cryptococcus neoformans in pigeon excrement in the city of Pelotas, State of Rio Grande do Sul. Rev Soc Bras Med Trop. 2010 Mar-Apr;43(2):198-200.
12. Ferreira, Vivian Lindmayer and Tania de Freitas Raso Survey of Cryptococcal Antigens in Urban Pigeons (Columba livia) in Sao Paulo State, Brazil. International Journal of Poultry Science 11 (1): 1-4, 2012
13. Ferreira-Paim K, Andrade-Silva L, Mora DJ, Pedrosa AL, Rodrigues V, Silva-Vergara ML. Genotyping of Cryptococcus neoformans isolated from captive birds in Uberaba, Minas Gerais, Brazil. Mycoses. 2011 Sep;54(5):e294-300.
14. Filiu W.F., Wanke B., Anguena S.M., Vilela V.O., Macedo R.C., Lazera M. (2002). Avian habitats as a source of Cryptococcus neoformans in the city of Campo Grande, Mato Grussu do Sul, Brazil. Rev. Sol. Brazil Medicine Tropical. 35:591-595.
15. Granados DP, Castañeda E. Isolation and characterization of Cryptococcus neoformans varieties recovered from natural sources in Bogotá, Colombia, and study of ecological conditions in the area. Microb Ecol. 2005 Feb;49(2):282-90. Epub 2005 Jun 17.
16. Grundet S., Mayser P., Redmann T., Kaleta E.F., (2005). Mycological examination on the fungal flora of the Chicken comb. Mycoses, 48:114-119.
17. Haag-Wackernagel, D. and H. Moch, 2004. Health hazards posed by feral pigeons. J. Infect., 48: 307- 313.
18. Hamasha AM, Yildiran ST, Gonlum A, Saracli MA, Doganci L. Cryptococcus neoformans varieties from material under the canopies of eucalyptus trees and pigeon dropping samples from four major cities in Jordan. Mycopathologia. 2004 Aug;158(2):195-9.
19. Horta JA, Staats CC, Casali AK, Ribeiro AM, Schrank IS, Schrank A, Vainstein MH. Epidemiological aspects of clinical and environmental Cryptococcus neoformans isolates in the Brazilian state Rio Grande do Sul. Med Mycol. 2002 Dec;40(6):565-71.
20. Ior, C.A, G. Ayanbimpe, L.D. Ior, J. Okechalu, G. O.Chris-Otubor. ENVIRONMENTAL ISOLATION OF Cryptococcus neoformans IN JOS. Scientific Research Journal (SCIRJ), Volume III, Issue VI, June 2015

21. Isfahani BN, Shadzi SH, Pour MC, Ilchi N. Isolation and detection of *Cryptococcus neoformans* from pigeon droppings: Isfahan and its suburb province pigeon towers. J Res Med Sci. 2001;6:20–2.
22. Kangogo M, Boga H, Wanyoike W, Bii C. ISOLATION AND CHARACTERISATION OF CRYPTOCOCCUS NEOFORMANS AND CRYPTOCOCCUS GATTII FROM ENVIRONMENTAL SOURCES IN NAIROBI, KENYA. East Afr Med J. 2014 Aug;91(8):281-5.
23. Kemoi, E K. ISOLATION AND CHARACTERIZATION OF YEAST FROM Gallus gallus DROPPINGS IN KABIGERIET VILLAGE, OLENGURUONE. Master of Science (microbiology) in the school of pure and applied sciences, Kenyatta University, October, 2012
24. Kemoi EK, Okemo P, Bii CC. PRESENCE OF CRYPTOCOCCUS SPECIES IN DOMESTIC CHICKEN (GALLUS GALLUS) DROPPINGS AND THE POSSIBLE RISK IT POSED TO HUMANS IN KABIGERIET VILLAGE, NAKURU COUNTY, KENYA. East Afr Med J. 2013 Jun;90(6):202-6.
25. Khosravi AR. Isolation of Cryptococcus neoformans from pigeon (Columbia livia droppings in northern Iran. Mycopathologia. 1997;139(2):93-5.
26. Kielstein, P. (1996), Untersuchungen zum ökologischen Verhalten von *Cryptococcus neoformans*. Mycoses, 39: 113–117.
27. Kuchak. A., Afshari S, Shokohi T, Aghili R, Badali H. Epidemiology and molecular characterization of Cryptococcus neoformans isolated from pigeon excreta in Mazandaran province, northern Iran. J Mycol Med. 2012 Jun;22(2):160-6.
28. Kuroki M, Phichaichumpon C, Yasuoka A, Chiranairadul P, Chosa T, Sirinirund P, Miyazaki T, Kakeya H, Higashiyama Y, Miyazaki Y, Ishida Y, Kohno S. Environmental isolation of Cryptococcus neoformans from an endemic region of HIV-associated cryptococcosis in Thailand. Yeast. 2004 Jul 30;21(10):809-12.
29. Laubscher W.D.F., Viljoen B.C., Albertyn J., (2000). The yeasts flora occurring in the trachea of Broiler Chicken. Food techno. Biotechnol. 38:77-80.
30. Lugarini, C., C.Z. Goebel, L.A.Z. Condas, M.D. Muro, M.R. Farias, F. Montiani-Ferreira and M.H. Vainstein, 2008b. Cryptococcus neoformans Isolated from passerine and psittacine bird excreta in the State of Parana, Brazil. Mycopathologia, 166: 61-69.
31. Lugarini, C., L.A.Z. Condas, G.C.G. Soresini, R.C.F. Santos, M.D. Muros, M. Ono, M.R. Faria and F. Montiani-Ferreira, 2008a. Screening of antigenemia and isolation of Cryptococcus neoformans and C. gattii from cloaca and crop of birds in the state of Parana, Brazil. Pesq. Vet. Bras., 28: 341-344.
32. MALIK, R., M. B. KROCKENBERGER*, G. CROSS*, R. DONELEYy, D. N. MADILLz, D. BLACK§, P. MCWHIRTER}, A. ROZENWAX, K. ROSE**, M. ALLEYyy, D. FORSHAWzz, I. RUSSELL-BROWN§§, A. C. JOHNSTONEyy, P. MARTIN*, C.R. O'BRIEN* & D. N. LOVE. Avian cryptococcosis Medical Mycology 2003, 41, 115–124
33. Mendes JF, Albano AP, Coimbra MA, Ferreira GF, Gonçalves CL, Nascente Pda S, de Mello JR. Fungi isolated from the excreta of wild birds in screening centers in Pelotas, RS, Brazil. Rev Inst Med Trop Sao Paulo. 2014 Nov-Dec;56(6):525-8.
34. Molter CM, Zuba JR, Papendick R. Cryptococcus gattii osteomyelitis and compounded itraconazole treatment failure in a Pesquet's parrot (Psittrichas fulgidus). J Zoo Wildl Med. 2014 Mar;45(1):127-33.
35. Musgrove MT, Jones DR, Hinton A Jr, Ingram KD, Northcutt JK. Identification of yeasts isolated from commercial shell eggs stored at refrigerated temperatures. J Food Prot. 2008 Jun;71(6):1258-61.
36. Rad, Samiee F. Isolation of Cryptococcus neoformans from pigeon excreta in Qazvin. Life Sci J 2013;10(1):214-219] (ISSN:1097-8135). http://www.lifesciencesite.com. 32

37. Rosario, Acosta B, Colom MF. Pigeons and other birds as a reservoir for Cryptococcus spp. Rev Iberoam Micol. 2008 Mar;25(1):S13-8.
38. Soltani M, Bayat M, Hashemi SJ, Zia M, Pestechian N. Isolation of*Cryptococcus neoformans* and other opportunistic fungi from pigeon droppings.*Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences*. 2013;18(1):56-60.
39. Takahara DT, Lazéra Mdos S, Wanke B, Trilles L, Dutra V, Paula DA, Nakazato L, Anzai MC, Leite Júnior DP, Paula CR, Hahn RC. First report on Cryptococcus neoformans in pigeon excreta from public and residential locations in the metropolitan area of Cuiabá, State of Mato Grosso, Brazil. Rev Inst Med Trop Sao Paulo. 2013 Nov-Dec;55(6):371-6.
40. Tangwattanachuleeporn M, Somparn P, Poolpol K, Gross U, Weig M, Bader O. Prevalence and antifungal susceptibility of Cryptococcus neoformans isolated from pigeon excreta in Chon Buri Province, Eastern Thailand. Med Mycol J. 2013;54(3):303-7.
41. Teodoro VL, Gullo FP, Sardi Jde C, Torres EM, Fusco-Almeida AM, Mendes-Giannini MJ. Environmental isolation, biochemical identification, and antifungal drug susceptibility of Cryptococcus species. Rev Soc Bras Med Trop. 2013 Nov-Dec;46(6):759-64.
42. Yamamoto Y, Kohno S, Noda T, Kakeya H, Yanagihara K, Ohno H, Ogawa K, Kawamura S, Ohtsubo T, Tomono K, et al. Isolation of Cryptococcus neoformans from environments (pigeon excreta) in Nagasaki. Kansenshogaku Zasshi. 1995 Jun;69(6):642-5.
43. Yilmaz A, Göral G, Helvaci S, Kiliçturgay K, Gökirmak F, Töre O, Gedikoğlu S. Distribution of Cryptococcus neoformans in pigeon feces. Mikrobiyol Bul. 1989 Apr;23(2):121-6.
44. Xavier, T. Francis, A.Auxilia, M. Kannan, A. Freeda Rose and S.R. Senthil Kumar Perez and A. Mezzarl, Isolation and Identification of Cryptococcus neoformans from pigeon droppings in Tiruchirappalli district of Tamil Nadu, South India. Int.J.Curr.Microbiol.App.Sci(2013) 2(11): 404-409
45. ZARRIN, M , Masoomeh JORFI , Nasrin AMIRRAJAB , Morad ROSTAM Isolation of Cryptococcus neoformans from pigeon droppings in Ahwaz, Iran. Turk J Med Sci 2010; 40 (2): 313-316

.

- In particularly the epidemiological knowledge of cryptococcoses in avian species is important for the understanding of the pathogenesis of infection and the risk factors associated with this illness (Haag-Wackernagel and Moch, 2004; Lugarini et al., 2008a).
- The diseases aspects of cryptococcal infection are becoming better defined in humans while the life this fungus leads outside the human host remains less well recognized (Lin and Heitman, 2006).
- Further studies involving, at the same time, birds, their serum, their feces and the environment in which they inhabited, are essential for a better comprehension of the role of avian species in the spread and maintenance of Cryptococcus neoformans in the environment.
- These records are important in the medical literature since prevention of human's exposure to animal-related illnesses requires knowledge of all the different aspects of the zoonosis.

2.3. Avian Macrorhabdiosis

Synonyms:

- " Going Light Syndrome "
- " Easily Become "
- " Virgamycosis "
- " Megabacteriosis "
- " Proventricular Disease "
- " Debilitating Syndrome "
- " Glandular stomach inflammation "
- " Proventriculitis "

Aetiology:

Macrorhabdus ornithogaster Tomasz., Logan, K.F. Snowden, Kurtzman & Phalen 2003

Synonyms:

- Megabacteria
- Virgamycosis avigastricus
- Avian gastric yeast

Classification history:

- **DORRESTEIN (1980)** described ***Macrorhabdus ornithogaster*** on the basis of microscopic morphology as a yeast
- **HARGREAVES (1981)** confirmed the organism as a yeast by demonstrating the mycelial structures of the organism detected in a PAS-stained preparation
- **SCHWEIGHARDT und HOFFMANN (1984)** accepted the fungal nature of the organism.
- **SCHWEIGHARDT *et al.* (1984)** considered ***Macrorhabdus ornithogaster*** as a fungus on the basis of its staining behaviour in Gram, PAS und Grocott stains
- **VAN HERCK *et al.* (1984)** failed to demonstrate the presence of a nucleus by the electron microscope and cosequently considered the organism as a bacterium
- **TSAI et al. (1992)** recognized large bacilli but could detect the presence of a nucleus and consequently refused the classification of the organism as a bacterium and approved it as a fungus
- **LUBLIN *et al.* (1998)** did not accept this opinion
- **COOKE (2000)** considered ***Macrorhabdus ornithogaster*** as a fungus on the basis of its resistance to antibacterial antibiotics and sensetivity to antifungals.
- **HUCHZERMEYER und HENTON (2000)** reported the sensitivity of ***Macrorhabdus ornithogaster*** to some antibiotics but not antimycotics and considered the organism as a bacterium, irrespective of its unusual laege size

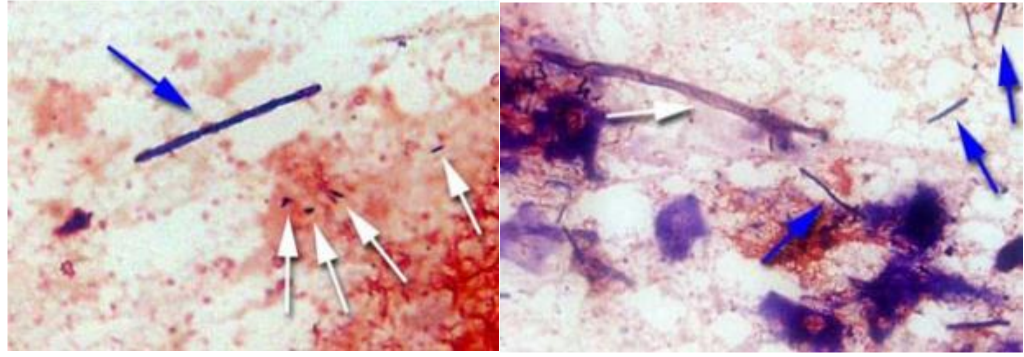
- **RAVELHOFER-ROTHENEDER *et al.* (2000)** carried out extensive studies that clearly demonstrated the presence of a nucleus, cell wall structures and budding typical of yeasts. Cosequently, they gave the organism the name „Fungoides proventriculi“
- **TOMASZEWSKI *et al.* (2003)** sequenced the ribosomal DNA of this organism and used this information to prove that it was a novel anamorphic ascomycetous yeast that belongs in its own new genus. ***Macrorhabdus***
- **Subsequently,** they proposed the name **Macrorhabdus ornithogaster** gen. nov., sp. Nov
- **The present status of the organism** from NCBI Taxonomy

  Macrorhabdus ornithogaster

  - Fungi
  - Ascomycota
  - Saccharomycetes
  - Saccharomycetales
  - Macrorhabdus

Morphology of *Macrorhabdus ornithogaster*

a. *Macrorhabdus ornithogaster* (previously known as megabacterium) is a unique, large, Gram-positive, bacillus-like structure, measuring 1 to 5 µm in width by 20 to 90 µm in length.
b. Isolation of this organism is extremely difficult which had impaired its taxonomic classification.
c. Electron microscopic studies have revealed the presence of eukaryotic nuclei with a nuclear membrane.
d. Optical flourescence studies have demonstrated that the organism’s cell wall contains components of cellulose and chitin that are often found in fungi.



Macrorhabdus ornithogaster is a large, Gram-positive, elongate structure (blue arrow). Notice the smaller bacterial flora (white arrows) in the background. Cloacal swab, Gram’s stain.Several M. ornithogaster (blue arrows) are present and stain Gram-positive (blue-purple). A larger piece of plant fiber (white arrow), often confused with M. ornithogaster, is present at the upper left. Cloacal swab, Gram’s stain.http://vetbook.org/wiki/bird/index.php?title=Macrorhabdus_ornithogaster

Natural hosts

The reported host range of M ornithogaster includes a wide range of psittacine birds, passerine birds, poultry, and other species. It has a worldwide distribution and it is found in both wild and captive birds

a. Psittacine birds

- lovebirds (Agapornis spp.) which are African species,
- Indian-ring necked parakeet (Psittacula krameri), an Asian species,
- parrolettes (Forpus spp.) which are South American species,
- the New Zealand red-crowned kakariki (Cyanoramphus novaezelandiae)
- budgerigar (Melopsittacus undulates),
- cockatiel (Nymphicus hollandicus),
- king parrot (Alisterus scapularis),
- red-winged parrot (Aprosmictus erythropterus),
- sulphur-crested cockatoo (Cacatua gtalerita),
- galah (Cacatua roseicapilla),
- white-tailed black cockatoo (Calyptorhynchus latirostris),
- Bourke's parrot (Neophema bourkii),
- scarlet-chested parrot (Neopehma splendida),
- princess parrot (Polytelis alexandrae),
- superb parrot (Polytelis swainsonii),
- mulga parrot (Psephotus vafrius) and the
- rainbow lorikeet (Trichoglossus)
- European goldfinch (Carduelis carduelis),
- green finch (Carduelis chloris), and
- sisken (Carduelis spinus),
- Begalese finch (Lonchura domestica),
- Pictorella finch (Heteromunia pectoralis),
- grey singing finch (Sirnus leucopygius) and the
- Australian finches zebra finch (Taeniopygia guttata),
- Gouldian finch (Erythrura gouldiae), and
- painted fire-tail finch (Emblema picta)

b. Other captive-raised gallinaceous birds that have been documented to be infected with M. ornithogaster include t

- Japanese quail (Coturnix japonica),
- grey partridge (Perdix perdix),
- Chukar partridge (Alectoris chukar),
- Ostrich (Struthio camelus)
- Greater rhea (Rhea americana)

c. **Poultry:** Macrorhabdus ornithogaster has been described in

- chickens,
- domestic ducks,
- turkeys and
- pigeons

Mode of infection:

- It is likely that most infections result from faecal-oral contamination from sick or subclinical birds shedding it in their faeces.
- In a controlled trial in chickens, infection was found to pass from experimentally infected chicks to uninfected chicks housed with them.
- It is also possible that altricial nestlings are infected when they are regurgitant fed by their parents.
- Worldwide dissemination is likely to have occurred as the result of the trade in cage birds (particularly canaries and budgerigars).

Incubation period:

- It is likely that colonization of the isthmus begins immediately upon exposure and heavy growth can be detected in experimentally infected birds by two weeks after infection.
- The time between infection and the development of signs, if they are going to occur at all, may range from a few weeks to years.

Morbidity:

- Little is known about the prevalence of infection in wild birds.
- In most instances, at least in cage birds, although many birds in a collection are infected only a small percentage of these birds will show signs of illness.

Mortality:

Birds with clinical signs that are not treated die. Mortality rates in infected flocks can vary significantly, from low rate in budgerigar and finch flocks to near 100% mortality in ostrich

Diagnosis

Clinical signs

- weight loss,
- vomiting,
- diarrhoea, appearing to eat but not ingesting food, whole seeds in the droppings, lethargy and being fluffed up
- Less commonly, birds may demonstrate melena.
- In ostrich chicks a stunting and runting syndrome was described
- The impact on chickens is unclear.
- Experimentally infected birds showed a reduced rate of gain, but no outward signs of illness

- Production chickens with natural infections all showed a variety of signs, but all flocks were impacted by a range of disease agents and it was not possible to determine which if any of the signs were caused by M. ornithogaster
- Finches are often found dead, but are thin suggesting that they had been ill, but this illness was not observed.

P.M. findings:

- Most birds will have significant pectoral muscle atrophy.
- Budgerigars and finches with M. ornithogaster infections may have a thickened wall to the proventriculus and gastric isthmus and have increased mucous in the lumen of the proventriculus.
- Ulceration of the proventriculus and less commonly the ventriculus may also occur.
- A scraping of the isthmus will reveal the organism, which may occur in large numbers

Demonstration of Macrorhabdus ornithogaster in the faeces

- Organisms are best demonstrated by mixing the faeces with 20 times their volume of saline, waiting 10 seconds for the larger debris to settle and doing a wet preparation of the surface film.
- Organisms can be seen with the stage diaphragm reduced to improve contrast using the 10X objective on the microscope.
- Organisms are strait-walled with rounded ends and are typically 3 to 4 micrometres wide and 20 to 80 micrometres long.
- Occasionally, branching (Y-shaped) forms are found in the faeces, but these are rare
- They can also be identified in faeces that are stained with quick stains used for cytology and with the Gram stain.
- They stain better and are more likely to stick to the slide if the slide is heat-fixed.
- They may stain incompletely with both stains, but are generally considered to be Gram positive.
- The cell wall contains chitin and they will stain with the Calcafluor white MR2 viewed with ultraviolet light (380-420 nm).
- They are also Periodic Acid Shift positive (Tomaszewski et al. 2003)

PCR based assay

- is available in the USA.
- Data on the sensitivity of this assay as compared to faecal examination has not been reported (Veterinary Medical Diagnostics, Milford, Ohio).

Histological findings:

- In mild infections there may not be any associated inflammation.

- In advanced cases the numbers of organisms increase and infection may extend into the proventriculus and into the koilin layer of the ventriculus.
- Organisms may penetrate deep between the glands in the isthmus and deep into the kolin,
- ulceration may occur.
- Inflammation is predominately lymphoplasmacytic, but may become heterophilic if there is ulceration

Isolation

- Macrorhabdus ornithogaster is readily grown in culture using cell culture media (e.g., Basal Eagle's Medium Eagle) adjusted to pH 3-4, containing 20% fetal bovine serum, and 5% glucose or sucrose under microaerophilic conditions at 42 C

Differential diagnoses

- The signs associated with M. ornithogaster infection are not specific and can occur with many other diseases, including
    - trichomoniasis
    - giardiasis,
    - bacterial and other fungal infections of the crop and stomach,
    - helminth infections of the digestive tract,
    - Bornavirus infection, crop and gastric foreign bodies and
    - heavy metal poisoning to name a few.

Treatment and Control

- The goals of treatment are to reduce the number of organisms and improve the general health and immunocompetence of the bird.
- Amphotericin (100 mg/kg, PO, bid for 30 days) has had the highest treatment success rate, but failures are common, especially with a shorter duration of treatment.
- Acidification of the proventriculus (apple cider vinegar, vitamin C) has been reported to create an environment less conducive to proliferation of *Macrorhabdus*.
- Voriconazole has been successful (anecdotal) at 10 mg/kg, PO, bid.
- Treatment with sodium benzoate in the drinking water has been anecdotally reported to be successful but still experimental.
- Sodium benzoate at 1 tsp/L water for 5 wk cleared the infection in nonbreeding budgerigars, but in budgerigars that were rearing chicks in high environmental temperatures >90°F, treatment with ½ tsp/L water resulted in neurologic signs and death of the adult budgerigars because of their increased water intake.
- The current recommendation for treatment of *Macrorhabdus* with sodium benzoate is ½ tsp (2.5 g) of sodium benzoate powder/L of water (used as only water source and made fresh daily). If the birds are not drinking the medicated

water, the dose should be decreased to ¼ tsp (1.25 g)/L of water, and slowly increased back to ½ tsp over the next few days.

- Feces should be rechecked at 14 days; if *Macrorhabdus* organisms are still present, the dose should be increased over several days to 1 tsp (5 g)/L.
- Feces should be rechecked at 30 days.
- The lower dose of ¼ to ½ tsp powder/L of water should be used in birds housed outdoors in summer (temperatures >90°F [32.2°C]) and in birds feeding chicks. Gloves should be worn when handling medication.
- Asymptomatic carriers are common. Artificial incubation of eggs and hand feeding nestlings can help establish a pathogen-free flock.

Reports

Baker (1985) reported that Going light' is a chronic but eventually fatal disease of budgerigars. Clinically the only consistent features are weight loss while maintaining a good food intake. The signs are caused by enteritis typified by lymphocyte and plasma cell infiltration and associated villous atrophy leading to a malabsorption syndrome.

Henderson *et al.* (1988) reported 18 budgerigars with clinical signs of 'going light'. The post mortem revealed ingluvitis caused by Trichomonas gallinae infection in seven birds, proventriculitis associated with the presence of megabacteria in eight birds and in three birds both conditions were present. Haematological examinations of blood taken from the living birds showed that those with T gallinae infection had normal white cell counts whereas those in which megabacteria were present had significant leucocytosis and heterophilia. Some birds in both groups were anaemic. The findings suggested that infection with megabacteria may be responsible for a proportion of cases of 'going light' in budgerigars and that haematological examination can establish this diagnosis in living birds.

Scanlan and Graham (1990) determined the cellular, cultural, and biochemical characteristics of eight isolates of a large Gram-positive bacillus that are commonly observed as apparently normal flora in the proventriculus of budgerigars (Melopsittacus undulatus) were determined. The bacterium was highly pleomorphic and changed markedly in both diameter and length when subcultured on agar media. The bacterium was facultative anaerobic and capnophilic, hemolytic on blood agar, and formed flat colonies with irregular edges after incubation for several days. All isolates grew on sodium azide agar but did not grow on MacConkey agar. The isolates were catalase-negative and oxidase-negative and did not reduce nitrate. All isolates failed to utilize arginine, lysine, ornithine or tryptophane but produced acid from glucose, galactose, levulose, maltose, melibiose, starch, and sucrose. All isolates produced acetoin from glucose and hydrolyzed esculin. The eight isolates could not be identified to either genus or species level based on the descriptions of currently classified organisms in the division Firmicutes as described in Bergey's Manual of Systematic Bacteriology.

Filippich and Hendrikz (1998) performed a study to measure the prevalence of megabacteria in budgerigar-breeding colonies and to evaluate possible methods to reduce the prevalence in 2 budgerigar (Melopsittacus undulatus) colonies with over 300 birds each. Overall the prevalence of megabacteria adjusted for colony differences was significantly higher ($P < 0.025$) in males compared to females. Age was not an influencing factor. After the initial survey, the prevalence in the offspring did not significantly ($P > 0.05$) decrease in the following two annual breeding seasons but it did significantly decrease after amphotericin B treatment. It was concluded that the practice of culling most birds with more megabacteria in faeces and discriminating against positive birds when selecting birds for breeding or culling birds on show quality does not decrease megabacteria prevalence in the offspring. However, a reduction in prevalence does occur with administration of amphotericin B. Birds may have amphotericin B-resistant organisms and these birds need to be identified and culled.

Werther *et al.* (2000) reportrd the occurrence of an megabacterium-like organism at small birds from the Northeast area of São Paulo State - Brazil. The results presented herein were obtained from 64 necropsy along four years (1994-1997). Sixty four birds (4 budgerigars Melopsittacus undulatus, 12 lovebirds Agapornis spp and 48 canaries Serinus canaria) were studied. About 56% of the examined birds showed at the proventricular mucus smear a presence of rod-shaped (similar to a toothpick) organisms, Gram positive and acidophilic in Giemsa staining, called megabacteria. Different kind of culture media was tested to replicated these organism in vitro. Also the dimension (large and width) of the fresh microorganism from the proventricular mucus and from the first culture was compared.

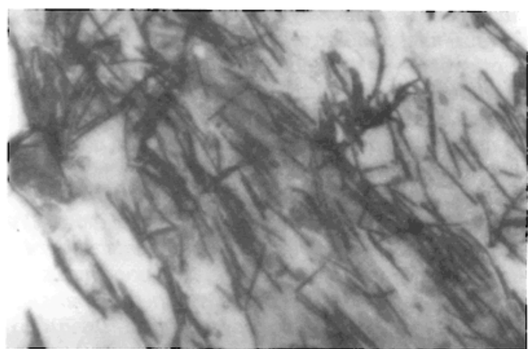


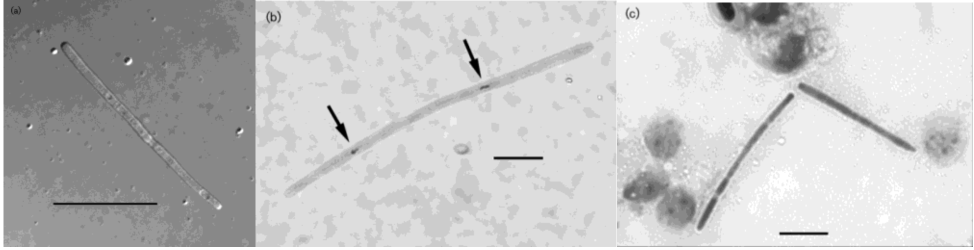
Figure 1 - Uniform elongated toothpicks-like structures observed in the smears of the proventricular mucus. Gram Stain, 40x.

Figure 2 - Photomicrograph of the proventriculus showing toothpick-like structures near the mucus glands openings. H&E Stain, 40x.

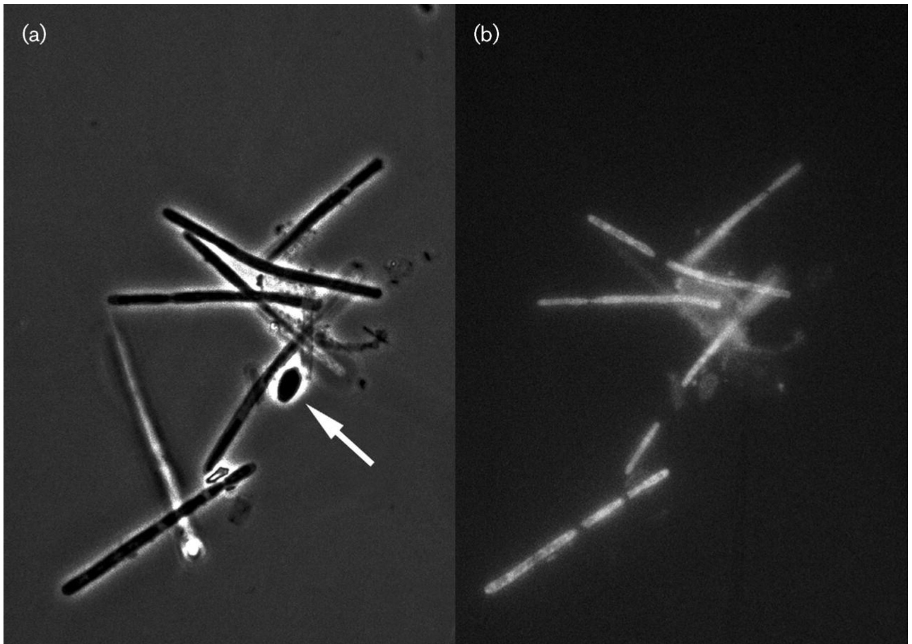
Werther *et al.* (2000)

Phalen and and Moore (2003) showed that the chicken can be used to amplify this organism and as a model to study its pathogenicity. An infection rate of 100% was achieved in day-old chicks orally inoculated with 10^5*M. ornithogaster* derived from the budgerigar (*Melopsittacus undulatus*). The organism was also determined to increase in number by greater than 10-fold 14 days after oral inoculation in these chicks. Chickens infected with *M. ornithogaster* demonstrated no sign of illness but had decreased feed conversion efficiency and consistent and characteristic histopathologic lesions in the proventriculus and isthmus of the stomach, suggesting that *M. ornithogaster* may represent a potential threat to the poultry industry.

Tomaszewski *et al.* (2003) reported that an organism commonly referred to as 'megabacterium' colonized the gastric isthmus of many species of birds. It is weakly Gram-positive and periodic acid-Schiff-positive and stains with silver stains. Previous studies have shown that it has a nucleus and a cell wall similar to those seen in fungi. Calcofluor white M2R staining suggests that the cell wall contains chitin, an eukaryote-specific substance, and rRNA in situ hybridization demonstrates that it is a eukaryote. To characterize this organism phylogenetically, DNA was extracted from purified cells. rDNA was readily amplified by PCR with pan-fungal DNA primer sets and primer sets derived from the newly determined sequence, but not with bacteria-specific primer sets. Specific primer sets amplified rDNA from isthmus scrapings from an infected bird, but not from a non-infected bird or other control DNA. The sequence was confirmed to derive from the purified organism by in situ rRNA hybridization using a specific probe. **Phylogenetic analysis of sequences of the 18S rDNA and domain D1/D2 of 26S rDNA showed the organism to be a previously undescribed anamorphic ascomycetous yeast representing a new genus. The name Macrorhabdus ornithogaster gen. nov., sp. nov. was proposed for this organism.**



Micrographs of *Macrorhabdus ornithogaster* gen. nov., sp. nov. in scrapings of the isthmus from a budgerigar: wet mount (a), acid-digested and Geimsa-stained (b) and Gram-stained (c). Structures believed to be nuclei are seen in the Geimsa-stained organism (b, arrows). Bars, 30 µm (a) and 10 µm (b, c). **Tomaszewski *et al.* (2003)**

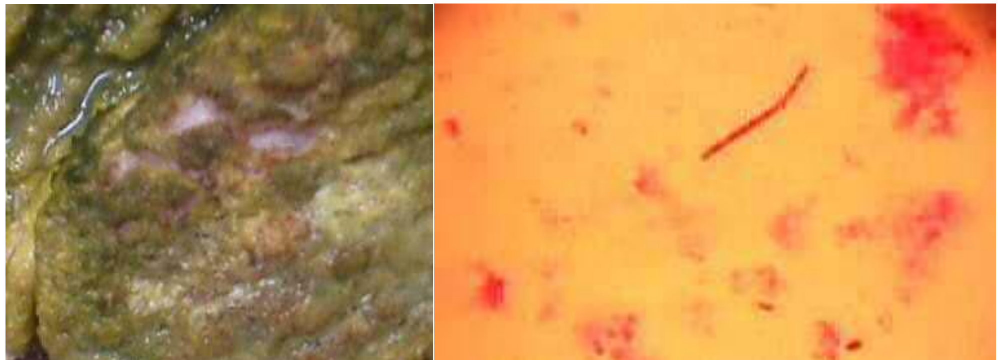


Bright-field (a) and PNA fluorescence *in situ* hybridization (b) images of cells of *M. ornithogaster* gen. nov., sp. nov. and *C. albicans* (arrow). The *M. ornithogaster*-specific probe for rRNA is selectively localized in the cytoplasm of *M. ornithogaster* cells. **Tomaszewski *et al.* (2003)**

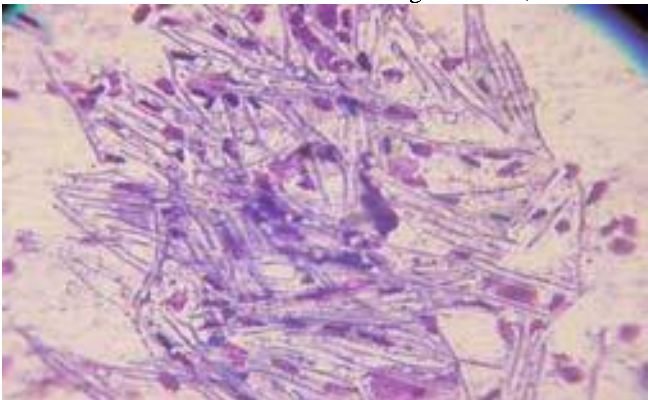
Marlier *et al.* (2006) conducted 312 post-mortem examinations of 178 canaries (Serinus canarius domesticus), 40 parakeets (Melopsittacus undulatus, Nymphicus hollandicus) and 94 parrots (Amazona aestiva, Psitaccus erithacus) were conducted at the Birds and Rabbits Service of the University of Liège, Belgium. After a detailed gross examination, tissue samples were collected for virological and/or bacteriological and/or parasitological examination to complete the diagnosis. In all cases, a microscopic examination of the proventricular mucus layer was undertaken for the detection of the anamorphic ascomycetous yeast Macrorhabdus ornithogaster, which causes the non-zoonotic but important disease in cage birds known as megabacteriosis. At the time of death, megabacteriosis was diagnosed respectively in 28% of canaries and 22.5% of budgerigars (P value for Fisher's exact test=0.5576), but was not diagnosed in parrots (P value for Fisher's exact test <0.0001). The incidence of megabacteriosis significantly increases along the years (P value for chi2 test <0.0001, Cramer's coefficient=0.3405). The most common gross lesions seen at necropsy of the 59 megabacteriosis cases was proventricular dilatation (86.1%). All the birds diagnosed as typical megabacteriosis cases were free of Salmonella spp. infections and of any parasitic infections. Four megabacteriosis cases (three canaries, one parakeet) were not included in statistical analysis as salmonellosis, pseudotuberculosis, coccidiosis and chlamydophilosis were diagnosed concomitantly in these birds. With the exception of megabacteriosis, the most frequent causes of death were protozoan (coccidiosis, lankesterellosis) infections (18.4%) and salmonellosis (17.1%) in canaries, and psittacosis (31.5%) and viral hepatitis (26.3%) in parakeets. In parrots, the most common causes of death were psittacosis (28.6%) and aspergillosis (28.5%).

Martins *et al.* (2006) mentioned that, since 2000, *Macrorhabdus ornithogaster* "megabacteriosis" has been diagnosed in the avian diseases laboratory in a diversity of avian species and varied spectrum of disease. The disease in some species (chickens, turkeys, guinea fowls) was clinically characterized by emaciation, prostration, loss of appetite, cachexia and death, with a typically chronic course. A more acute disease was observed in finches (canary-*Serinus* and zebra-*Taeniopygia*) and budgerigars (*Melopsittacus undulatus*). The large rod shaped organism, visible from 100 times magnification, with and without staining, could be detected in sick and also in reasonably normal individuals of some species, such as chickens, turkeys, quails and pigeons. In rheas (*Rhea americana*), ostriches (*Struthio camelus*), canaries, zebra-finches, guinea-fowl (*Numida meleagris*) and budgerigars. The disease was severe, causing to up to 100% mortality. The infection could be detected in some species along with other infectious or disease problems, such as endoparasites (helminths, coccidia) and ectoparasitism (order *Mallophaga* or/and order *Acarina*). The cultivation of *M. ornithogaster* was successfully achieved in solid and liquid media, originated from chickens (four isolates), guinea fowl (1 isolate), chuckar partridge (1 isolate) and canary (1 isolate). A very interesting finding at microscopy was motility of *M. ornithogaster*, as detected both in cultures obtained on agar for pathogenic fungi and passaged into thioglycolate broth, as well as on samples observed in wet preparations from *in vivo*. Differences in colony aspects were noted among the isolates. Experimental infections were attempted in chicken and japanese

quail, using a chicken isolate, allowing the detection of the organism in the proventriculus and liver in apparently normal birds. One chicken isolate was injected intraperitoneally in Balb/c mice and resulted in 100% mortality.

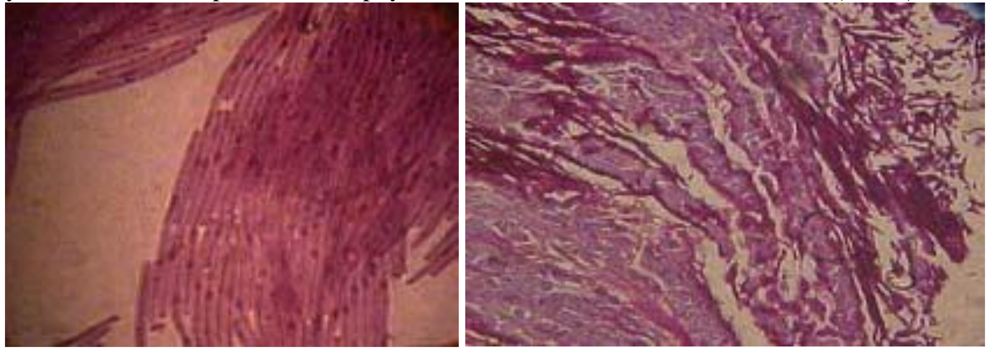


Ostrich At necropsy, ulcers on the gizzard mucosa, Impression smears stained by Giemsa. long rod-shaped organisms were observed at 400 and 1000 times magnification, **Martins *et al.* (2006)**



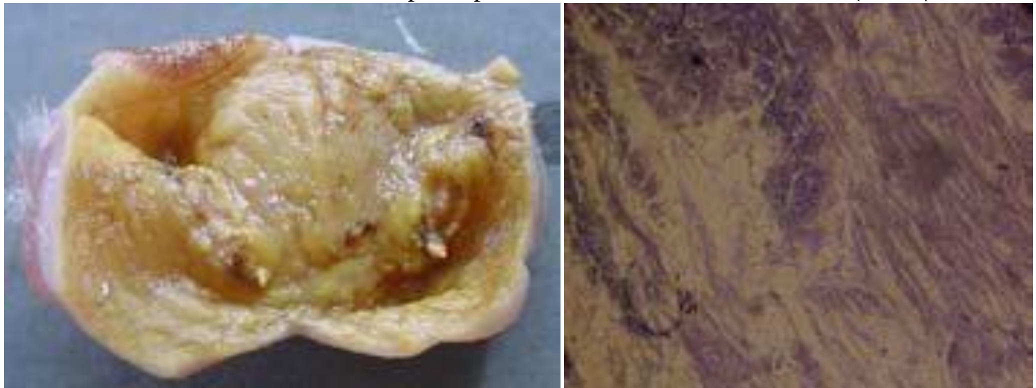
Budgerigar Impression smears stained by Giemsa showing very large rod-shaped organisms were observed **Martins *et al.* (2006)**

Canary: Emaciated birds presented atrophy of the breast muscles **Martins *et al.* (2006)**



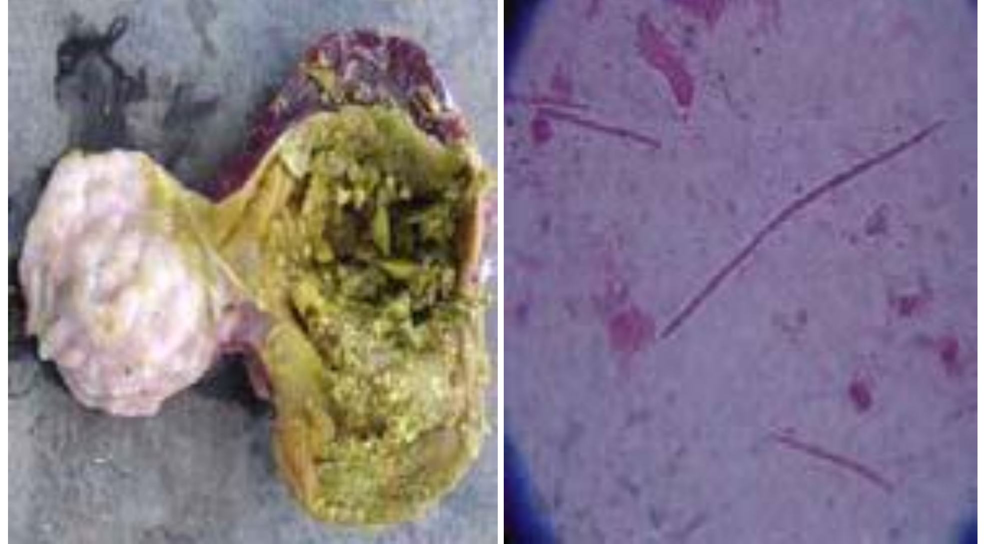
Giemsa-stained impression smears show very large rod-shaped organism, packed and disposed in palisade. Histologic sections of proventriculus showing the colonization into the crypts of the proventricular mucosa **Martins *et al.* (2006)**

A zebra finch: emaciation and death, atrophied pectoral muscles **Martins *et al.* (2006)**



At necropsy, gizzard ulcerations. Histopathology (H&E) of the gizzard mucosa enabled the visualization of massive clumps of re-ornithogaster attached to the epithelium **Martins *et al.* (2006)**

Broiler chickens emaciation, prostration, Proventricular: enlarged, hypertrophic and irregular **Martins *et al.* (2006)**

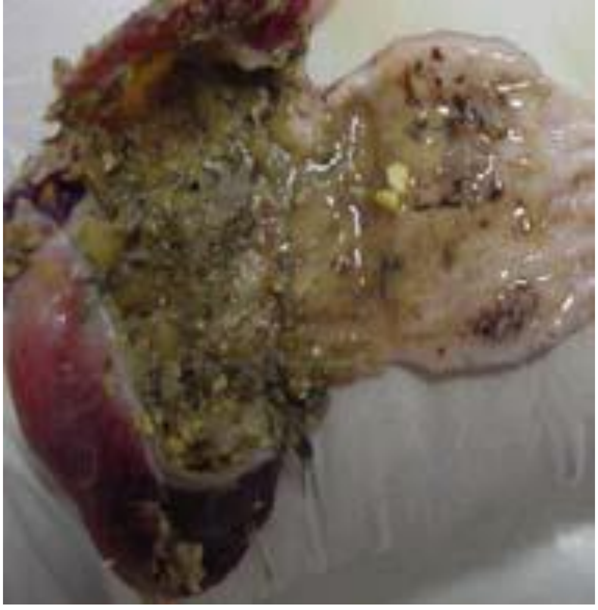


Proventricular: enlarged, hypertrophic and irregular). Impression smears stained by Giemsa revealed very large rod-shaped organisms **Martins *et al.* (2006)**

Quails: enlarged proventriculus with milky secretion and eroded gizzard koilin layer, where the organism could be detected were observed **,** In the liver hemorrhages were seen **Martins *et al.* (2006)**

A rhea: severe emaciation, Hemopericardium and cachexia demonstrated by the reduction of the cardiac fat. **Martins *et al.* (2006)**

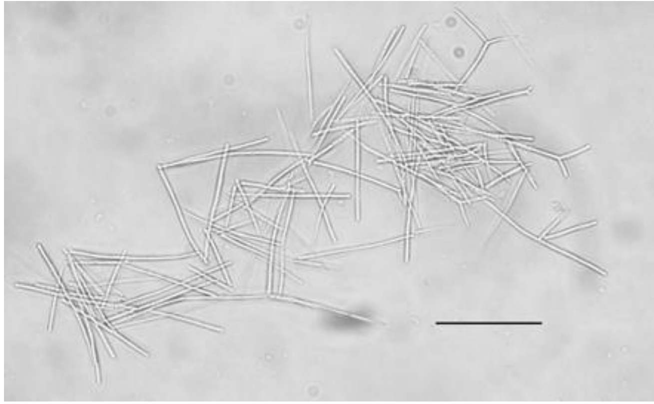
Ulcerations in the proventriculus and ventricular impaction were observed and mucosal slide smears enabled the visualization of the large organism **Martins *et al.* (2006)**



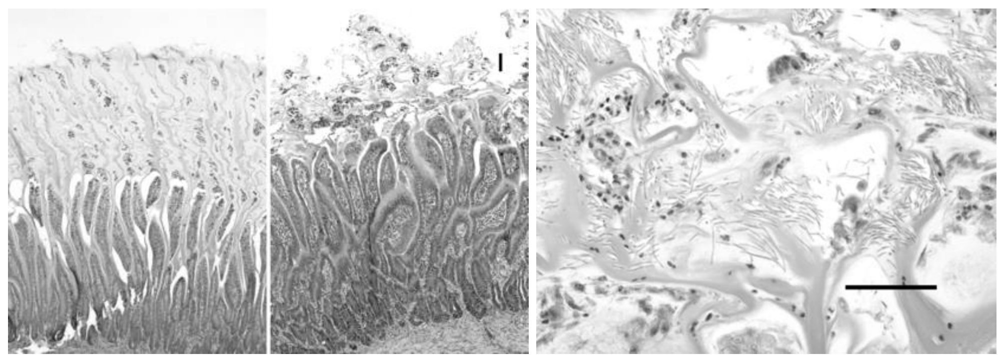
A free-range flock of guinea fowl showing ulcerations on the gizzard mucosa and petechial hemorrhages on the proventricular mucosa **Martins *et al.* (2006)**

Hannafusa *et al.* (2007) examined multiple liquid and solid media of varying pH, sugar concentration, and fetal bovine serum (FBS) concentrations, incubated at various temperatures in room air or microaerophilic conditions, for their ability to support the growth of M. ornithogaster, obtained from a budgerigar (Melopsittacus undulatus). Optimum growth conditions were found to be Basal Medium Eagle's, pH 3 to 4, containing 20% FBS, and 5% glucose or sucrose under microaerophilic conditions at 42 degrees C. Using these conditions, M.ornithogaster was repeatedly passaged without loss of viability. Polyclonal isolates of M. ornithogaster consistently assimilated glucose, sucrose, and trehalose. M. ornithogaster did not grow with prolonged exposure to atmospheric oxygen, but growth in microaerophilic conditions was moderately enhanced by preincubation with atmospheric oxygen for 24 hours. An isolate of M. ornithogaster was found to be infective to day-old chickens, reduce their rate of weight gain, and induce a mild to moderate heterophilic inflammation of the isthmus. M. ornithogaster was reisolated from the chicks 7 days after infection, fulfilling Koch's postulates. A 761-bp sequence of 18S rDNA from this isolate was

compared to the originally reported M. ornithogaster sequence and was found to be 97% identical.



Kohler illumination of a cluster of *Macrorhabdus ornithogaster* grown in optimized medium under microaerophilic conditions at 42°C. Bar = 60 µm **Hannafusa *et al.* (2007)**



Photomicrographs of hemotoxylin and eosin-stained sections through the gastric isthmus of a 7-day-old control (**left**) and *Macrorhabdus ornithogaster* infected chicken (**right**). The koilin of the infected chicken is disrupted by heavy growth of *M. ornithogaster*. In addition, there is an increased cellularity to the lamina propria of the infected bird, and there are microabscesses in the koilin. Bar = 100 µm. Photomicrograph of a hemotoxylin and eosin-stained section of the koilin layer of a 7-day-old chicken infected with *Macrorhabdus ornithogaster.* Numerous *M. ornithogaster* are present in the koilin. Bar = 100 µm **Hannafusa *et al.* (2007)**

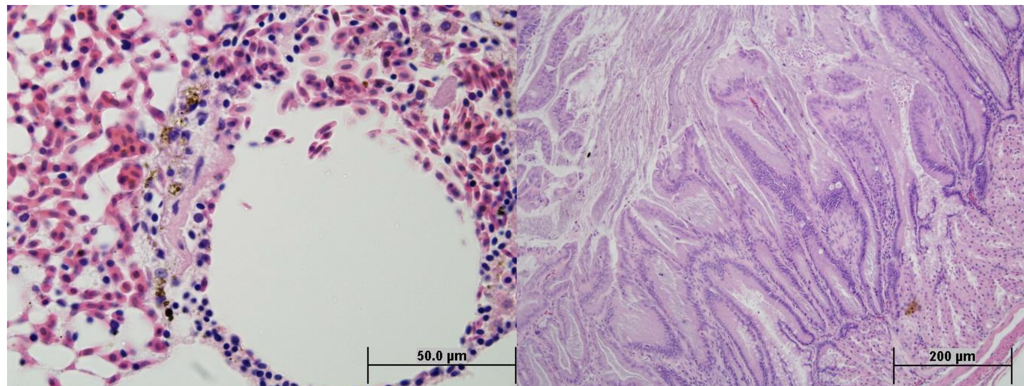
Hanka (2008) reported that a significant correlation between single clinical signs and the degree of infestation exists only in few birds. For all orders of birds can be ascertained a significant correlation between the degree of infestation and an increased accumulation of mucus in the proventriculus, redness or petechia of its mucous membrane, dilatation or thickening of the mucosa and conspicuously dark intestinal contents. Average linear measurements of Macrorhabdus ornithogaster of almost all birds range between 26 µm and 45 µm. In contrast, Macrorhabdus ornithogaster in cockatiels are significantly shorter and have an average length of 21 µm. Age and gender of the affected animals have no significant effect on the rate and degree of infestation of Macrorhabdus ornithogaster. Also, no correlation is noticeable concerning the season of the death and the degree of infestation. The birds of the order Galliformes show a weak significant accumulation of deaths during the autumn and winter months were an exception here. However, a significant correlation is clearly established between the nutritional condition and the degree of infestation with Macrorhabdus ornithogaster in the orders Galliformes, Psittaciformes and

Passeriformes. The average body weight of budgerigars free of Macrorhabdus ornithogaster amounts to 38.3 grams, that from infected budgerigars amounts to 33.4 grams. Canaries free of agents have an average body weight of 19.6 grams, and infected only 15.7 grams. Diagnostically, it is important that only a low proportion of the Macrorhabdus ornithogaster-positive birds (8 animals of 63 examined) are also histologically positive following H&E and PAS staining. Macrorhabdus ornithogaster is considered as a facultative pathogenic agent for birds with the likely exception of budgerigars and birds of the family Carduelidae. The isolation and propagation of Macrorhabdus ornithogaster in liquid and solid synthetic media failed in all instances.

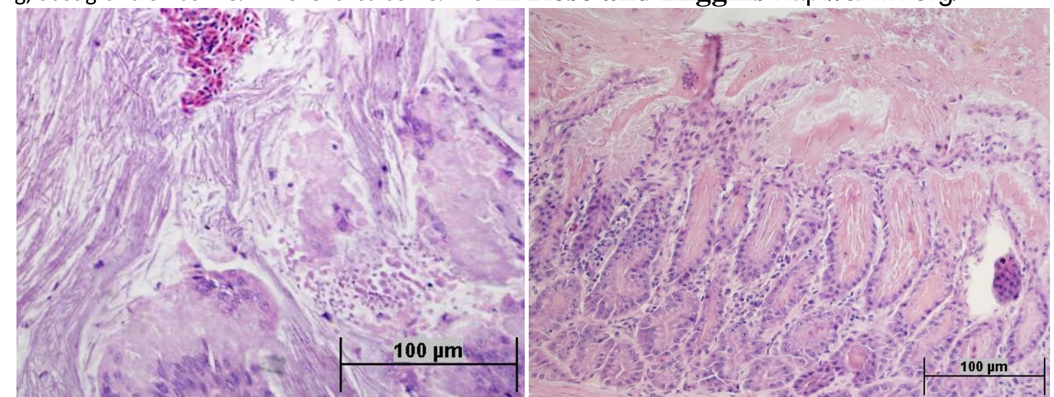
Jansson *et al.* (2008) diagnosed proventriculitis and chronic respiratory disease in two flocks of gray partridges (Perdix perdix) on unrelated Swedish game bird farms. Affected birds showed loss of condition, respiratory signs, and flock mortality rates of 50 and 98%, respectively. The proventricular lesions were associated closely with fungal organisms that were microscopically indistinguishable from the ascomycetous yeast Macrorhabdus ornithogaster (former provisional name "megabacterium"). At necropsy, the proventriculi were swollen and hyperemic, and viscous mucus adhered to the mucosa. Proventricular hemorrhages were commonly detected, and one bird had proventricular rupture and peritonitis. Microscopically, mild to severe subacute to chronic lymphoplasmacytic proventriculitis, microabscesses, necrosis, epithelial metaplasia, disrupted koilin, ulcers, and hemorrhages were observed. Transmission electron microscopy of the proventricular microorganisms revealed a membrane-bound nucleus, vacuoles, ribosomes, microtubules in parallel arrays, and a two-layered cell wall but no mitochondria. Scanning electron microscopy of the proventricular epithelium demonstrated masses of organisms with occasional constrictions in parallel arrangement. Many of the birds also suffered from concurrent respiratory bacterial infections and/or gastrointestinal candidiasis. The clinical course and gross and microscopic proventricular lesions were similar to those described in psittacine and passerine pet birds colonized by M. ornithogaster-like microorganisms but differed from published case reports and experimental infections of chickens in which the clinical signs and lesions have been considerably milder. The findings presented in this paper suggest that mycotic proventriculitis, presumably associated with M. ornithogaster, may be a serious but possibly opportunistic, although unusual, disease problem in gray partridges on game farms.

Rose and Higgins (2009) reported megabacteriosis ('ARWH case 1168.1') in a. female adult zebra finch (Poephila guttata) found dead on the ground. Lesions were not evident within oesophagus, brain, myocardium, skeletal muscle, small intestine, pancreas, ovary, kidney. The pulmonary parenchyma was markedly congested. Small dust granulomas were scattered throughout the atrial interstitium. The proventricular glands were distended with large numbers of large, filamentous, septate organisms. The intestinal lumen also contained many of these organisms and abundant sloughed epithelial cells. These organisms were also evident within the deep aspect of the ventricular crypts along the proventricular/ventricular junction. The organisms were multifocally evident within the koilin layer and superficial epithelium throughout the ventriculus, They commented that the bird was suffering from extensive gastric megabacteriosis. The diagnosis was confirmed through the application of special stains to recut sections of the proventriculus. Megabacteria stain positively with Gram's and PAS stains. Megabacteria are often associated with chronic weight loss in psittacines. This bird was in good body condition, thus, the significance of this finding is uncertain. The sloughing of epithelial cells into the proventricular lumen and

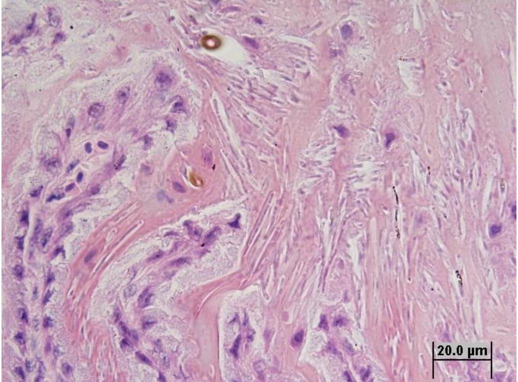
ventricula koilin layer suggests that the infection may have resulted in increased cell turnover or cell death.



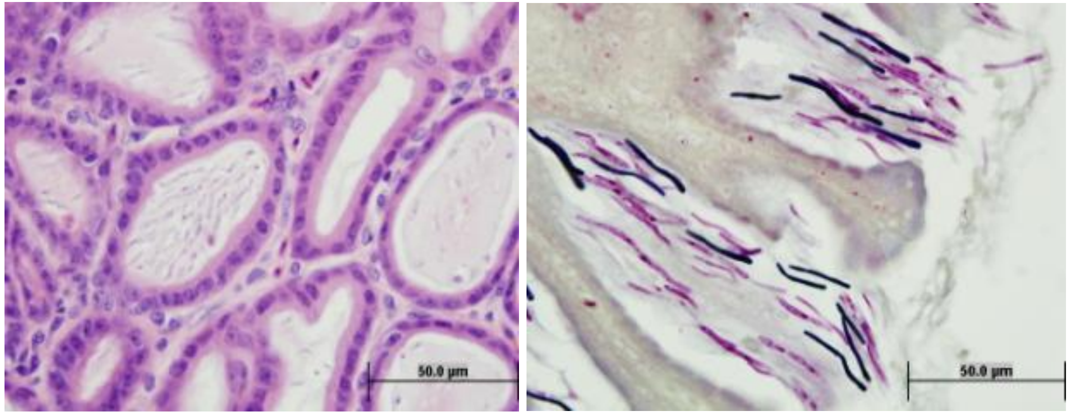
Lung, dust granulomas. H&E Proventriculus H&E 40xm **Rose and Higgins** http://arwh.org/

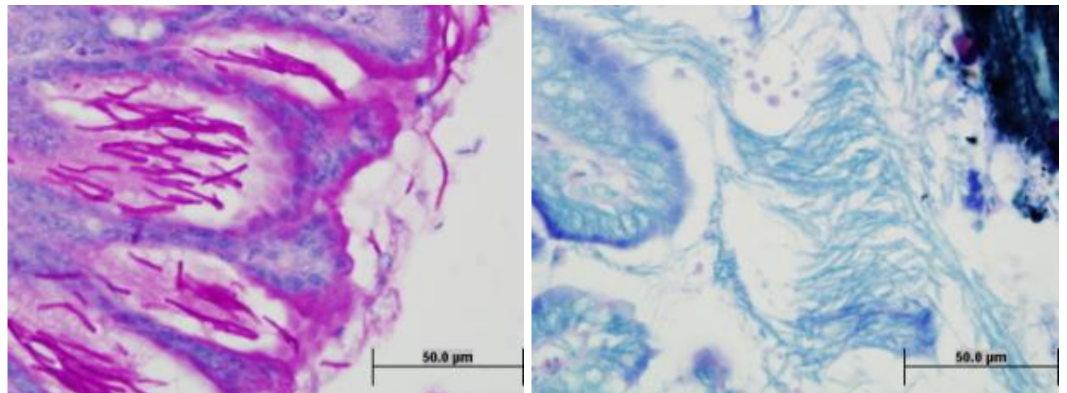


Proventriculus H&E 100Ventriculus (gizzard), mucosa and koilin lining. H&E 40x **Rose and Higgins** http://arwh.org



Ventriculus, mucosa and koilin lining. H&E 100x **Rose and Higgins** http://arwh.org

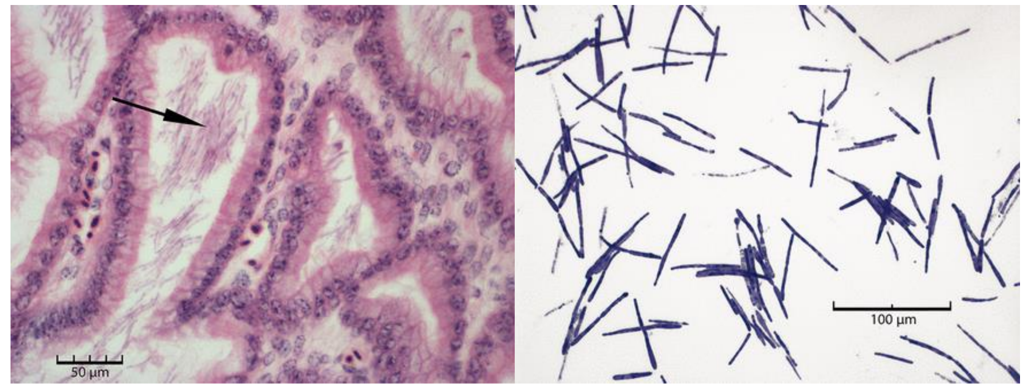




Staining characteristics of Megabacteria. a) H&E; b) Brown and Brenn's Gram's (BB), c) Periodic acid Schiff (PAS), d) Zeihl Neilsen (ZN). **Rose and Higgins** http://arwh.org

Behnke and Fletcher (2011) conducted a field investigation on a flock of adult hobby chickens showing intermittent signs of enteritis. Roosters examined in the initial field visit and postmortem had cecal worms, roundworms, tetratrichomonads, and coccidiosis. Macrorhabdus ornithogaster was diagnosed histologically in the mucosal isthmus of the proventriculus and ventriculus. Three roosters and two hens were examined in a follow-up investigation of the flock conducted 9 days later. Macrorhabdus ornithogaster was confirmed in one hen.

Hanafusa *et al.* (2013) carried out an experimental study to determine if Macrorhabdus ornithogaster, a yeast recovered from the junction of the proventriculus and ventriculus of the stomach of parrots and other birds, can infect mice. Fifteen healthy ARC (S) female mice (age 10 weeks) were incoculated with M. ornithogaster by gavage at (two different dosage rates [n = 5]) or intraperitoneal injection (n = 5) (one dosage rate). They were euthanized 5 days later and examined for gross and microscopic evidence of infection. Macrorhabdus ornithogaster was not found to colonize the stomach, peritoneum, or viscera of the challeneged mice. The results of this study showed that M. ornithogaster was not able to infect mice by the oral and intraperitoneal routes of administration and suggested that infection in mammals is unlikely to occur. *They stated that M. ornithogaster* is a slender and long microorganism (2 µm wide and up to 80 µm long) composed of two to six cells. Filamentous bacteria and chains of large rod-shaped bacteria can superficially resemble it. However, this yeast has some very distinctive staining characteristics that distinguish it from other microorganisms. It stains positively with the Gram stain, but unlike bacteria in which the stain is found in the cell walls, the stain variably accumulates in the cytoplasm of *M. ornithogaster* and is not found in the cell wall. The yeast also stains with the Periodic Acid Shift stain and the positive response obtained with calcafluor distinguishes it from bacteria. Genetically, it is readily separated from bacteria, as its ribosomal DNA can be amplified by PCR using panfungal and *M. ornithogaster*-specific primers.



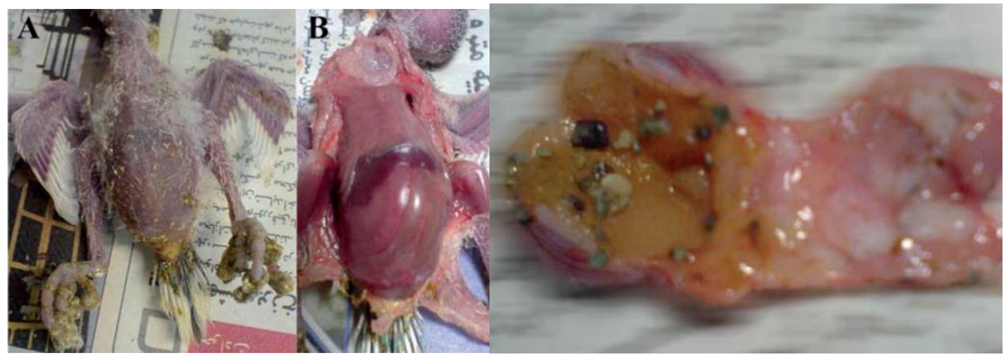
Haematoxylin and eosin stained section through the junction of the proventriculus and ventriculus (isthmus) of the stomach of a canary. *Macrorhabdus ornithogaster* are seen as tightly packed slender rod-shaped organisms that that are moderately eosionphilic. In this section they fill the spaces within the mucosal glands (Arrow). Gram stain of *Macrorhabdus ornithogaster* grown in culture. This organism is considered to be Gram positive, but often stains incompletely or faintly. **Hanafusa *et al.* (2013)**

Lanzarot *et al.* (2013) performed a work to assess the prevalence and patterns of fecal shedding of M. ornithogaster in a colony of healthy canary birds (Serinus canaria) bred in captivity. Fresh fecal samples from 39 canaries (17 males and 22 females) were cultured in liquid media for M. ornithogaster enrichment. Only two clinically healthy females were fecal culture-positive for the yeast, which represents an overall prevalence of 5.13% in the sampled population. A close surveillance of the two culture-positive canaries, which included periodical microscopic examination of fresh stool samples, showed prolonged fecal shedding of M. ornithogaster. Nevertheless, both animals remained asymptomatic throughout the study period. To the best of our knowledge, this is the first study reporting the continuous shedding of M. ornithogaster by clinically healthy canaries.

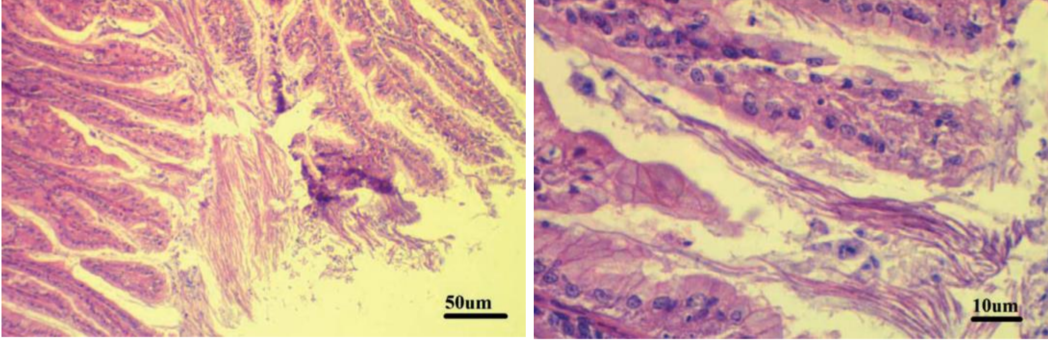
Australian Registry of Wildlife Health (2014) reported that 9 cases of ***M. ornithogaster*** infections were reported wild galah with a chronic wasting disease and 7 captive birds. They included a king quail (Coturnix chinensis), a zebra finch, a double-barred finch (Taeniopygia bichenovii), a plum-headed finch (Neochmia modesta), two red-browed finch (Neochnia temporalis) and a chestnut-breasted manikin (Lonchara castaneothorax) which are all native Australian species. Lastly, infection was detected in a Java sparrow (Lonchura oryzivoma) an Indonesian species.

Madani *et al.* (2014) investigated the occurrences of acute macrorhabdosis resulting in severe mortality in budgerigar fledglings and the effect of different treatment regimens for the control of the disease. The budgerigar (Melopsittacus undulates) flock consisted of over five hundred breeding adults. The morbidity of chicks reached 90% with more than 50% mortality. The significant clinical and pathological findings included distended abdomen, diarrhoea, ingluvitis, proventriculitis, and mild enteritis. Severe M. ornithogaster infection was diagnosed based on cytologic and histologic investigations. Three weeks of nystatin medication in the feed and vinegar administration in the drinking water led to moderate improvement of the flock mortality. After the initial treatment, 500 mg/Lsodium benzoate was administered in

the drinking water for four weeks. The second treatment regimen was promisingly effective in reducing mortality. However, some sick and retarded birds with M. ornithogaster with positive proventricular smears at necropsy were found in the flock. Consequently, a higher dosage of 1 gr/Lin drinking water for another four weeks was recommended. After the eight weeks of treatment, no new cases were found in the flock and all dropping samples became negative for the presence of M. ornithogaster. Based on these preliminary findings, sodium benzoate can be an efficient and inexpensive alternative to the previous labour intensive and expensive treatment using amphotericin B.



Heavy plantar contamination of the feet in a two-weekold budgerigar affected by severe macrorhabdosis. Diarrhoea and khaki coloured dropping can be a clinical sign of macrorhabdosis in budgerigars. B) Distended abdomen and diphtheric ingluvitis in budgerigar hatchling. Typical thickening of crop and mucosal diphtheric lesion can be the result of severe macrorhabdosis.Proventricular lesion of acute macrorhabdosis in a budgerigar fledgling. Increased thickness of proventricular wall, enormous mucoid content, mucosal hyperaemia and a focal haemorrhage in the isthmus between the proventriculus and the gizzard can be observed.



Proventricular mucosa affected by acute macrorhabdosis. Aggregation of a massive number of rod to filamentous shaped *M. ornithogaster* resembling a broom stick and their invasion of the submucosa are evident. H&E staining. Bar = 50⌠m. Colonization and invasion of *M. ornithogaster* within proventricular mucosa.

Phalen (2014) reviewed an update on the diagnosis and management of *Macrorhabdus Ornithogaster* in avian species. He mentioned that Macrorhabdus ornithogaster is an anamorphic Ascomycetes yeast that grows only at the junction of the proventriculus and ventriculus in birds. It can infect many species of birds. There is convincing evidence that M ornithogaster can cause disease in its host but it is also clear that many birds live with this organism without obvious signs. The only effective treatments for M ornithogaster are a few antifungal drugs and these drugs do not always lend themselves to large-scale flock treatment. Direct observation of the organism in the feces is a specific but somewhat insensitive means of diagnosis. At least three antifungal drugs are reported to be effective for treatment of M. ornithogaster but resistance to one or more of these drugs may occur

Babazadeh *et al.* (2015) described the Megabacteriosis and staphylococosis in a dead canary referred to the clinic of veterinary medicine, university of Tehran, Iran. The proventriculus was dilated and erosive lesions were seen. In wet smear prepared from proventriculus, Macrorhabdus ornithogaster was observed by light microscope. A purulent mass was detected in metatarsal joint, the gram staining of suspected mass determined the bird suffered from staphylococosis. Prescribed drugs for Megabacteriosis and Staphylococosis were nystatin and enrofloxacin respectively.

Borrelli *et al.* (2015) carried out a study to propose the use of a new rapid and user-friendly diagnostic tool for the detection of Macrorhabdus ornithogaster infection in birds. The current report focuses on the diagnostic feasibility of different methods, with particular emphasis on the application of the mini-Flotac technique for the diagnosis of M. ornithogaster infection. The mini-Flotac method is particularly tailored for epidemiological monitoring and surveillance, where large numbers of fecal samples must be rapidly, yet reliably, examined. Gram staining, as the standard method, was used to validate the reliability of the mini-Flotac method. This tool has not yet been used in avian species or in the diagnosis of yeast infections. In our study, M. ornithogaster showed excellent performance in a flotation assay, which had not been demonstrated previously. Our results suggest that the mini-Flotac method is a valid, sensitive, and potentially low-cost alternative technique for use in the diagnosis of this yeast infection in birds.

Legler *et al.* (2015) mentioned that the colonization of the gastric ascomycetous yeast Macrorhabdus (M.) ornithogaster could be associated with a chronic wasting disease in several bird species in captivity. In the wintering season 2012/13 injured Eurasian Siskins (Carduelis spinus, n = 8) from the area of Hannover, Lower Saxony, Germany were examined microbiologically and pathologically. In six out of eight injured Eurasian Siskins M. ornithogaster was detected. The yeast was diagnosed microscopically in wet smears from the gastric isthmus and/or in faecal samples. Histopathological examination (n = 4) of the macroscopically slightly enlarged proventriculus in infected birds demonstrated the growth of M. ornithogaster in the mucosal surface and in the ducts of the glands without an inflammatory reaction. As a possible sign of a lowered fitness, all six infected siskins had a reduced body weight (mean: 11.8 ± 1.64 g) in the lower normal weight range compared to the two injured Eurasian Siskins without M. ornithogaster (15.0 g) as well as to data from the literature. Concurrent intestinal bacterial infections comprised Escherichia coli, Clostridium perfringens or Salmonella Typhimurium, that are regarded as an abnormal bacterial flora for Eurasian Siskins.

References

1. Australian Registry of Wildlife Health (1914) http://arwh.org/
2. Baker JR. Clinical and pathological aspects of “going light” in exhibition budgerigars (Melopsittacus undulates). Veterinary Record 1985;116:406-408.
3. Babazadeh, D. , Samereh Ghavami , Hossein Nikpiran , Nima Dorestan. Acute Megabacteriosis and Staphylococosis of Canary in Iran J. World's Poult. Res. 5(1): 19-20, March 25, 2015
4. Behnke EL, Fletcher OJ. Macrorhabdus ornithogaster (Megabacterium) infection in adult hobby chickens in North America. Avian Dis. 2011 Jun;55(2):331-4.

5. Borrelli L, Dipineto L, Rinaldi L, Romano V, Noviello E, Menna LF, Cringoli G, Fioretti A. New Diagnostic Insights for Macrorhabdus ornithogaster Infection. J Clin Microbiol. 2015 Nov;53(11):3448-50.
6. Brian Stockdale,B. Macrorhabdus Ornithogaster (Megabacteria) – New name for an old disease
7. Cooke, S. W. (2000): Role of megabacteria in mammals. The Veterinary Record 146, 444..
8. Dorrestein GM, Zwart P, Buitelaar MN. Problems arising from disease during the periods of breeding and rearing canaries and other aviary birds. Tijdschr Diergeneeskd. 1980 Jul 1;105(13):535-43.
9. Filippich LJ, Hendrikz JK. Prevalence of megabacteria in budgerigar colonies. Aust Vet J. 1998 Feb;76(2):92-5.
10. *FILIPPICH*, *L. J. PARKER*, *M. G. Megabacteria* in wild *birds in ...* A. *Megabacteria in birds in Australia*. *Australian Veterinary*. *Practitioner*. *1993*. 23(2): 71-72,
11. Gerlach H: Megabacteriosis. Sem Avian Exotic Pet Med 10:12-19, 2001.
12. Huchzermeyer FW, Henton MM, Keffen RH: High mortality associated with megabacteriosis of proventriculus and gizzard in ostrich chicks. Vet Rec 133:143-144, 1993
13. Hanafusa Y, Costa E, Phalen DN. Infection trials in mice suggest that Macrorhabdus ornithogaster is not capable of growth in mammals. Med Mycol. 2013 Aug;51(6):669-72.
14. HANKA ,K. Untersuchungen zum Nachweis von Macrorhabdus ornithogaster bei Vögeln der Ordnungen Galliformes, Psittaciformes, Passeriformes, Anseriformes und Columbiformes sowie Versuche zur Anzüchtung des Erregers der Macrorhabdiose in vitro. INAUGURAL-DISSERTATION , Faculty of Vet. Med. Justus-Liebig-Univ. Gießen 2008
15. Hargreaves, R. C. (1981): A fungus commonly found in the proventriculus of small pet birds. Proceedings of the 30th Western Poultry Disease Conference and 15th Poultry Health Symposium, University of California, Davis, 1981, S. 75-.
16. Hannafusa Y, Bradley A, Tomaszewski EE, Libal MC, Phalen DN. Growth and metabolic characterization of Macrorhabdus ornithogaster. J Vet Diagn Invest. 2007 May;19(3):256-65.
17. Henderson GM, Gulland FM, Hawkey CM. Haematological findings in budgerigars with megabacterium and Trichomonas infections associated with 'going light'. Vet Rec. 1988 Nov 5;123(19):492-4.
18. Huchzermeyer, F. W., Henton, M. M. (2000): Megabacteria in mammals. TheVeterinary Record 146, 768.
19. Jansson DS, Bröjer C, Mattsson R, Feinstein R, Mörner T, Hård af Segerstad C. Mycotic proventriculitis in gray partridges (Perdix perdix) on two game bird farms. J Zoo Wildl Med. 2008 Sep;39(3):428-37.
20. Lanzarot P, Blanco JL, Alvarez-Perez S, Abad C, Cutuli MT, Garcia ME. Prolonged fecal shedding of 'megabacteria' (Macrorhabdus ornithogaster) by clinically healthy canaries (Serinus canaria). Med Mycol. 2013 Nov;51(8):888-91.
21. Legler M, Stelter R, Jung A, Wohlsein P, Kummerfeld N. First detection of Macrorhabdus ornithogaster in wild Eurasian Siskins (Carduelis spinus) in Germany. A case study. Tierarztl Prax Ausg K Kleintiere Heimtiere. 2015;43(3):161-5.
22. Lublin, A., Mechani, S., Malkinson, M., Weisman, Y. (1998): A five-year survey of megabacteriosis in birds of Israel and a biological control trial. Proceedings of the Association of Avian Veterinarians, S. 241-245.
23. Madani, S. A., Amir Ghorbani , Fatemeh Arabkhazaeli. Successful treatment of macrorhabdosis in budgerigars (Melopsittacus undulatus) using sodium benzoate Journal of Mycology Research. Vol 1, No 1: Page 21-27, September 2014 JMR (Sep.2014), 1(1) 21

24. Marlier D, Leroy C, Sturbois M, Delleur V, Poulipoulis A, Vindevogel H. Increasing incidence of megabacteriosis in canaries (Serinus canarius domesticus). Vet J. 2006 Nov;172(3):549-52.
25. Martins N.R.S, Horta A.C, Siqueira A.M, Lopes S.Q, Resende J.S, Jorge M.A, Assis R.A, Martins N.E, Fernandes A.A, Barrios P.R, Costa T.J.R and Guimarães L.M.C. (2006). *Macrorhabdus ornithogaster* in ostrich, rhea, canary, zebra finch, free range chicken, turkey, guinea-fowl, columbina pigeon, toucan, chucker partridge and experimental infection in chicken, Japanese quail and mice. *Arquivo Brasileri*ro de *Medicina Veterinaria Zootecnia*.58:291-298.
26. Phalen. D.N., Update on the Diagnosis and Management of*Macrorhabdus Ornithogaster* (Formerly Megabacteria) in Avian Patients. Vet Clin Exot Anim 17 (2014) 203–210
27. Phalen, D. N. and Robert P. Moore. Experimental Infection of White-Leghorn Cockerels with Macrorhabdos ornithogaster (Megabacterium). Avian Diseases 47(2):254-260. 2003
28. Ravelhofer-Rotheneder, K. (2002): Verschiedene Aspekte zur Diagnostik und Pathogenität einer "Megabakterien"- Infektion beim Wellensittich. XIII. DVG-Tagung über Vogelkrankheiten, München, 21./22. Februar 2002, S. 66-73.
29. Rose,K. and D Higgins (2009) Megabacteriosis in a Zebra Finch (*Poephila guttata*) (ARWH Case 1168.1). Australian Registry of Wildlife Health, Taronga Conservation Society. http://arwh.org/sites/default/files/files uploads/Case%201168.1%20Megabacteriosis%20in%20a%20Zebra%20Finch.pdf
30. Scanlan CM, Graham DL. Characterization of a Gram-positive bacterium from the proventriculus of budgerigars (Melopsittacus undulatus). Avian Dis. 1990 Jul-Sep;34(3):779-86.
31. Schweighardt, H., Pechan, P., Lauermann, E. (1984): Leichtwerden, Hinfälligkeit und Erbrechen als Ausdruck einer spezifischen, pilzbedingten Drüsenmagenentzündung bei Wellensittichen und Kanarienvögeln. Kleintierpraxis 29, 439-442.
32. Tsai, S. S., Park, J. H., Hirai, K., Itakura, C. (1992): Catarrhal proventriculitis associated
33. Tomaszewski EK, Logan KS, Snowden KF, Kurtzman CP, Phalen DN. Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast,Macrorhabdus ornithogaster gen. nov., sp. nov. Int J Syst Evol Microbiol. 2003 Jul;53(Pt 4):1201-5.
34. van Herck, H., Duijser, T., Zwart, P., Dorrestein, G. M., Buitelaar, M., van der Hage, M. H. (1984): A bacterial proventriculitis in canaries. Avian Pathology 13, 561-572.
35. Werther, K, Schocken-Iturrino, R P, Verona, CES, & Barros, LSS. (2000). Megabacteriosis Occurrence in Budgerigars, Canaries and Lovebirds in Ribeirao Preto region - Sao Paulo State - Brazil. *Revista Brasileira de Ciência Avícola*, *2*(2), 183-187.

2.4.Avian Rhodotorulosis

Rhodotorulosis (mycotic dermatitis): Rhodotorulosis is caused by pink yeast *Rhodotorula*, the yeast cells common contaminants and are infrequently associated with disease conditions (Vazquez, 2011).

- The fungus has been isolated from poultry litter and pigeon faecal droppings and is of public health concern.
- *Rhodotorula glutinis* produces dermatitis in broiler chicken, while *Rhodotorula mucilaginosa* cause dermatitis of feathers (Beemer *et al.,* 1970; Hubalek, 1978; Chauhan and Roy, 1996; Alvarez-Perez *et al.,* 2010).
- *Rhodotorula* species predominantly associated with trachea of fowls and has even been isolated from digestive organs (crop).
- Birds die suddenly with crop highly distended and filled with feed.

Reports:

Aruo (1980) described necrotizing cutaneous rhodotorulosis in chickens in Uganda

Beemer *et al.* (1970) reported an outbreak of dermatitis, where 5-20% of the plucked chickens had skin lesions on the back and thighs. *Rhodotorula mucilaginosa* was isolated and the condition could be reproduced in healthy chickens by the application of a suspension of the organism. Filtrates of culture fluid or smaller doses of the organisms were ineffective. Unhygienic conditions are considered to be a predisposing factor as the outbreak ceased after the poultry runs were cleaned and had not reappeared

Page *et al.* (1976) reported an outbreak of dermatitis involving Rhodotorula glutins in broiler-type chickens in south-eastern United States

Wirth and Goldan (2012) published an updated paper focusing on the general epidemiological aspects of Rhodotorula in humans, animals, and the environment. They mentioned that Rhodotorula species have emerged as opportunistic pathogens that have the ability to colonise and infect susceptible patients. Rhodotorula species are ubiquitous saprophytic yeasts that can be recovered from many environmental sources. Several authors describe the isolation of this fungus from different ecosystems, including sites with unfavourable conditions. Compared to R. mucilaginosa, R. glutinis and R. minuta are less frequently isolated from natural environments. Among the few references to the pathogenicity of Rhodotorula spp. in animals, there are several reports of an outbreak of skin infections in chickens.

References:

1. Aruo, S.K., 1980. Necrotizing cutaneous rhodotorulosis in chickens in Uganda. Avian Dis., 24: 1038-1043
2. Beemer, A. M., S. Schneerson-Porat and E. S. Kuttin. Rhodotorula mucilaginosa Dermatitis on Feathered Parts of Chickens: An Epizootic on a Poultry Farm, *Avian Diseases*, 14, 2 (May, 1970), 234-239
3. Wirth, Fernanda Wirth and Luciano Z. Goldani, "Epidemiology of Rhodotorula: An Emerging Pathogen,"Interdisciplinary Perspectives on Infectious Diseases, vol. 2012, Article ID 465717, 7 pages, 2012. doi:10.1155/2012/465717 and Luciano Z. Goldani, "Epidemiology of Rhodotorula: An Emerging Pathogen,"Interdisciplinary Perspectives on Infectious Diseases, vol. 2012, Article ID 465717, 7 pages, 2012. doi:10.1155/2012/465717
4. Page, R K , O J Fletcher, C S Eidson, G E Michaels. **Dermatitis produced by Rhodotorula glutins in broiler-age chickens.** Avian Dis 1976 Apr-Jun;20(2):416-21

3. Avian Mycosis Caused by Moulds *106*

3.1. Avian aspergillosis

Synonyms:

Pneumonomycosis, Bronchomycosis, Cytomycosis, Brooder pneumonia, and in part Pseudotuberculosis and Mycosis; in ostriches Chick fever and Yellow liver. Pneumonie du couveuse (Fr.).

Avian aspergillosis is an infectious fungal disease of wild and domestic birds caused by Aspergillus species. It is characterized by primary involvement of respiratory tract, formation of yellow cheesy plaques and hard nodular masses in the lungs and air sacs, though other organs may also be generally involved. The disease is non-contagious and usually occur either in epizootic (acute) or sporadic (chronic) form.

Historical:

Aspergillosis was one of the first diseases described for wild birds; Aspergillosis was noted in

- a **scaup** in 1813
- a **European jay** in 1815.
- **Loons** and marine birds that are brought into rehabilitation,
- **captive raptors**,

- **penguins** being maintained in zoological parks and other facilities commonly die from aspergillosis
- the "green mould" seen in the air-sac and bronchial lesions (Owen, 1832) and many of the early accounts are difficult to interpret for this reason.
- Aspergillosis was first described in a wild duck in 1833
- Aspergillosis was reported in turkeys as early as 1898.

Aetiology:

i. ***Aspergillus fumigatus*: was reported by:**

Walker (1915). Ainsworth and Rewell (1949), Eggert and Barnhart (1953), O'Meara and Chute (1959), Beer (1963), Refai and Rieth (1966), Olson (1969. PALYA and BALOGH (1971), Refai (1971), Ghori and. Edgar (1973), Saif and Refai (197, Yamada *et al.* (1977), Zink *et al.* (1977), Bassiyoni *et al.* (1981), Richard and Thurston (1983), Veselský *et al.* (1984), Chaudhary *et al.* (1988), Okoye *et al.* (1989a), Okoye *et al.* (1989a), Flach *et al.* (1990), Pal *et al.* (1990), Peden and Rhoades (1992), Singh *et al.* (1993). Beckman *et al*. (1994), Kunkle and Rimler (1996), Richard *et al.* (1996). Jensen *et al.* (1997). Kunkle and Rimler (1998a,b). German *et al.* (2002), Akan *et al.* (2003), Bhattacharya (2003), Lair-Fulleringe *et al.* (2003), Atasever and Gümüşsoy (2004), Yokota *et al.* (2004), Cortes *et al.* (2005), Femenia *et al.* (2007), Tokarzewski *et al.* (2007), Xavier *et al.* (2007), Abou-Rawash *et al.* (2008), Beernaert *et al*. (2008), Khosravi *et al.* (2008), Akkoc *et al.* (2009), Shathele *et al.* (2009), Singh *et al.* (2009), ARAGHI *et al*. (2010), Alvarez-Perez *et al.* (2010), Lisa *et al.* (2010), Olias *et al.* (2010), Arné e*t al.* (2011), Olias *et al.* (2011), Van Waeyenberghe *et al.* (2011), M i l o š et *al.* (2011), Ceolin *et al*. (2012), Kureljušić *et al.* (2012), Korniłłowicz-Kowalska and Kitowski (2013), Reza et *al.* (2013), Spanamberg *et al.* (2013), Thierry *et al*. (2013), Abdulrahman *et al.* (2014), Araghi *et al.* (2014), Burco *et al.* (2014), Cafarchia *et al.* (2014), Kwanashie *et al.* (2014, Fischer and Lierz. (2015)

ii. ***Aspergillus flavus*: was reported by:**

Ainsworth and Rewell (1949), PALYA and BALOGH (1971), Refai (1971), Saif and Refai (1977), Richard and Thurston (1983), Okoye *et al.* (1989a), Barton *et al.* (1992), Perelman and Kuttin (1992). de Wit *et al.* (1993), Martin *et al.* (2007), Xavier *et al.* (2007), Stoute *et al.* (2009), Tijani *et al.* (2010), Hadrich *et al.* (2013a), Hadrich *et al.* (2013b)m Cafarchia *et al.* (2014)m Kwanashie *et al.* (2014)

iii. ***Aspergillus niger*: was reported by:**

Perelman and Kuttin (1992), Akan *et al.* (2003), ARAGHI *et al*. (2010), Araghi *et al.* (2014), Kwanashie *et al.* (2014)

iv. ***Aspergillus terreus*: was reported by:**

Kwanashie *et al.* (2014)

v. ***Aspergillus nidulans*: was reported by:**

Ainsworth and Rewell (1949)

vi. **Aspergillus species: were reported by:**

Harold *et al.* (1968), Redig *et al.* (1972), Zink *et al.* (1977), Corkish (1980), Dyar *et al.* (1984), Redmann and Schildger (1989), Julian and Goryo (1990), Perelman *et al.* (1993), Richard and DeBey (1995, *TÜRKÜTANIT* (1999), Souza *et al.* (2000), Throne Steinlage *et al.* (2003), Beytut *et al.* (2004). Copetti *et al.* (2004), Balseiro *et al.* (2005), Cacciuttolo *et al.* (2009), Jacobsen *et al.* (2010), França *et al.* (2012), **Nouri** *et al.* (2013), Sultana *et al.* (2015), Tarello (2016)

Hosts:

Domesticated and caged birds; fowl, turkey, duck, goose, pigeon, canary, budgerigar, parrot, ostrich. Captive wild birds, especially water birds; duck, geese, penguin, stork, flamingo, cormorant, parrot, hawk, owl, pheasant, peafowl. Free-living wild birds; wood-pigeon, seagull, rook, pheasant, sparrow, swan, jay, grouse, Manx shearwater, thrush.

a. **Chickens: reported by:**

Eggert and Barnhart (1953), O'Meara and Chute (1959), Saif and Refai (1977), Yamada *et al.* (1977), Corkish (1980), Bassiyoni *et al.* (1981), Veselský *et al.* (1984), Okoye *et al.* (1989a). Redmann and Schildger (1989), Julian and Goryo (1990), Pal *et al.* (1990), Hamet *et al*. (1991, Barton *et al.* (1992). de Wit *et al.* (1993), Beckman *et al*. (1994), Akan *et al.* (2003), Throne Steinlage *et al.* (2003). Femenia *et al.* (2007). Martin *et al.* (2007), Zafra *et al.* (2008), Cacciuttolo *et al.* (2009), Islam *et al.* (2009), Arné e*t al.* (2011), M i l o š et *al.* (2011), Ceolin *et al*. (2012), França *et al.* (2012), Spanamberg *et al.* (2013). Thierry *et al*. (2013), Abdulrahman *et al.* (2014), Kwanashie *et al.* (2014), Singh *et al.* (2014), Sultana *et al.* (2015)

b. **Turkeys: reported by:**

Refai and Rieth (1966), PALYA and BALOGH (1971), Richard and Thurston (1983), Dyar *et al.* (1984), Okoye *et al.* (1989a), Peden and Rhoades (1992), Richard and DeBey (1995), Richard *et al.* (1996), Jensen *et al.* (1997), Kunkle and Rimler (1998a,b). Lair-Fulleringe *et al.* (2003), Ozmen and Dorrestein (2004), Cortes *et al.* (2005), Mukaratirwa (2006), Femenia *et al.* (2007), Singh *et al.* (2009), Stoute *et al.* (2009), Olias *et al.* (2010), Arné e*t al.* (2011), Kureljušić *et al.* (2012), Melloul *et al.* (2014)

c. **Pigeons: reported by:**

Tokarzewski *et al.* (2007), Beernaert *et al*. (2008

d. **Ducks: reported by:**

Zinkl *et al.* (1977), Graczyk *et al.* (1997), Graczyk *et al.* (1998), Bhattacharya (2003). Tell *et al.* (2006), Hurley-Sanders *et al.* (2015)

e. **Geese: reported by:**

Beer (1963), Harold et al. (1968), PALYA and BALOGH (1971), Okoye *et al.* (1989a), *TÜRKÜTANIT* (1999), Ramisz *et al.* (2001), Beytut *et al.* (2004)

f. **wild birds reported by:**

i. **Captive wild birds:** Ainsworth and Rewell (1949), Ivey (2000). Cacciuttolo *et al.* (2009)
ii. **Canaries: Nouri *et al.* (2013), Reza et *al.* (2013)**
iii. **Cranes:** Hausmann ***et al.* (2015), Schwarz *et al.* (2016)**

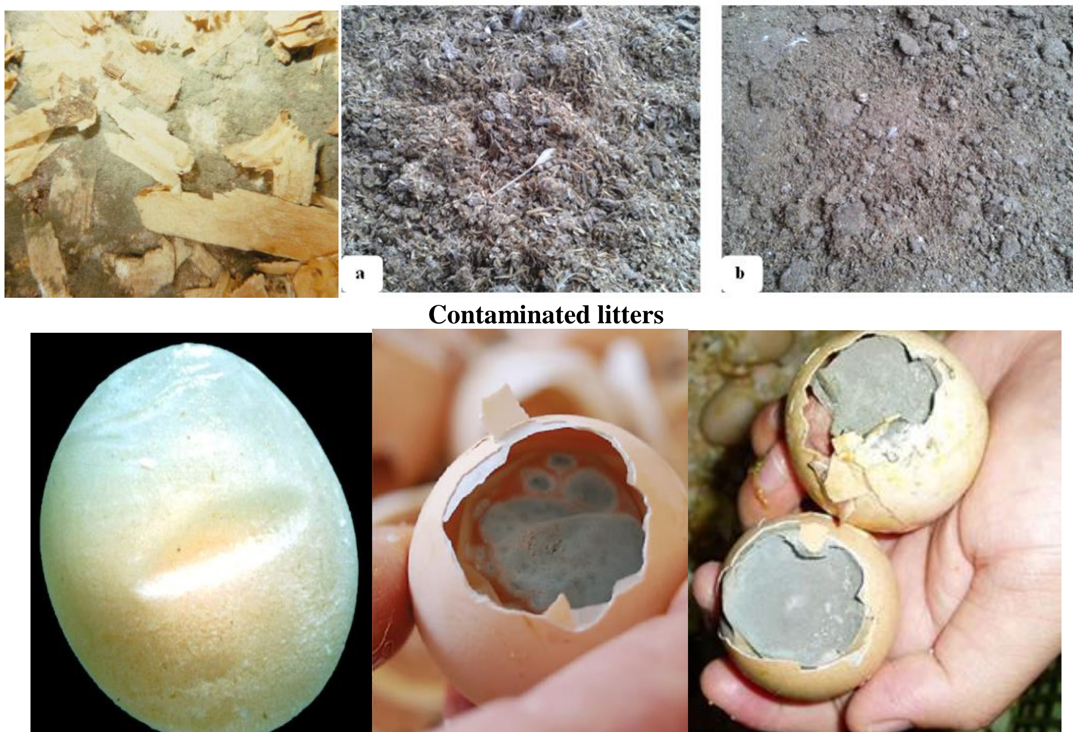
iv. **Crows:** Zinkl *et al.* (1977)
v. **Falcons:** Arca-Ruibal *et al.* (2006), Kummrow *et al.* (2012), Fischer and Lierz. (2015), Tarello (2016)
vi. **Goshawks:** Redig *et al.* (1972)
vii. **Griffons:** Li *et al.* (2015)
viii. **Kiwi:** Glare *et al.* (2014)
ix. **Lovebirds: Guilherme *et al.* (2014)**
x. **North Island robin: Low *et al.* (2005)**
xi. **Ostrich:** Walker (1915), Perelman and Kuttin (1992), Yokota *et al.* (2004), Khosravi *et al.* (2008), Akkoc *et al.* (2009), Shathele *et al.* (2009), ARAGHI *et al*. (2010), Tijani *et al.* (2010), İÇEN *et al.* (2011), Araghi *et al.* (2014), Azizi *et al.* (2014)
xii. **Penguins:** Flach *et al.* (1990), German *et al.* (2002). Xavier *et al.* (2007)
xiii. **Quail:** Olson (1969), Ghori and. Edgar (1973), Chaudhary *et al.* (1988)
xiv. **Seabirds:** Balseiro *et al.* (2005)
xv. **Swans:** Souza *et al.* (2000), Abou-Rawash *et al.* (2008)
xvi. **Vultures**: Jung *et al.* (2009)
xvii. **White stork:** Olias *et al.* (2011)
xviii. **Wild starling:** Atasever and Gümüşsoy (2004)
xix. **Rhea:** Copetti *et al.* (2004)

Sources of infection

- Aspergillus species develop and sporulate easily in poor quality bedding or contaminated feedstuffs in indoor farm environments.
- The spores Aspergillus can easily be spread by draught or the wind. The spores are found in low numbers throughout the whole environment. Aspergillus spores survive and grow in a wide range of conditions, but especially on organic matter, like egg yolk, cardboard boxes and wood. Growth in the spores is initiated by conditions of high humidity and temperature (37 - 45 ˚C). Cycles of high and low humidity optimise the growth of the fungus (mycelium) and the spread of its spores. The hatchery therefore provides optimum environmental conditions for Aspergillus to thrive.

- Aspergillus spores can enter the hatchery either directly via the eggs, or via incoming air. When the hatchery environment is contaminated by a high level of Aspergillus, the spores can easily enter the air handling unit and ventilation system. The climate, temperature and humidity in ventilation ducts is ideal for the propagation of Aspergillus, especially when organic matter (debris) has accumulated.

- Aspergillus species can penetrate egg shells under ideal growth conditions and thus infect the embryos. Such eggs will often appear green when candled (the embryo will be dead). Infected embryos may hatch with well- developed lesions.
- If infected eggs break in the hatchery, large numbers of spores are released which contaminate the hatchery environment and air systems can lead to severe outbreaks in very young birds (less than 3 weeks of age). Eggs punctured for in-ovo injection are particularly susceptible to contamination. Even low-level contamination of hatchers or air systems can result in mortalities of 50% or greater when in-ovo injection is used.



Contaminated litters

Contaminated eggs http://www.ava.org.af/books/Aspergillosis.pdf, www.issr-journals.org

Transmission and Predisposing Factors

- All birds are susceptible to aspergillosis. It is reported in domestic birds like poultry, duck, and quails as well as in wild birds
- Inhalation of conidia or spores from contaminated feed, fecal material, soil and contamination of egg in ovo, infect the developing embryo. Higher susceptibility of birds to aspergillosis may be attributed to anatomic and physiologic characteristics of the avian respiratory system.
- The small non-expanding lungs and nine air sacs constitute a primary nidus for infection because the air (or conidia) reaches the caudal air sacs before it pass through those part of the lungs in which the gas exchange takes place
- Higher body temperature also allows quick fungal growth.
- Other factors include
    - chronic stress,
    - unsanitary conditions,

- overcrowding,
- malnutrition,
- vitamin deficiencies especially vitamin A
- overuse of certain medications (corticosteroids)
- respiratory irritants (disinfectant fumes and aerosol sprays).
- birds that are otherwise ill or are very young or old are also susceptible to aspergillosis.

Experimental infections were carried out by:

Bassiyoni *et al.* (1981), Chaudhary *et al.* (1988), Julian and Goryo (1990). Peden and Rhoades (1992), Perelman *et al.* (1993), Kunkle and Rimler (1996), Kunkle and Rimler (1998a,b), Atasever and Gümüşsoy (2004), Tell *et al.* (2006), Femenia *et al.* (2007), Beernaert *et al.* (2008), Jacobsen *et al.* (2010), Lisa *et al.* (2010), Thierry *et al*. (2013), Melloul *et al.* (2014)

Pathogenesis and immune response

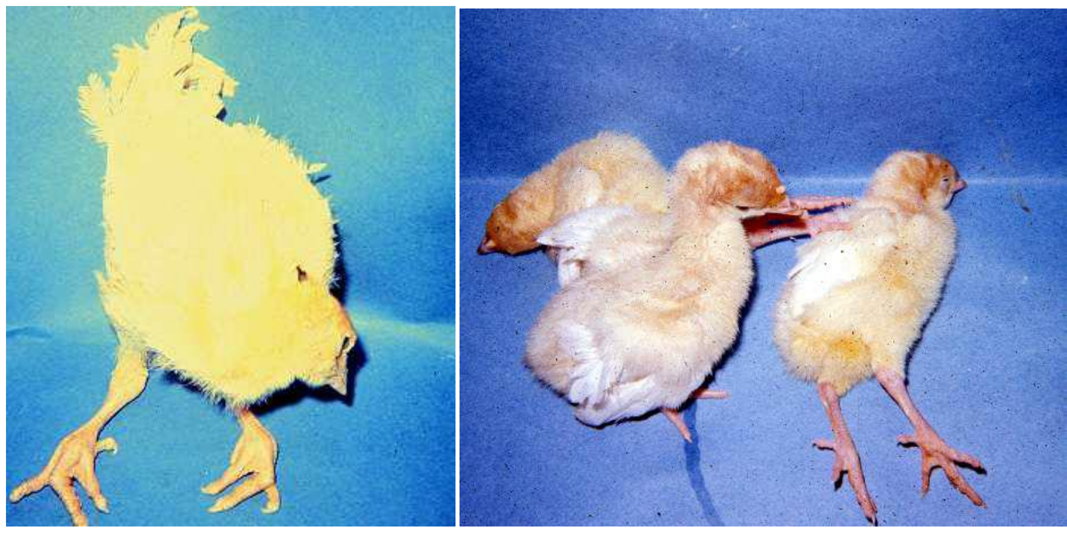
- Aspergillosis is caused by inhalation of overwhelming numbers of small, hydrophobic fungal spores (conidia) into the lungs
- During inhalation, spores initially enters the bird through the nares (two holes in the beak leading to respiratory system), trachea and to the primary bronchi (mesobronchi) which deliver the inhaled air to the posterior thoracic and abdominal air sacs.
- The inhaled air reaches the posterior air sacs prior to contacting epithelial surfaces in the lungs.
- Air sacs are particularly prone to infection due to epithelial surface nearly devoid of a mucociliary transport mechanism and absence of macrophages.
- **Conidia** of small size (2-3 microns) enter and germinate in the lungs and air sacs.
- **Conidia** come in contact with the sticky mucus lining the respiratory tract
- **Condia** may be engulfed by the espiratory epithelial cells as part of the innate immune system.
- **Conidia** germinate externally forming **hyphae**, penetrate and damage the cells
- **Hyphae** invade, via spaces between and within the epithelium, cause cilia loss and cell detachment, serosal inflammation and superficial necrosis in the air sacs and adjacent
- **Hyphae** are tissue and angio invasive and have a unique capacity to survive and proliferate within the host
- **Hyphae** pass from the abluminal to the luminal surface and cause endothelial cell injury
- **Hyphae** fragments can break off and circulate in the bloodstream resulting in disseminated lesions, involving pneumatic bone, peritoneum, internal organs or the CNS
- **Macrophages** phagocytose conidia in an actin-dependent manner through the recognition of pathogen-associated molecular patterns by host cell pattern recognition receptors, (TLR2 and TLR4) and the C-type lectin receptor dectin-1
- A proinflammatory response is generated characterized production of cytokines and chemokines
- **Dectin-1** specifically binds to fungal carbohydrates (1, 3)- glucan, which results in phagocytosis, activation of macrophages and generation of proinflammatory

Clinical Signs

- **Acute aspergillosis**
  - results from inhaling an overwhelming number of spores
  - usually develops in young birds,less than a week old.
  - generally have a acute or peracute infection resulting in high morbidity and mortality.
  - clinical signs include
    - difficult breathing, decrease or anorexia,
    - polydypsia,
    - cyanosis,
    - foetid diarrhea
    - emaciation.
  - birds may die suddenly without showing any clinical sign.

- **Chronic aspergillosis**
  - may take weeks or months to develop.
  - is much more common in older birds.
  - clinical signs include:
    - inappetence, emaciation, dyspnea, gasping, increased thirst, fever, diarrhea and signs of nervous involvement
    - green coloration in urates and hepatomegaly can be seen.
    - respiration may be noiseless and syrinx involvement leads to wheezing, rattling or clicking sound.
    - nares may become plugged or discharge with rhinitis
    - opthalmitis and keratitis (periorbital and eyelid swelling with cheesy yellow exudates in the conjunctival sac)
    - necrotic granulomatous dermatitis.
    - wing droop can be observed when pneumatic bones such as the humerus gets involved
  - birds may die due to severe respiratory involvement.

Gasping chicks www.thepoultrysite.com



Nervous signs

Neurological signs associated with Aspergillosis infection 1 - Dr. Kylie Hewson

Brooder pneumonia

- A severe respiratory disease of birds that takes the form of an acute rapidly fatal pneumonia in young chickens and turkeys.
- It may cause devastating loss of birds in hatcheries.
- It occurs in chicks, contaminated *in ovo* or during hatching, causing death of the embryo (dead-in-shell)
- highly fatal in the first ten days of life and results in a major respiratory distress , mortality rate may rise slightly or increases suddenly, peaks during a few days, and then returns to initial state,
- respiratory signs include dyspnoea, gasping, hyperpnoea with panting, nonproductive coughing, wheezing, cyanosis and sometimes nasal discharge
- The disease tends to produce two distinct phases of mortality.
- In the first three weeks of life,
    - mortality rates ranging from 5% to 50% may occur as a result of an acute respiratory condition.
    - Difficult and open-mouthed breathing of hatchery infected chicks can be observed within the first five days. These "gaspers" suffer from obstruction of the respiratory airways.
- Infected survivors may develop chronic symptoms,
    - mortality rates of up to 5%.

- may include a variety of nonspecific clinical signs, as dyspnoea, depression, dehydration, and emaciation
- may result in failing respiratory function, blindness, neurological dysfunction or duodenal dropsy.

Lesions

- **Pulmonary aspergillosis:*reported by:***
  TÜRKÜTANIT (1999), Beytut *et al.* (2004), Copetti *et al.* (2004), Mukaratirwa (2006), Zafra *et al.* (2008), Akkoc *et al.* (2009). Jung *et al.* (2009), Shathele *et al.* (2009), Singh *et al.* (2009) , Tijani *et al.* (2010), Arné e*t al.* (2011), İÇEN *et al.* (2011), Olias *et al.* (2011) , Ceolin *et al*. (2012), França *et al.* (2012) , Guilherme *et al.* (2014)
- **Thoracic aspergillosis:** Low *et al.* (2005)
- **tracheal aspergillosis :** Pal *et al.* (1990)**,** Barton *et al.* (1992), Singh *et al.* (1993), Singh *et al.* (2014)
- **Airsaculitis : Richard *et al.* (1996),** Spanamberg *et al.* (2013)
- **Eye aspergillosis:** Beckman *et al*. (1994)
- **CNS:** Ozmen and Dorrestein (2004)

Respiratory System

- **Peracute aspergillosis**
  - complete congestion of lung and no formation of nodules.
- **Acute aspergillosis**,
  - lungs show the most striking lesions which are characterized by marked congestion and often studded with milliary yellow nodules
  - air sacs are usually thickened with small whitish-yellow plaque-like lesions.
- **Chronic aspergillosis**
  - typical granulomatous lesions.
  - variable sized nodules or multiple plaques that may be disseminated throughout the air sacs
  - lesions are especially observed in the periphery of the lungs and caudal thoracic and abdominal air sacs and may show sporulating fungal colonies.
  - serous membranes of the air-sacs with yellowish-white plaque-like lesions or raised white nodules.
  - trachea and bronchi may become blocked either by mucoid discharge or by the yellowish-white plaque-like lesions or raised white nodules.

Nasal aspergillosis

- causes exudative rhinitis
- malformation of the nostrils and beak
- rhinosinusitis, with almost complete destruction of the premaxilla and deformation of the upper beak

Digestive system

- plaque-like lesions may occur in the mouth, gizzard and intestines
- involvement of the rib bone beneath the lung
- liver, kidney, spleen, heart may be involved with formation of nodular granulomatous lesions

Cerebellum and cerebrum

- abscesses may occur with or without pulmonary and other lesions.
- circumscribed white to greyish areas were observed
- granuloma formation was also seen

Reporoductive system

- nodular lesions in the ovaries
- salphingitis
- white to grayish nodules on the serosal surface of the oviduct

Skin

- mycotic pododermatitis
- foot pads may show keratinized epidermal disruption, encrustations and acute inflammation
- epidermal cysts associated with A. fumigatus have been described in the comb
- mycotic dermatitis was also reported in domestic fowl

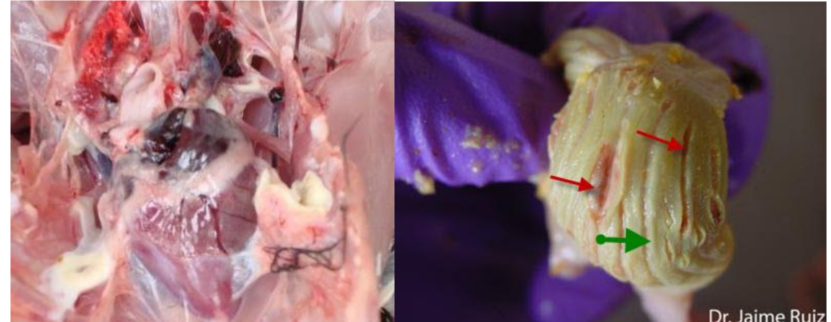
Eyes Lesions

- were observed in the eyes of baby chicks and in
- mycotic keratitis may lead to periorbital swelling, swollen and adhered eyelids with turbid discharge, cloudy cornea and cheesy yellow exudates within the conjunctival sac
- blepharitis and dermatitis involving the eyelids and the head were recorded

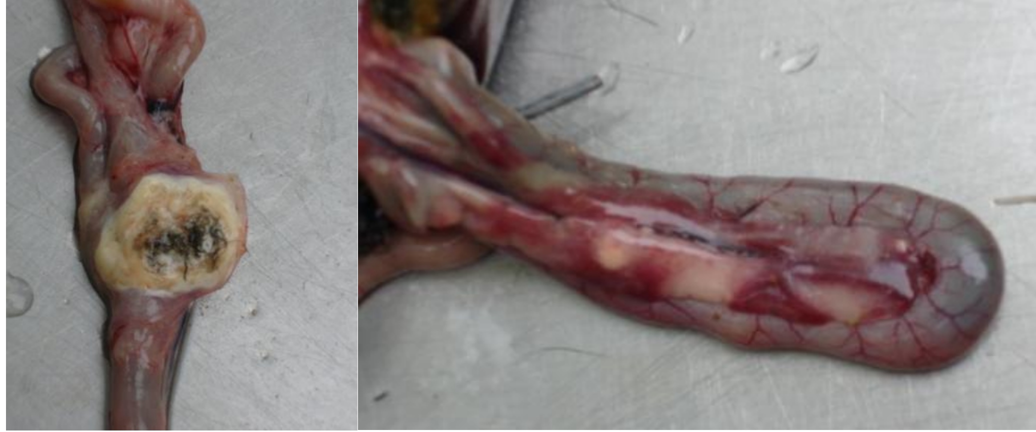
Bones and Joints

- involvement of ribs of broiler was observed
- osteo-arthritis and granulomatous osteoarthritis of the hip joints with necrosis of the femur head was observed in turkey

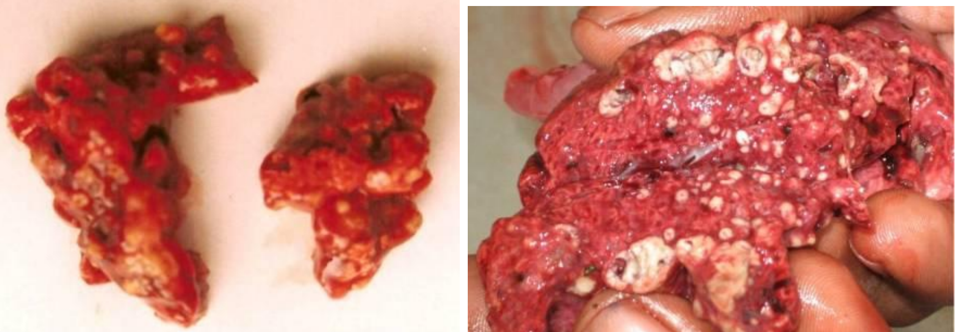
Gallery



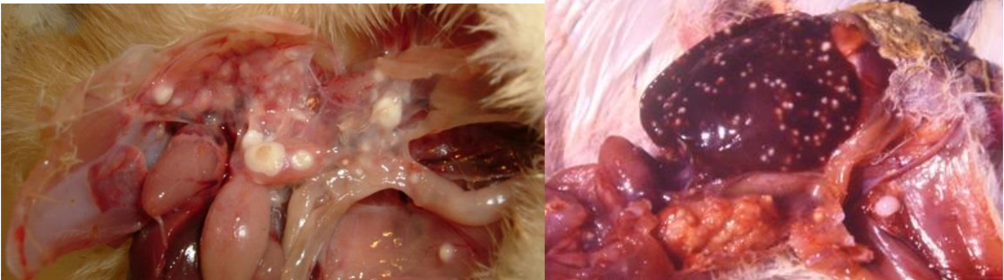
Aspergillosis: Brooder pneumonia: Multiple "disc-shaped" granulama in thoracic airsacs in chicken. Gizzard showing erosions (red arrows)and numerous fissures (green arrow)on its inner surface. **VPP 321: Avian Pathology**



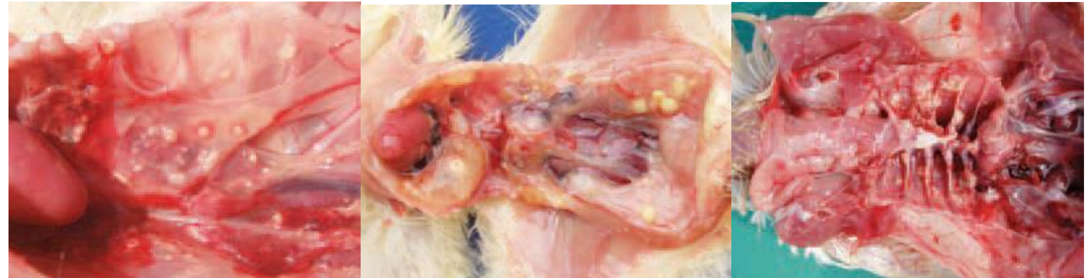
Brooder pneumonia: Intestine showing a foci of granulama exhibiting grey coloured hyphae of the fungus - *Aspergillus sp.,*in chicken. Brooder pneumonia: Pancreas showing multifocal paler necrotic areas and more vascularisation of duodenal serosa in *Aspergillus sp.,* infection of chicken. **VPP 321: Avian Pathology** .



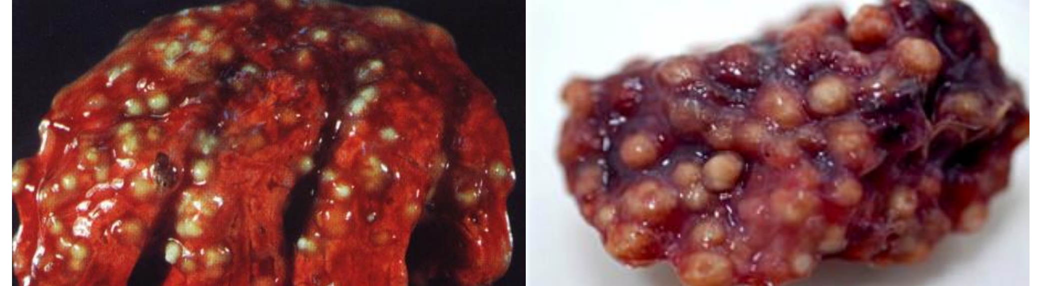
Brooder pneumonia: Lungs showing numerous granulamatous " saucer shaped"nodules due to *Aspergillus sp.,* infection in pigeon. Brooder pneumonia: Cut section of lungs showing numerous granulamatous nodules in varying sizes due to *Aspergillus sp.,*infection in a turkey. **VPP 321: Avian Pathology**



Left: Aspergillosis in the air sacs of a chick, www.sandhillvet.co.uk
Right: Multiple nudules in the liver of 3 weeks old poult, due to aspergillosis., www.poultrymed.com



Aspergillus granulomas of the serous coats:www.thepoultrysite.com



Lung nodules =aspergillosis, OldVe T.com Multiple nodular lesions in the lung of a duck. Parasitology-Mycology, ENVA

Aspergillosis lesion on the eyelid **Dr. Kylie Hewson**

Aspergillosis lesions in the brain -, Aspergillosis lesions in the peritoneal cavity - **Dr. Kylie Hewson**

Aspergillosis in wild birds:

A. fumigatus has been isolated from lesions in wild birds since the early 1800s. Major die-offs of free-ranging wild birds have been reported from waterfowl, gulls, and corvids following dumping of mouldy waste grains in areas where birds feed.. Aspergillosis has also been reported in penguins, raptors, migratory , waterfowl, psittacines and zoologic specimens, such as flamingos.

i. Aspergillosis in penguins

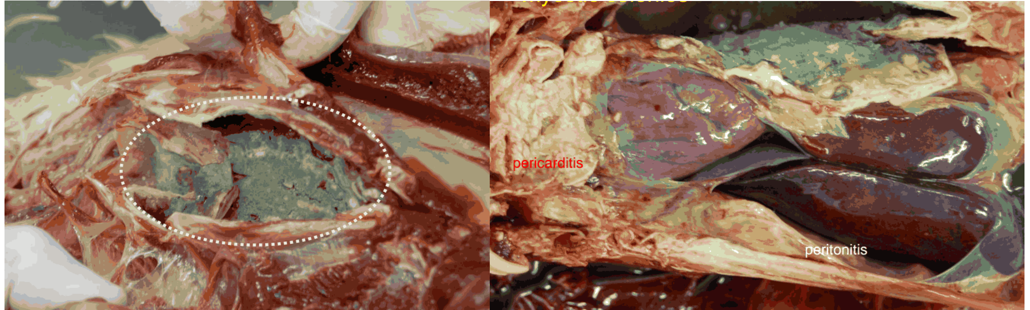
On Sunday, November 10th, 2013, staff at the Calgary Zoo made the difficult decision to euthanize a 14-year-old male Gentoo penguin Houdini. He had been sick for almost a month. The results of a necropsy confirmed severe aspergillosis – a fungal infection that affects the respiratory system and is one of the most common causes of death in captive penguins and has been recorded in wild penguins. Penguins are among several birds that are exquisitely sensitive to acquiring the infection, with increased disease noted when the spore levels become concentrated and/or the birds immune systems are depressed as when they moult.

Disseminated Aspergillosis in a Little Penguin, arwh , , forum.backyard poultry.com

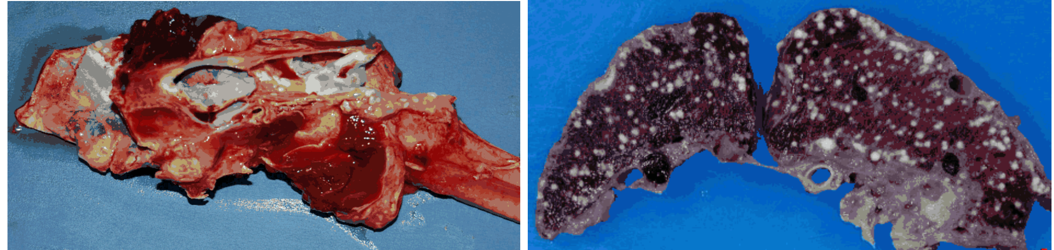
The necropsy of penguin showed that airsacs were diffusely thickened, opaque, and studded with multiple off-white to pale green dull. Similar plaques were scattered across coelomic viscera serosal surfaces and adherent to the lungs which were diffusely dark red purple and wet.

The histopathology revealed that granulomatous, heterophilic, and necrotizing inflammation with myriad intralesional fungal hyphae morphologically consistent with *Aspergillus* spp. were present in the lungs, airsacs, kidneys, oviduct, trachea, mesentery, and serosal surfaces of coelomic viscera. There was significant atrophy of adipose tissue, skeletal muscle, liver, and pancreas

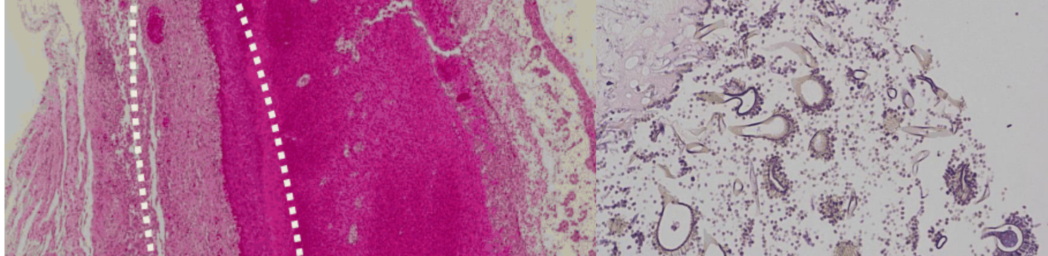
In a retrospective studies in Gifo University, Yanai reported that, 10 of 42(28.3%) cases of death in penguins in Japan were due to aspergillosis.



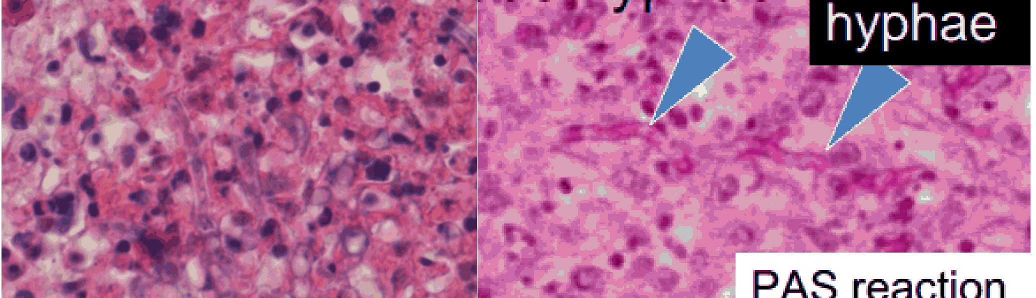
Aspergillosis (air sacs) in an adult male Macaroni penguin, Yanai, Gifu University



Multiple necrotic areas around oesophagus and lungs,Yanai,Gifu multi-focal necrosis in the lungs,Yanai, Gifu



Histological sections: thickened air sac with conidial heads of A. fumigatusin the lumen of the air sac,Yanai, Gifu



ii. Aspergillosis in parrots

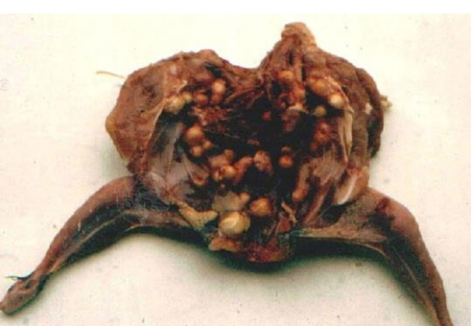
Aspergillosis is less common in companion parrots; however, disease is more prevalent in African grey parrots (*Psittacus erithacus*), Amazon parrots (*Amazona* spp.), Pionus parrots (*Pionus* spp.) and macaws. The likelihood of a fungal infection is increased if the bird is housed in an environment in which there is poor sanitation, high relative humidity and high temperatures, which can increase the load of fungal spores. A bird with a weakened immune system due to steroid administration or concurrent illness (particularly when treatment involves long-term antibiotics) is also at greater risk for aspergillosis.

- Like humans, parrots and other large birds cough when they experience a respiratory irritation. Coughing can be normal when it only occurs every now and then, but when parrot's cough becomes chronic, it could be a sign of aspergillosis. Spores released by the aspergillus can get trapped in the bird's respiratory system, causing the cough.
- In addition to coughing, when aspergillus spores get into parrot's throat and lungs its voice may change and it may refuse to talk entirely.
- Spores released by aspergillus affect different parts of the respiratory system in different ways. Such symptoms include labored breathing or sudden fits of suffocation. Your bird's neck may turn blue as it tries to talk or cough. This is indicative of choking, but the bird could also be suffocating on an aspergillus spore. In some instances, parrots are known to suddenly drop dead after inhaling an aspergillus spore.

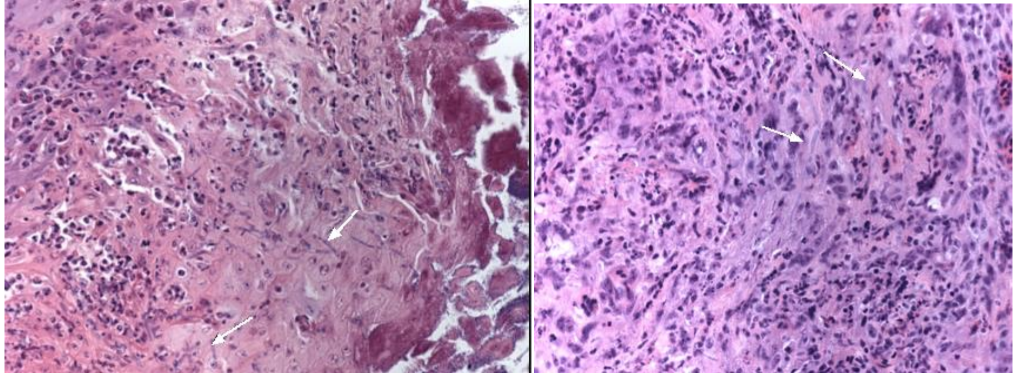
African Grey parrot Amazon parrots Pionus parrots Blue Yellow Macaw

iii. Aspergillosis in quails

Aspergillosis in quail is charachterized by the formation of yellowish white nodular growth in lungs and intercostal areas with thickened air sacs. Histopathologically, lungs show severe congestion with focal haemorrhages, multiple granulomatous inflammation with caesative necrotic areas in centre. Various fungal elements like conidia, long septate hyphae with mononuclear and heterophilic infiltration are seen in these areas. Microbiological study reveals velvety bluish green colony of Aspergillus fumigatus.



Nodules in the air sacs and on the peritoneal serosa in a case of aspergillosis in a common quail. www.fmv.utl.



Aspergillosis in quail crop , heart , www.rarc.wisc.edu

iv. Aspergillosis in ostrich

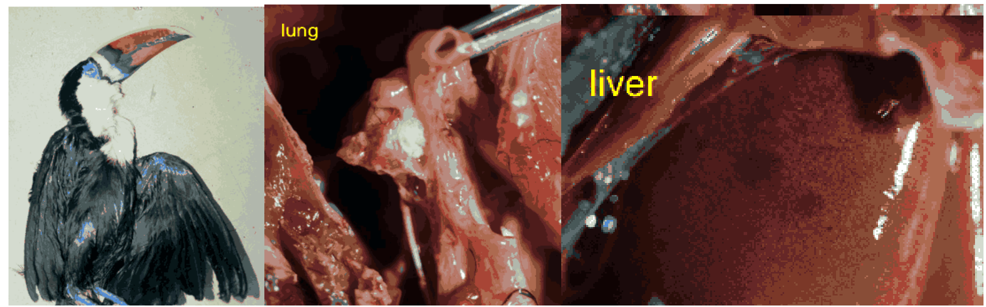
Aspergillosis in ostrich was reported by Perelman and Kuttin (1992), Katz *et al.* (1966), Pérez, *et al.* (2003)Yokota *et al.* (2004), SANCAK A.A. and PARACIKOLU (2005), Khosravi (2008), Shathele et al. (2009) and Tijani *et al*. (2012). ARAGHI *et al.* (20014) describes an aspergillosis outbreaks in ostrich flocks of eastern Iran during 2010–2012. They reported that signs of respiratory involvement, anorexia, depression, progressive emaciation and decreased production were the most commonly seen in affected farms. Morbidity rate was 43% and 54.53% in breeding birds and chickens, respectively. *Aspergillus fumigatus* and *Aspergillus niger* were identified.

www.doctorfungus.org, ostrich c with respiratory aspergillosis.

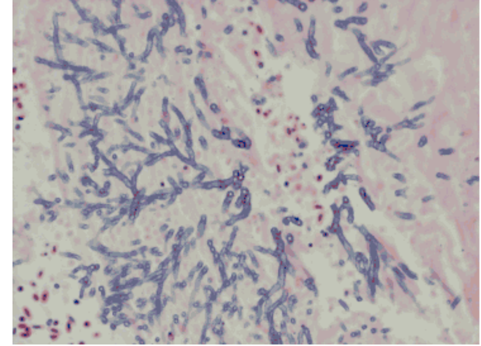
v. Aspergillosis in red-tailed hawk (*Buteo jamaicensis*).

Aspergillosis in red-tailed hawk Multiple granulomas in the lungs *A. fumigatus* spores in air sacs,MVS

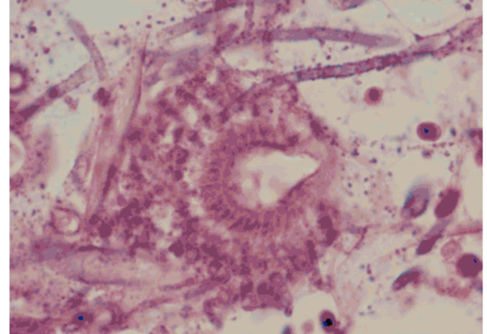
vi. **Aspergillosis in Aspergillosis in Red-billed Toucan** (Yanai, Gifu Univ)



Aspergillosis in Red-billed Toucan, Yanai, Gifu Univ.: multifocal lesions in the lung and liver

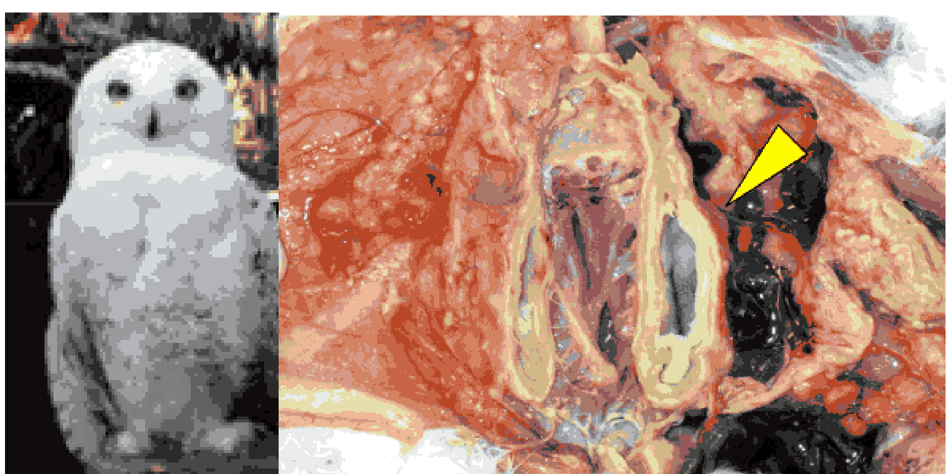


Hyphae radiating from a central necrotic nodule



Conidia heads of Aspergillus in a pulmonary cavity

vii. **Aspergillosis in Snow Owl**

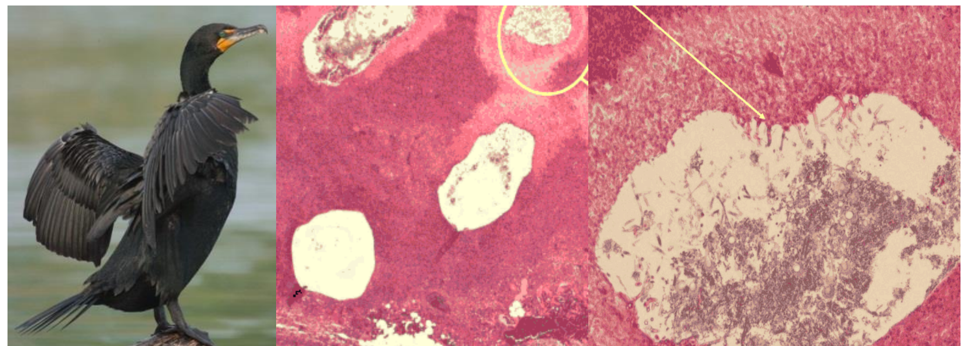


Severely thickened air sacs of Snow Owl caused by A. fumigatus, Yanai, Gifu

viii. **Aspergillosis in Goshawk**

Aspergillosis in Goshawk, Yanai, Gifu

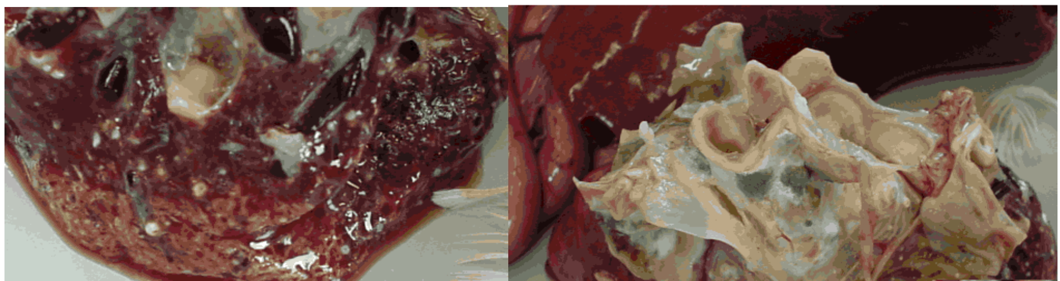
ix. Aspergillosis in Cormorants


Aspergillosis in Cormorants, multifocal necrosis with Aspergillus hyphae in the lung

x. Aspergillosis in swans

Souza *et al.* (2005) reported on mortality in wild swans in Northwest Washington State due to aspergillosis. On Jan. 27, 2014, 149 dead swans have been found in Whatcom County. The death was attributed also here to aspergillosis. a disease which is being blamed for the deaths of trumpeter swans spending the winter on Whatcom County's Wiser Lake. Yani (2013) reported as aspergillosis in whooper swans.

Whooper swan, multifocal white nodules in the air sacs with frequent fungi, Yanai, Gifu



Multiple mycotic lesions in the lungs and larynx of a whooper swan, Yanai, Gifu

xi. Aspergillosis in kiwi

The Wellington Zoo reported on 30 September 2013 that, eight young rowi—the rarest species of kiwi—have died from respiratory tract infections. The kiwi were being treated for nematodes (a type of worm) in Wellington Zoo when they started to show signs of respiratory problems.The respiratory tract infection was caused by the fungus *Aspergillus spp.* which is commonly found in the environment. It is thought that the birds' weakened immunity from the nematodes made them susceptible to this.

Travis *et al.* (2014) reported on isolation and odentification of *Aspergillus* spp. from Brown Kiwi (*Apteryx mantelli*) Nocturnal Houses in New Zealand.

A Rowi kiwi chick, Eight Kiwi chicks have died from respiratory tract infections at Wellington Zoo.

Histopathology

Histopathological studies of avian aspergillosis wee carried out by:

Ozmen and Dorrestein (2004), Tokarzewski *et al.* (2007), Abou-Rawash *et al.* (2008), Khosravi *et al.* (2008), Zafra *et al.* (2008), Cacciuttolo *et al.* (2009), Islam *et al.* (2009), Singh *et al.* (2009), Stoute *et al.* (2009), ARAGHI *et al*. (2010), Jacobsen *et al.* (2010), Lisa *et al.* (2010), Tijani *et al.* (2010), İÇEN *et al.* (2011), Kureljušić *et al.* (2012). **Nouri** *et al.* (2013), Reza et *al.* (2013), Abdulrahman *et al.* (2014), Araghi *et al.* (2014), Azizi *et al.* (2014), Guilherme *et al.* (2014), Singh *et al.* (2014), Li *et al.* (2015), Sultana *et al.* (2015)

- well-organised granulomatous reactions develop, encapsulated by outer thick fibrous layer, in non-aerated as well as aerated parenchyma.
- adjoining tissues may show neither exudative inflammation nor vascular lesions
- **in superficial diffuse form**,
  - non-encapsulated pyogranulomatous reaction containing fungal elements predominates in air sacs and lungs
  - pyogranuloma is characterized by a centre with variable amounts of septate, dichotomously branching hyphae containing large numbers of conidiophores and conidia. These hyphae were surrounded by a cliff of radially arranged macrophages, heterophils, foreign body giant cells and lymphocytes (
  - multinucleated cells phagocytized fungal elements
- **in case of severe inflammation**,
  - parabronchioles obscured with eosinophilic necrotic material containing degenerated heterophils, erythrocytes and exfoliated epithelial cells
  - mixed types of both tissue reactions in the same tissue section were also reported
- **in case of angioinvasive pulmonary aspergillosis**
  - vascular invasion by fungal hyphae involving numerous small to large veins of lungs and air sacs.
  - both alveolar epithelium and the blood vessel wall may be severely damaged by penetrating fungal hyphae.
  - vessels may be thrombosed as a result of fungal hyphae invasion and intramural host reaction
- Aspergillosis may be an acute, rapidly fatal disease or a more chronic disease. Both forms of the disease are commonly seen in free-ranging birds, but the acute form is generally responsible for large-scale mortality events in adult birds and for brooder pneumonia in hatching birds.

  - **Acute aspergillosis** has been found in free ranging waterfowl. The circumstances of these events are uniformly associated with birds feeding in waste grain and in silage pits during inclement weather.
  - **Chronic forms of aspergillosis** have been described in wild birds since at least 1813. Typically, the lungs and air sacs are chronically infected, resulting in a gradual reduction in respiratory function. Eventual dissemination of the fungus to the liver, gut wall, and viscera is facilitated by infection of the extensive system of air sacs that are part of the avian respiratory system.

- Most reported mortalities of free-ranging wild birds involve isolated mortalities found during postmortem evaluations rather than mortalities found during major die-offs

Description of main Aspergillus species isolated from cases of avian aspergillosis

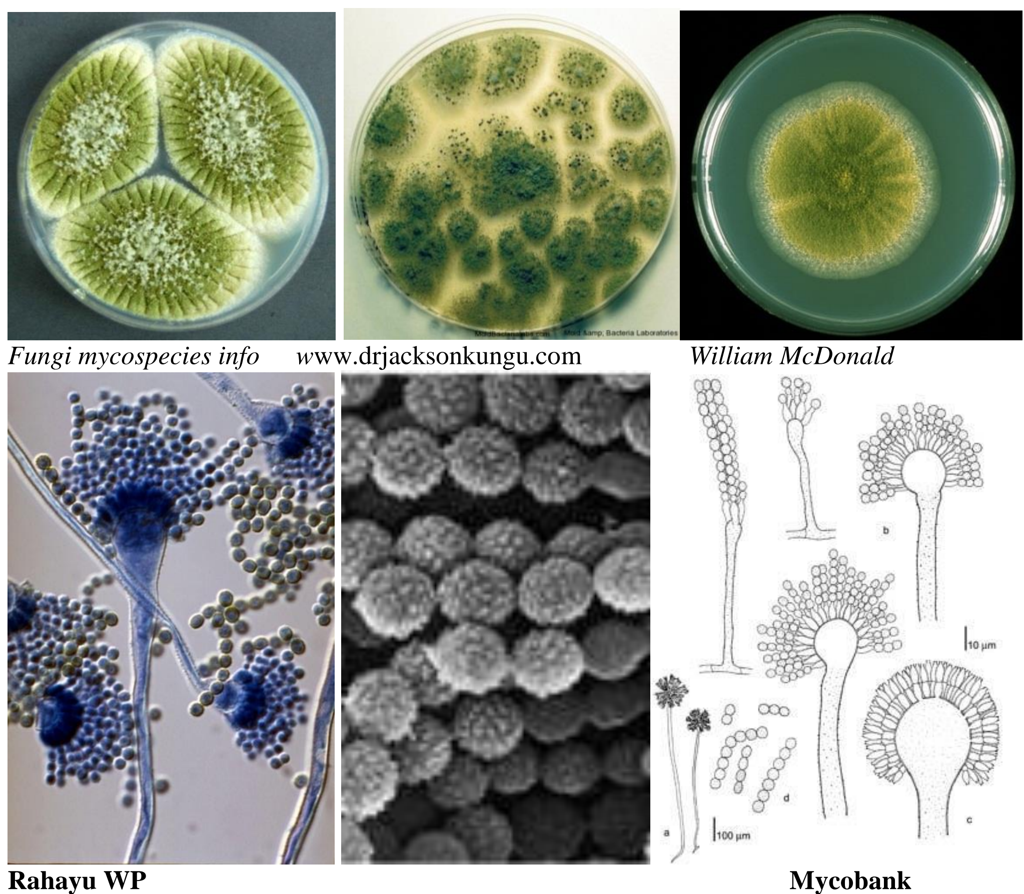
i. Aspergillus fumigatus Fresenius, 1863.

Colony diam (7 d): CYA25: 21-67 mm; MEA25: 25-69 mm; YES25: 48-74 mm; OA25: 34-62 mm, CYA37: 60-75 mm, CREA: poor growth, no or very weak acid production. Colour: greyish turquoise or dark turquoise to dark green to dull green. Reverse colour (CYA): creamy, yellow to orange. Colony texture: velutinous, st. floccose. Conidial head: columnar. Conidiation: abundant, rarely less abundant. Stipe: 50-350 × 3.5-10 µm. Vesicle diam, shape: 10-26 µm, pyriform to subclavate, sometimes subglobose, but rarely globose. Conidia length, shape, surface texture: 2-3.5(-6) µm, globose to ellipsoidal, smooth to finely rough

Aspergillus fumigatus, Mycoba

ii. Aspergillus flavus Link, 1809

A. flavus is known as a velvety, yellow to green or brown mould with a goldish to red-brown reverse. On Czapek dox agar, colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, mostly 300-400 um in diameter, later splitting to form loose columns .The conidiophores are variable in length, rough, pitted and spiny. They may be either uniseriate or biseriate. They cover the entire vesicle, and phialides point out in all directions. Conidia are globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 mm in diameter. Based on the characteristics of the sclerotia produced, A. flavus isolates can be divided into two phenotypic types. The S strain produces numerous small sclerotia (average diameter ,400 mm).

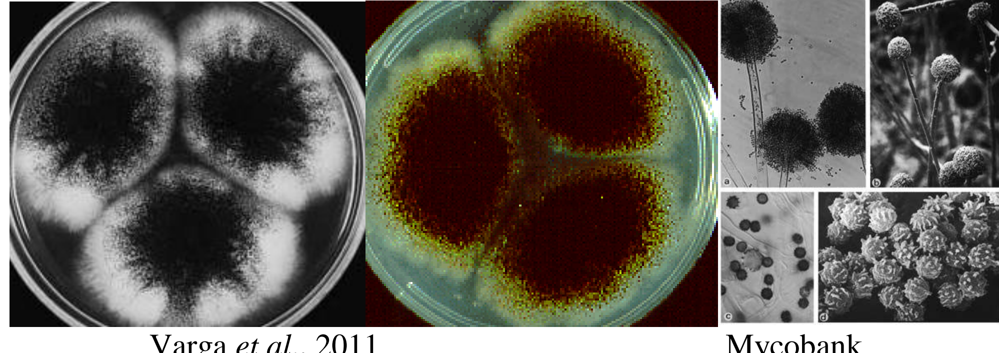


Fungi mycospecies info www.drjacksonkungu.com *William McDonald*

Rahayu WP **Mycobank**

iii. *Aspergillus niger* van Tieghem 1867

On Czapek dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm x 15-20 um in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5.0 um in diameter), dark brown to black and rough-walled.

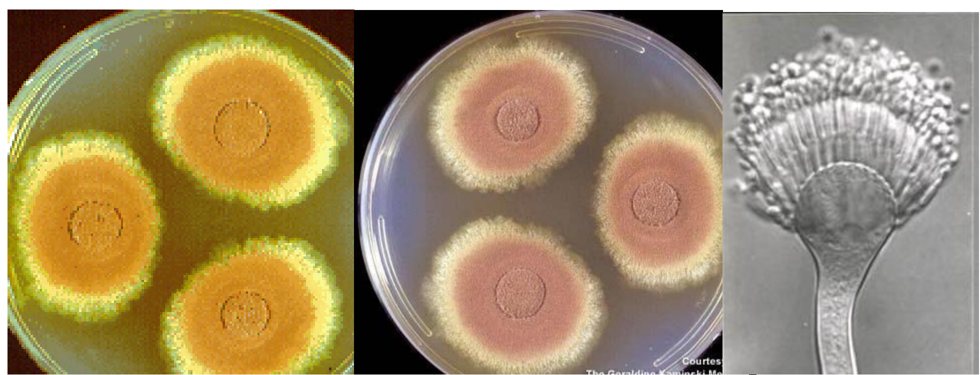


Varga *et al*., 2011 Mycobank

iv. *Aspergillus terreus* Thom, (1918)

Colonies on potato dextrose agar at 25°C are beige to buff to cinnamon. Reverse is yellow and yellow soluble pigments are frequently present. Moderate to rapid growth

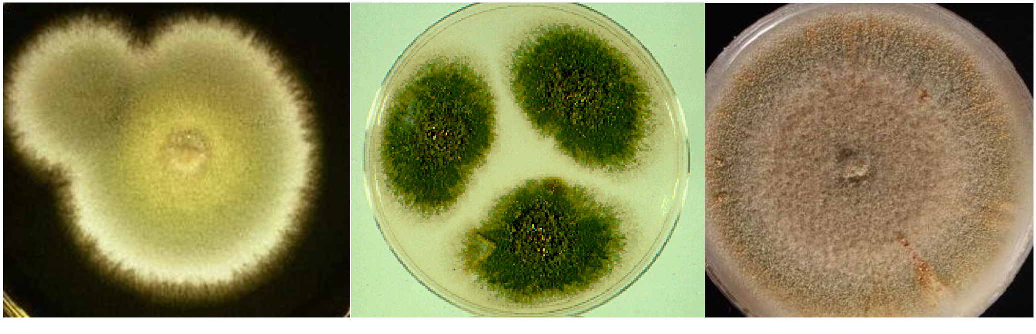
rate. Colonies become finely granular with conidial production. Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle). Conidiophores are smooth-walled and hyaline, 70 to 300µm long, terminating in mostly globose vesicles. Conidia are small (2-2.5 µm), globose, and smooth. Globose, sessile, hyaline accessory conidia (2-6 µm) frequently produced on submerged hyphae.



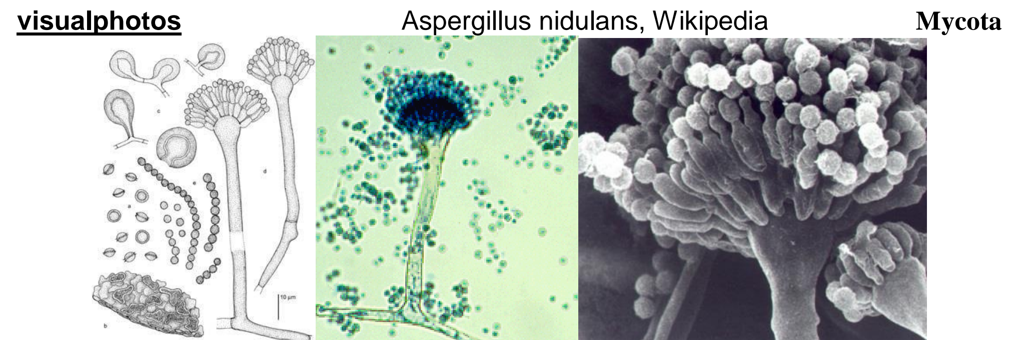
A. terreus mycology.adelaide.edu.au www.mold.ph Mycobank

v. *Aspergillus nidulans* (Eidam) G. Winter (1884)

Colonies on potato dextrose agar at 25°C are dark green with orange to yellow in areas of cleistothecial production. Reverse is purplish to olive. Exudate is usually present and may be brown to purplish. Growth rate is slow to moderate in comparison with other clinically significant *Aspergillus* species. Hyphae are septate and hyaline. Conidial heads are columnar. Conidiophores are brown, short (60-150 µm in length), and smooth-walled. Vesicles are hemispherical, small (8-12 µm in diameter), with metulae and phialides occurring on the upper portion. Conidia are globose (3-4 µm) and rough. *A. nidulans* is a homothallic species capable of producing the teleomorph (sexual stage) without mating studies. The ascomycetous telemorph (*Emericella nidulans*) produces brown to black globose cleistothecia (100-250 µm) that are engulfed with globose Hülle cells. Ascospores are reddish brown, lenticular (4 x 5 µm), with two longitudinal crests



visualphotos Aspergillus nidulans, Wikipedia Mycota



Mycobank www.mycology.adelaide.edu SEM Micrograph Gallery, public.gettysburg.edu

Diagnosis

- **Antemortem diagnosis** of aspergillosis can be very difficult since the signs of disease mimic those of many other illnesses, especially in the chronic form. Cases of aspergillosis in birds are often diagnosed based on postmortem findings of white caseous nodules in the lungs or air sacs of affected birds

- **Direct Microscopy (Wet Smear Examination)**
  - by preparing a wet smear.
  - a nodule can be dissected out and crushed on a slide beneath a cover slip in a drop of 20% potassium hydroxide and lactophenol cotton blue.
  - the lactophenol cotton blue stains the fungal hyphae.
  - Wet mounts can also be prepared from sputum or nasal swabs in either 10% KOH and Calcofluor or Parker ink and/or Gram stain.

- **Histopathological Examinations**
  - tissue samples (lungs, trachea, pharynx and thoracic air sacs as well as other organs) fixed in 10% neutral buffered formalin are processed and embedded in paraffin blocks
  - Aspergillus hyphae are stained poorly in H and E stained sections.
  - Differential stains such as Periodic acid-Schiff (PAS), Bauer's and Gridley's stains differentiate and easily identify the hyphae and mycelia.
  - Special stains for fungus Grocott’s and Gomori Methanamine Silver stain should be employed to detect the presence of fungal hyphae

- **Culture**
  - Small pieces of lesions aseptically removed are placed onto plates or slants containing malt agar, Sabouraud's glucose agar or antibiotics and incubated at 37ºC for 24 hours.
  - Species of Aspergillus can be identified by observing the characteristic conidial head and colony

- **Immunohistochemistry**
  - Immunohistochemistry with monoclonal or polyclonal antibodies can be used to identify

- **Serology**
    - A number of serological test have been applied in the diagnosis of aspergillosis. It includes counter
        - immunoelectrophoresis (CIE),
        - agar gel immunodiffusion (AGI)
        - enzyme-linked immunosorbent assays (ELISA).
    - **in acute cases**, antibody titre is low and thus detection of circulating Aspergillus antigen in the serum may be useful
    - **in chronic cases,** antigen levels may be low, detection of antibodies may be useful.
    - serological tests have not been validated in poultry and are not currently used in farms to investigate Aspergillosis outbreaks.

- **Polymerase chain reaction assays** (PCR)
  Application of PCR on heparinized whole blood, tracheal washings, air sac fluids, respiratory tract granulomas, or (biopsy) tissue samples from birds may be of value in diagnosing avian aspergillosis

Differential Diagnosis

- Avian aspergillosis signs are nonspecific and depend on the system involved.
- Pulmonary aspergillosis is usually differentiated from other avian respiratory diseases by the granulomatous lesions at necropsy, but needs to be differentiated from other mycoses and mycobacteriosis.
- Aspergillosis should be differentiated from chlamydiophylosis, tuberculosis, neoplasia, vitamin A deficiency, bacterial disease, candidiasis, ascitis, hepatomegaly and pneumonia.

Treatment

- Treatment for aspergillosis is complicated and relies on the use of antifungal medication.
- The success of treatment depends upon the location and extent of the infection.
- The most potent drugs may not reach the fungal granulomas or the walled-off fungus by the inflammatory response.
- The best treatment results if the granulomatous lesions are debrided and a topic treatment in conjunction with a systemic therapy is given.
- The drugs used include
    - itraconazole,
    - fluconazole,
    - clotrimazole,
    - miconazole,
    - ketoconazole and
    - amphotericin

Economic losses due to aspergillosis

- Direct losses related to mortality, in spontaneous outbreaks, mortality ranged between 4.5% and 90%, whereas age of diseased birds varied from 3 days to 20 weeks
- Feed conversion and growth rate in recovering birds remain poor.
- Airsacculitis is a major reason for carcass condemnation at slaughter inspection
- In turkey production the disease occurs late in the growing cycle or primarily affects costly breeder toms

Prevention and Control

- No commercial vaccine against aspergillosis is available till date.
- Some autogenous vaccines have been applied but with little information about this vaccine.
- Although numerous antifungal protocols have been proposed to cure birds with aspergillosis, treatment of the disease in poultry farms is virtually impossible
- **Preventative measures**
    - reduction of predisposing immunosuppressive factors such as malnutrition and stress should be encouraged.
    - standard of hygiene, nutrition and housing should be maintained.
    - mouldy litter or feed should be avoided.
    - feeders, waterers and incubators should be frequently cleaned and disinfected.
    - appropriate ventilation should be provided to maintain relative humidity so as to prevent wet litter.
    - environmental contamination should be control by sporadic or repeated antifungal treatment.
        - spraying of fungistatic agents like nystatin, thiabendazole or copper sulphate (at 1 gram per 2 litre of water daily morning for 3 days)

Reports:

Walker (1915) reported that aspergillosis (Chick Fever or Yellow Liver) among **ostrich chicks** reared on some farms in South Africa sometimes causes a mortality as high as 70 per cent. The disease has thus assumed considerable economic importance, inasmuch as prices as high as 200 to300 may be paid for adults, and 30 for three month-old chicks. In preliminary observations the author discovered that various moulds, notably of the Aspergillus type, were found in the lesions of chick fever and, in exposure experiments, were capable of transmitting the disease. On a plan outlined by the Director of Veterinary Research of the Union of South Africa, the author carried out further detailed investigations into the nature of the disease, the results of which are summarised in the following conclusions: - "1. *Aspergillus fumigatus* appeared in the ostrich, more particularly in *the* ostrich chick, from a few days to about 3-4 weeks after hatching, and was responsible for the disease in ostrich chicks commonly known as ' Yellow Liver or Chick Fever.' "2. ***Aspergillus***

fumigatus was the commoner and more pathogenic species. "3. Outbreaks usually appeared in an epidemic form and were more prevalent amongst chicks artificially hatched and reared. "4. *Aspergillus fumigatus* infection occurred in the air chamber of the egg, and was common in straw and other vegetable matter and in soil which has been fertilized with decomposed vegetable matter, such as stable manure, etc. "5. Infected eggs were the chief source of infection of incubators, the liberation of *Aspergillus fumigatus* from the air-chamber taking place either at the time of hatching or when infected eggs were opened in the incubators. "6. Aspergillosis was contracted naturally from: -"(a) Infected eggs just previous to or at time of hatching. "(b) Infected bedding used in chicks' sleeping boxes. "(c) Infected incubators. "7. Aspergillosis may be transmitted artificially by inhalation and ingestion and intravenous inoculation of cultures. "8. Aspergillus infection occurred chiefly through the respiratory tract, the lungs and air-sacs being the seat of infection. "9. Infection may occurred through the digestive tract."10. *Aspergillus fumigatus* was transmitted from infected to clean eggs through the unbroken shell. "11. The contents of unbroken eggs may escape through the intact shell. In such cases the bacteria which existed in the contents were probably a source of infection of eggs, more particularly those in contact. "12. Spores of *Aspergillus fumigatus* vegetated after passing through the intestinal canal. "Prevention consisted in the use of: - "(1) Non-infected incubators. "(2) Non-infected bedding in the chicks' sleeping boxes. "(3) Non-infected eggs for incubation purposes."Boiling water has given satisfactory results in the sterilization of cultures of ***Aspergillus fumigatus***.".

Ainsworth and Rewell (1949) diagnosed 78 cases as aspergillosis in **captive wild birds**. Cultures were made from 68 cases; 45 yielded pure growths of ***Aspergillus fumigatus*****,** three were ***A. flavus***, one was ***A. nidulans***. There were no anatomical differences in the disease produced by these fungi.

Eggert and Barnhart (1953) isolated ***Aspergillus fumigatus*** readily on Sabouraud's agar from the lungs of **four-day-old chicks** submitted for examination from a hatchery at Richmond, Virginia. This was believed to be the first report of the transmission of aspergillosis through the eggshell during incubation.

Moore (1953) stated that aspergillosis is becoming recognized as a disease of considerable economic importance. Aspergillosis (brooder pneumonia) is usually thought of as being a disease affecting the respiratory tract of young birds. In recent years it has been diagnosed in a number of breeding flocks, where the males are more frequently affected than the females.

O'Meara and Chute (1959) reported that **chicks** in the process of hatching and up to 2 days of age were easily infected with ***Aspergillus fumigatus*** spores by contaminating the forced draught incubator with wheat which had been inoculated with the fungus. Older chicks were more resistant.

Beer (1963) used an improved form of swab and selective cultural conditions for isolation of ***Aspergillus fumigatus*** from 86 of 1188 **Pink-footed Geese** (*Anser brachyrhynchus* Baillon), in four of 61 Canada Geese (*Branta c. canadensis* L.) and 13 of 102 Herring Gulls (*Larus a. argentatus* L.) caught in Britain. The Pink-footed Geese, migrating from Iceland, appear to become infected shortly after arrival. In the Solway, a higher level of infection was found than elsewhere. The fungus was shown

to be present in the natural habitats of these birds and it is suggested that infection usually occurs from a heavy but local growth of the fungus in a micro-habitat associated with the birds' food

Refai and Rieth (1966) reported an outbreak of brooder pneumonia in turkey farms in Nile Delta in Egypt. The mortality rate reached 52.8% in Bronze turkey chicks and 66.3% in Dutch turkey chicks. The deaths started in 3 days old chicks and continued up to the age of 28 days. The mycological examination of the lungs of dead chicks revealed the isolation of ***Aspergillus fumigatus***, which was recovered also from the walls of the incubators.

Harold *et al.* (1968) described an epizootic in **Canada geese** (Branta canadensis) at the Swan Lake National Wildlife Refuge, Chariton County, Missouri, in 1966. Approximately 2,000 geese died during the first 18 days of October. Losses were localized and did not spread to 75,000 other geese on the refuge. Respiratory distress was common among the few sick geese that were observed in the field. All except two geese examined at the laboratory had varying degrees of pulmonary aspergillosis. Three distinct phases of mycotic pneumonia were recorded

Olson (1969) reported 2 epizootics of aspergillosis in the same colony of **Japanese quail (***Coturnix cotumix japónica*). The clinical signs were inappetance, depression and accelerated breathing. The gross lesions consisted of yellow caseous nodules (up to 2 mm diam.) in the lungs and air sacs, and focal areas of malaria in the infected brains. Pure colonies of ***A. fumigatus*** were cultured from imprints of sliced nodules from the lungs.

PALYA and BALOGH (1971) described an outbreaks of pulmonary aspergillosis with 1-25% morbidity, in which 10-50% of the birds that died showed nervous symptoms and meningoencephalitis and intra-cranial lesions in geese and turkeys. Symptoms generally appeared late; most birds showed respiratory difficulties but in some cases only nervous signs appeared (incoordinated movement, twisting of neck and head, paralysis of limbs), along with listlessness and diarrhoea. Death ensued within 1-5 days and at post mortem pulmonary aspergillosis was seen in 90% of the lungs and in 10-50% of birds yellow to grey necrotic areas were seen in the brain; in a few cases foci were found in the liver and kidneys and ulcers were seen on the walls of the glandular stomach and bursa of Fabricius. ***Aspergillus flavus*** was isolated from the lesions in 1 flock of geese and from rations and litter. From a flock of **turkeys *A. fumigatu*s** was recovered from lesions and litter. The disease was reproduced experimentally by intranasal inoculation of *A. flavus* spores in 3 goslings and 4 turkey poults. It was concluded that the spores can sporulate on the catarrhal-mucous fluid of the nasal cavity and find their way to the meninges and propagate in the brain, although spread may take place directly to the brain through the lymphatic system.

Refai (1971) isolated ***A. fumigatus, A. flavus. A. terreus, A. niger, A. glaucus, Paecilomyces. Fusarium ansd Stemphylium*** species from dead-in-shell embryos (500 samples) and poultry feeds (47 samples). He concluded that contaminated feeds were the main source of infection in poultry farms.

Redig *et al.* (1972) sampled **goshawks** (Accipiter gentilis atricapillus) at Hawk Ridge in Duluth, Minnesota for prevalence of fungi of the genus Aspergillus. Fungi of this genus were recovered from 26 of 49 birds (53%) in 1972 and 4 of 45 (7%) birds in

1973. Aspergillosis was confirmed at necropsy in three wild goshawks in 1972, but none in 1973. The disease was further confirmed at necropsy in 8 of 12 (67%) goshawks trapped in the fall and retained for falconry in 1972 and in 2 of 17 (12%) such birds in 1973. We suggest tha t the stress of intraspecific agonistic behavior in conjunction with a high density of goshawks and greatly reduced prey base may increase the susceptibility of these hawks to aspergillosis.

Ghori and. Edgar (1973) reported that **Coturnix quail** exposed to ***Aspergillus fumigatus*** spores at hatching suffered significantly greater mortality from the ensuing disease than did chickens or turkeys exposed at the same time. Chickens were the least affected of the three. Survivors sacrificed at 28 to 39 days following exposure and that had lesions experienced significant growth depression.

Saif and Refai (1977) reported the frequent isolation of ***Aspergillus flavus*** and ***Aspergillus fumigatus*** from the yolks of dead-in-shell embryos and from nodular lesions in lungs and/or air sacs of dead birds in turkey and chicken farms, respectively. Both Aspergillus species and other fungi were isolated from the egg incubators, egg stores, feeds and litters.

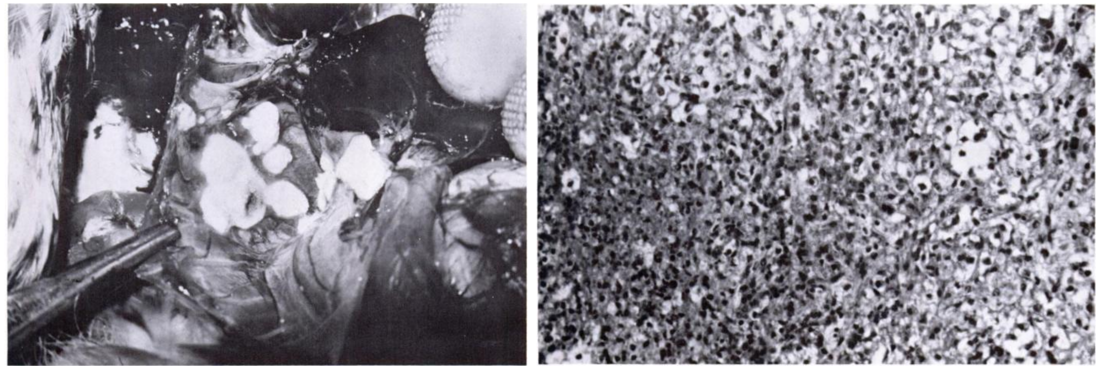
Yamada *et al.* (1977) described **dermatitis** producing black necrotic foci in at least 10% o of 70-day-old broiler chickens dealt with in a chicken-processing plant in Kagoshima Prefecture. From these foci was isolated ***Aspergillus fumigatus*** almost in a pure form. It was not found in any visceral organ. Histopathological examination revealed changes of granulomatous necrotic dermatitis and the presence of hyphae of this fungus in the tissue.

Zink *et al.* (1977) reported the death of 1,000 to 1,500 **common crows** (Corvus brachyrhynchos) of aspergillosis in south-central Nebraska. The birds were debilitated, lethargic and dyspneic. On necropsy, typical lesions of aspergillosis were found in the lungs and air sacs. Histopathologic examination revealed granulomatous lesions around fungal hyphae in the respiratory system.

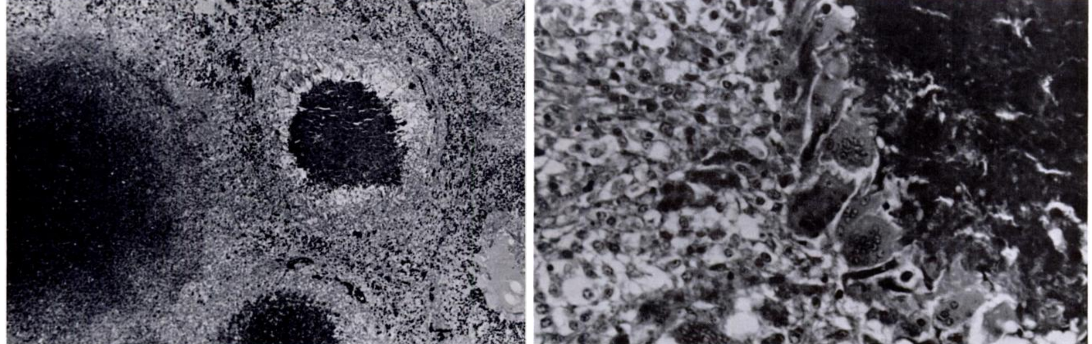
Zink *et al.* (1977) reported a case of aspergillosis in a **Brahmini duck** *(Tadoroma ferruginea)*, which presented irregular, firm, greyish nodules in the lung tissue. The size of nodules varied from pin head to pea size. Nodules on culture yielded ***Aspergillus fumigatus***. Histopathological study of lung tissues revealed giant cells and granulomatous inflammation in haematoxyline and eosin (H & E) preparation. Periodic acid scruff (PAS) and GOMORI'S GROCOTT methenamine silver (GMS) stain treated sections, showed numerous septate branching fungal mycelia. The relation of this fungus in causation of the disease in birds has been discussed.

Adrian *et al.* (1978) described the history, gross necropsy, histopathology and predisposing factors of two **epornitics** of acute aspergillosis in **mallard ducks** in north central Colorado. Most mortalities were picked up from the shore of the lakes during the die-offs. A few that were floating, dead or moribund on the water were picked up by a boat or a Labrador retriever. A total of 270 mallards were picked up from 28 through :30 October 1975, hut due to predation and/or delay only 128 were suitable for necropsy. l)ucks were picked up at Woods Lake. from 10 through 14 October 1976: 117 birds were found and all were fresh enough to be examined at necropsy. Samples of moldy corn were picked up from the surrounding corn fields and ensilage pits. The great majority of the birds in both epornitics were in good-to-

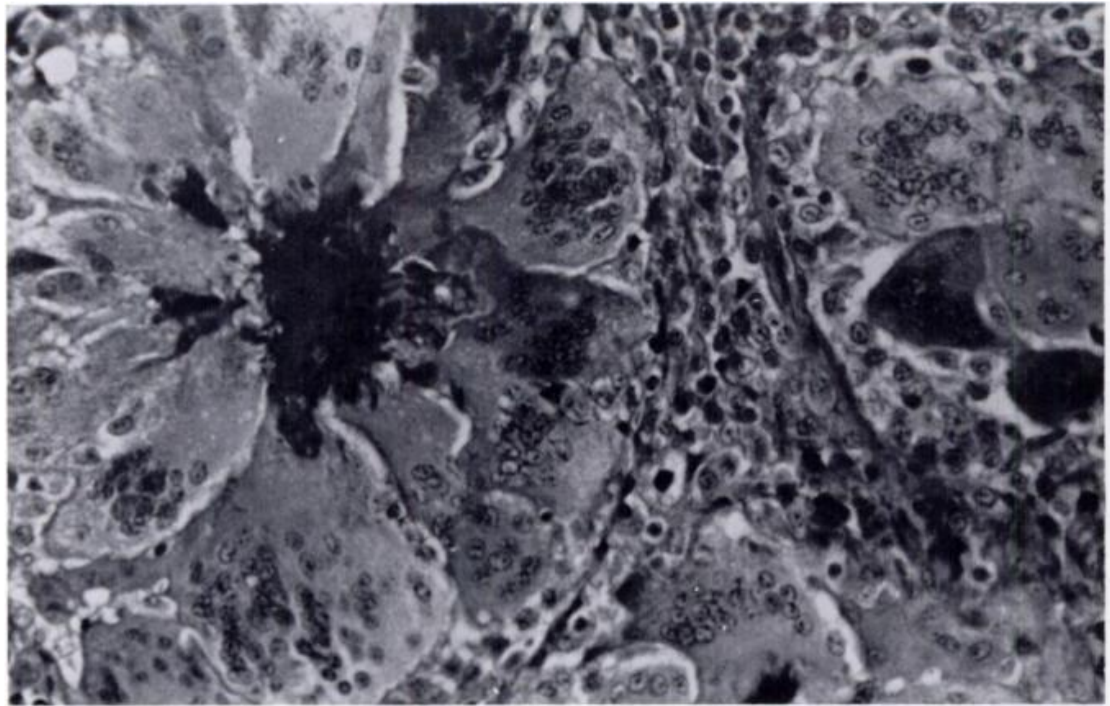
excellent body condition. A few were in poor condition. Aspergillus lesions were remarkably similar in all birds examined. The organs predominantly affected were the lungs and air sacs. The lungs were dark red, wet and contained many small, white miliary nodules disseminated throughout the lung parenchyma. A slightly higher prevalence of white nodules were present around the mesobronchus of the abdominal air sac in the posterior ventral aspect of the lungs. Seventy percent of the birds had white-to-yellow plaques in the interclavical, anterior and posterior thoracic, and abdominal air sacs. These plaques varied in texture from cotton-like, with visible hyphae and fruiting bodies, to firm, flat, yellow plaques. The size and number of mycotic plaques on the air sacs varied from bird to bird, but all had the white nodular lung lesions. The birds with clear air sacs appeared to have more lung damage than birds with both lung and air sac involvement.



Chronic type *Aspergillus* plaques in the air sacs of a mallard., Acute mycotic foci within the lungs. Note the central core of necrosis with the reaction around the periphery composed predominantly of macrophages and heterophils. (H & E *x* 250). **Adrian *et al.* (1978)**



Subacute mycotic foci with a central core of necrotic debris surrounded by multinucleated giant cells with many macrophages and a few heterophils. (H & E Journal of Wildlife Diseases Vol. 14, April, 1978 **215** X **160).** Subacute mycotic nodule. (H & E X 400). **Adrian *et al.* (1978)**



Chronic mycotic nodule with a small central core of necrotic debris with a zone of predominantly large multinucleated giant cells with a few macrophages on the periphery. (H & E X 400). **Adrian *et al.* (1978)**

Corkish (1980) reported aspergillosis in a house that contained about 30,000 **Warren** and 17,000 **Hisex** replacement **pullets** aged 5-6 weeks and the birds were in the top two tiers of four blocks of three-tier rearing cages. The environment was controlled but because it was winter and only minimal ventilation was necessary, the air inlets in the roof had been left closed in order to maintain the temperature. Birds in block 1 had been placed in cages 2 days before those in the other blocks, all having been previously vaccinated against Marek's disease at the hatchery. On the farm, they had been vaccinated against infectious bursal disease (IBD) by spray at 2 weeks and given combined Newcastle disease (ND) and infectious bronchitis (IB) vaccines by spray at 3 weeks. At the end of week 5, the mortality in block 1 rose for about 5 days and then fell; 98 birds died, while in the same period only 20, 15 and 36 birds died in blocks 2, 3 and 4 respectively. Slight respiratory signs were said to have been noticed in birds in block 1 about this time. Nine birds from block 1, three of which were alive, were submitted for post-mortem examination. The live birds had a dry, wheezing respiration. Gross lesions were confined to the trachea in all birds. There was a slight excess of mucus and in the upper/ mid portion caseating nodules were adherent to the wall, ranging from one or two to more than a dozen. A crush preparation of nodules revealed masses of fungal hyphae. On culture a fungus was isolated which on the basis of its rapid growth, colonial morphology on Sabouraud's medium and microscopic appearance of conidiophores on a wet preparation made from the culture, was identified as A fumigatus. Haematoxylin and eosin stained histological sections of nodules showed a marked granulomatous reaction with giant cell formation and in very severe cases this reaction involved the epithelium, submucosa, cartilage and muscle with virtual occlusion of the lumen. Grocott-Gomori Hexamine silver stained sections showed the presence of fungal hyphae in some of these **granulomas.**

Bassiyoni *et al.* (1981) infected 159 one-day old, Fayoumt chicks, one hundred . 2-weeks old Fayoumt chicks and twenty four 5-month old Nicole chickens were experimentally infected by ***C***. The first group was divided into 3 equal subgroups (A,B,C). Birds of subgroups A and B were infected intranasally with $4x10^6$,. birds of subgroup B received 2 weeks later a second similar dose intranasally and those of subgroup C were kept as control. The 2-weeks old chicks were divided into 2 subgroups, one subgroup was infected intranasally with $4x10^6$ and the other was kept as control. The Nicole chickens were divided into 4 equal subdroups, the first 3 subfroupe were infected with $4x10^6$ spores intranasally, via abdominal air sacs or ds of the subgroups A and B in the one-day old group showed symptoms of depression, ruffled feathers, nasal discharge, sneezing and gasping on the 10th day after infection. In dead birds, the lesions were confined to the respiratory organs. The lungs were pneumonic and air sacs were turbid in young birds, while yellowish white hatd nodules observed in the lungs and air sacs of10-14 days old birds .

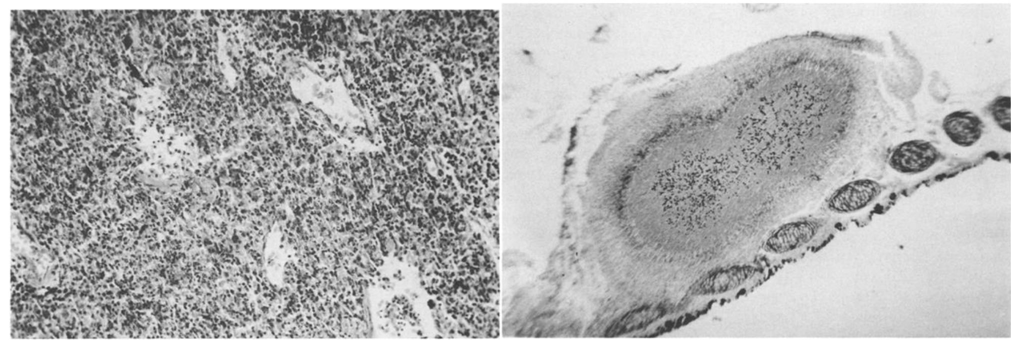
Richard and Thurston (1983) exposed 3-week-**old turkey poults** to aerosolized spores of either ***Aspergillus fumigatus or A. flavus*** for 15 min. Cultures of blood samples yielded the organisms. Aspergillus fumigatus was also isolated from brain and liver tissue samples taken immediately after exposure. Exfoliated cells from the lungs of 10 poults exposed to aerosolized spores of A. fumigatus were allowed to attach to glass slides in tissue culture. Several types of the fixed and stained cells had attached or ingested spores of A. fumigatus. Macrophages were the predominant cell type with ingested spores, although other cell types may be involved in the transport of spores of A. fumigatus into the blood stream after aerosol exposure.

Dyar *et al.* (1984) reported severe mortality that occurred in a flock of 16,000 **turkey poults** after fresh litter consisting of poplar, oak, sweetgum, and pine shavings was added to the brooder house. Signs and lesions seen were consistent with aspergillosis. Mortality for the first 21 days of brooding was 305 poults. New litter was added on day 21; 6,041 poults died during the next 24 days. Mold counts were done on the original litter and added litter. The mold counts for the original litter was 1.0 X 10(5) organisms per gram of litter. The mold count for the added litter was 2.5 X 10(6) organisms per gram of litter. After the added litter had been treated with nystatin and copper sulfate, the mold count dropped to less than 1 X 10(4) organisms per gram of litter. Mortality was also reduced but not to pre-infection levels. Rales, mucus rhinitis, and air-sacculitis due to Escherichia coli developed. Despite treatment, performance of the flock remained poor.

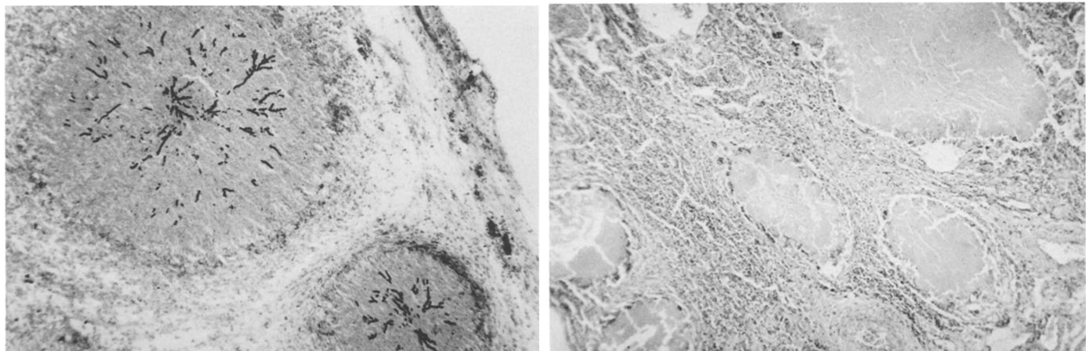
Veselský *et al.* (1984) described an enzootic of **chick** mycosis, caused by the spores of the fungus ***Aspergillus fumigatus***. The mycotic infection affected the respiratory tract of the birds; pathological changes were located mainly in the region of the trachea. The changes had the nature of diphtheroid necrotic inflammation destroying the mucous membrane and causing almost an obstruction of the trachea. Deposits of granulomatous inflammation, containing fungus elements, were detected in the peritracheal tissue, and in individual birds also in the lungs. Litter contaminated with Aspergillus was the source of infection.

Chaudhary *et al.* (1988) reported that intratracheal inoculation of young quail chicks with ***Aspergillus fumigatus*** spores resulted in the development of characteristic gross and microscopic lesions. The lesions were restricted to respiratory tract and there was no dissemination of infection to other tissues of the body. Gross changes in lungs and air sacs were observed within 24 hours and continued up to 20 days while in trachea

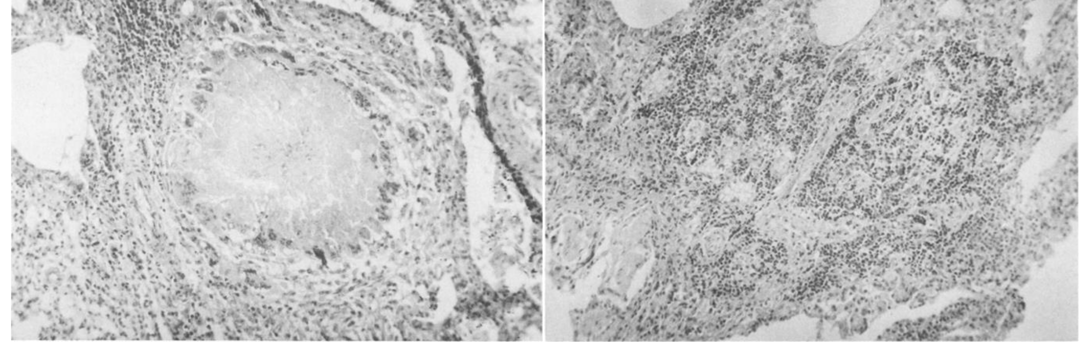
these were noticed from the 3rd to the 9th day post-infection. The lesions, in general, included congestion and focal haemorrhages in the first 2 days followed by the development of varying-sized greyish-white nodules in the lungs, air sacs and trachea. Microscopic changes consisted of congestion, haemorrhages and a diffuse cellular infiltration in the first 2 days followed by granulomatous reaction with well developed granulomas in lungs, air sacs and trachea. Spores and developing hyphae of Aspergillus could be demonstrated in sections from 24 hours to 20 days of infection. Reisolation of the fungus was consistently achieved from the lungs, air sacs and trachea up to 14 days



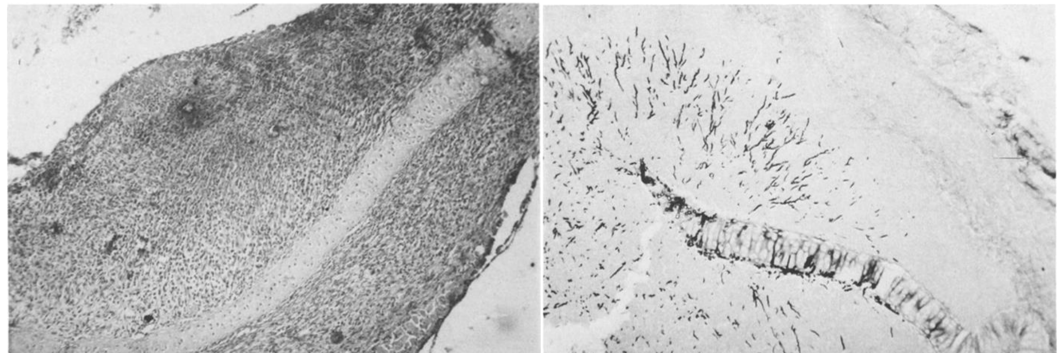
Lung (24 hr PI). Diffuse pneumonia characterized by congestion, sero-fibrinous exudate and infiltration of macrophage~ and heterophils. H & E • Lung (3 DPI). A well developed granuloma containing fungal spores and hyphae in the wall of primary bronchus. OMS • 9,24 **Chaudhary *et al.* (1988)**



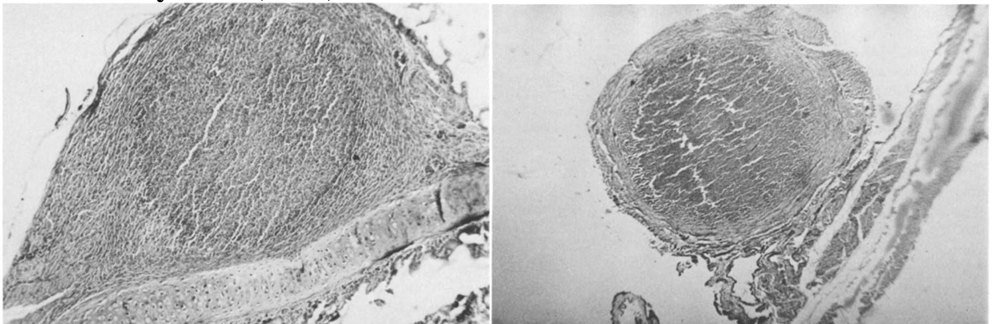
Lung (7 DPI). *A. fumigatus* spores and radiating hyphae in the centre of granulomas. GMS • Lung (14 DPI). Multiple granulomas replacing the lung parenchyma. H & E x23,1.ophils. The bronchioles were almost free from exudate.In chicks killed on the 21st and 28th day of infec tion, Lung (14 DPI). Multiple granulomas replacing the lung parenchyma. H & E x23,1. **Chaudhary *et al.* (1988)**



Lung (14 DPI). A typical granuloma to show giant cells at the periphery of central caseated mass. H & E x46,2 Lung (35 DPI). Mononuclear cell infiltration and mild fibroplasia. H & E • **Chaudhary *et al.* (1988)**



Trachea (3 DPI). Granulomatous reaction involving the mucosa and submucosa. H & E x 23,1. Trachea (5 DPI). Fungal hyphae in granulomatous tissue. The tracheal cartilage is penetrated by fungus. GMS x23,1. **Chaudhary *et al.* (1988)**



A typical granuloma in the wall of trachea of a naturally dead (6 DPI) quail chick. H & E x23,~.
Air sac (7 DPI). A typical mycotic granuloma. The central necrotic mass is surrounded by a narrow zone of mononuclear cells and a fibrous capsule. H & E x 9,24 **Chaudhary *et al.* (1988)**

Okoye *et al.* (1989a) performed a study to supply information on **Aspergillus fumigatus** infection of poultry in Nigeria. The disease in **broiler chicks** was characterized by gasping, droopiness, emaciation and heavy mortality while affected grower chickens showed emaciation, weakness, diarrhoea and 17 per cent mortality. The disease was sporadic in laying flocks. Granulomatous nodules were observed in birds that died in each outbreak. The nodules were numerous and affected mainly the lungs and thoracic air sacs in the broiler chicks while only few large nodules were observed mainly in the abdominal air sacs in the layers.

Okoye *et al.* (1989b) described 2 outbreaks of **pulmonary aspergillosis** involving a flock of 66 **turkey poults** and a group of 12 **gosling**. The lungs showed multiple yellowish to greyish nodules. Histology demonstrated hyphae characteristic of ***Aspergillus fumigatus*** and ***A. flavus*** was recovered in culture. Sections of liver showed features of aflatoxicosis. Cultures of samples of litter and feed yielded A. flavus in quantity. Representative isolates from lung lesions, litter and feed were found to produce aflatoxin B1.

Redmann and Schildger (1989) studied the course of a spontaneous **outbreak of aspergillosis in a broiler flock.** Up to the 10th day of life the total mortality was 8%. Surviving broiler had an average body weight of 141 g on the 7th day of life, in contrast to the normal body weight of 150 g per bird. **Aspergillosis** was diagnosed in another three broiler flocks as well in an early stage of infection. Treatment with **Enilconazole** (Clinafarm Spray, Janssen, Neuss) in these flocks at a single time via spray in a dosage of 1.5 g enilconazole per 10 m2 housing ground obviously reduced mortality from the second day on after treatment. The average body weight of 7-day-

old broiler was in a normal range. The success of the treatment depends on an early diagnosis and immediate start of the therapy. The source of infection (hatchery, transport, litter etc.) should be discovered and eliminated to prevent flocks from further infections.

Flach *et al.* (1990) reported that aspergillosis was the commonest cause of death in **gentoo penguins** (Pygoscelis papua) at Edinburgh zoo from 1964 to 1988. Chicks were the most susceptible group and 14 per cent of hatched birds died of the disease (47 per cent of post mortem diagnoses) although in two years no cases were recorded and in two other years there were outbreaks which killed more than 50 per cent of the birds hatched. There was no correlation between the size of the colony and the prevalence of the disease but the climate during the rearing and weaning periods may have influenced the prevalence. The majority of cases of aspergillosis occurred between July and September and affected two- to three-month-old chicks shortly after they had been moved from their parents to a creche area for hand-feeding. No links were found between the occurrence of the disease and the date of hatching or the nest site, or whether the chicks were single, first or second-hatched twins, or with their weights, but the progeny of one parental pair were found to be overrepresented and more males than females died of the disease. Adult cases occurred sporadically and males were significantly more susceptible than females (P less than 0.05). Neither vaccination with a killed suspension of ***Aspergillus fumigatus*** nor therapeutic treatment with **ketoconazol**e were effective in reducing the incidence of aspergillosis, although the late diagnosis of the disease was probably the main cause for the failure of these treatments.

Julian and Goryo (1990) induced **experimentally pulmonary aspergillosis** in one-day-old **chicks,** which resulted in some, no clinical signs, or little damage, while others developed marked interstitial pneumonia. Chicks with severe lung damage died from respiratory failure (60%) or developed pulmonary hypertension followed by right ventricular hypertrophy and dilation, right ventricular failure from valvular insufficiency, portal hypertension and ascites (10.7%). Chicks that developed ascites before day 16 (13/31) had shown mouth-breathing and other signs of hypoxia and developed pulmonary hypertension, probably from hypoxia-induced polycythaemia. Most chicks that developed ascites after day 16 (18/31) .

Pal *et al.* (1990) recorded an unusual case of **mycotic tracheitis** in a young chick caused by ***Aspergillus fumigatus*** from Anand, India, during 1987. The pathogen was demonstrated in the tracheal exudate by potassium hydroxide technique and also recovered from the trachea on Sabouraud dextrose agar at 37 degrees C. The characteristic hyphae of Aspergillus spp. were demonstrated in tracheal mucosa in histopathological sections stained by periodic acid Schiff method. Interestingly, the lesions were confined only to the trachea. Environmental investigations established the source of infection in the farm where the litter was highly contaminated with A. fumigatus.

Hamet *et al.* (1991) demonstrated that the contamination of the **hatchery** originates on the **egg** shell and that each time the eggs are manipulated, spores of Aspergillus fumigatus are thrown into suspension in the air. Thus it seems necessary to bring eggs with as few as possible spores of ***Aspergillus fumigatus*** on their shell into the hatchery. Prophylaxis of aspergillosis should be foreseen from the conception of the hatchery: the ventilation system and the internal lay-out should be designed to prevent

dispersion and accumulation of Aspergillus fumigatus spores during the processing of the eggs through the hatchery.

Barton ***et al.*** **(1992)** documented a case of localized **tracheal aspergillosis** in 6 1/2-week-old single-comb **white leghorn pullets** caused by ***Aspergillus flavus***. Yellow caseous plaques adherent to the mucosal surface of the tracheas were observed grossly. In several tracheas, the plaques occluded the lumina, and the surrounding tracheal walls were reddened. Histologically, the mucosa was necrotic and infiltrated with macrophages, and fibroplasia was evident in the sub-adjacent tracheal wall. The lumen of the trachea was almost completely occluded by a combination of fungal mycelia and pyogranulomatous exudate. Portions of tracheal cartilage were elevated into the lumen of the trachea. Other than a sudden increase in mortality to 0.5% per day, there was no evidence of disease in the flock. Depletion of bursal lymphocytes, with concomitant cryptosporidiosis, was evident on histological examination. Acute infectious bursal disease was diagnosed in the succeeding flock at this ranch based upon serology and typical histology.

Peden and Rhoades (1992) used 16 ***Aspergillus fumigatus*** isolates of environmental, mammalian, and avian origin to assess: 1) **intra-air-sac inoculation** as a viable challenge alternative to aerosol exposure, and 2) isolate variability in pathogenicity. Development of lesions, antibody response in survivors, mortality, and weight gains were assessed. Turkey poults were challenged with equal numbers of viable conidia. Total number of conidia given per experimental group varied markedly and did not influence mortality. Antibody response as measured by the enzyme-linked immunosorbent assay and agar gel immunodiffusion test was erratic, although most poults with high antibody scores had marked lesions and low weight. Lesions were characterized by necrogranulomatous pneumonia and airsacculitis with marked visceral involvement. The source of the isolate was not a factor in mortality, although this was biased by the high numbers of isolates from birds with aspergillosis. The single environmental isolate produced no mortality.

Perelman and Kuttin (1992) reported a severe case of aspergillosis in a flock of 3- to 8-week-old **ostriches** at a farm in Israel. ***A. niger*** and ***A. flavus*** were isolated from the lungs of affected ostriches. A heavy contamination with both fungi was detected in the hatchery, suggesting a brooder borne infection. The clinical and pathological findings observed in this case are described.

de Wit ***et al.*** **(1993)** noted that in a flock of **chicks** the number of birds dying per day from infection with especially ***Aspergillus flavus*** increased up to 1% during the second half of the fattening period. Levels of Aspergillus flavus were measured before and after cleaning of the chicken house with the anti-mycotic agent **eniconazole** (Clinafarm, Janssen Pharmaceutical Company, BV). The cleaning and disinfection schedule followed reduced levels if Aspergillus flavus to zero.

Perelman ***et al.*** **(1993)** described a **model for aspergillosis** by injecting fungal spores into the lung. The model permits evaluation of anti-mycotic agents and their effect on the development of lesions in the infected lung, the spreading to the second lung and other organs. The therapeutic effect of the azole compounds **enilconazole,** ketoconazole, itraconazole and levamisole was determined. Itraconazole was found to be the most effective.

Singh ***et al.*** **(1993)** described an **outbreak of mycotic tracheitis** in 8000 2-month-old, female **White Leghorn** birds. The birds were showing difficult respiration and

there was mortality of 7-8 birds daily in the flock. On post-mortem examination of the affected birds, the trachea was found to be occluded with a white caseous mass. Microscopically hyphae were found invading the tracheal epithelium, cartilage and serosal layer along with infiltration of macrophages and lymphocytes. ***Aspergillus fumigatus*** was isolated in pure culture from the trachea. The birds responded to oral copper sulphate treatment. The ubiquitous occurrence of the organism and the conditions of the harvesting season have been found to be responsible for the outbreak of the disease.

Beckman *et al*. (1994) observed **eye infections** in single-comb white leghorn breeder chicks at 5 days of age, and morbidity increased from 0.05% to 1.5% after debeaking at 7 days of age. All chicks necropsied at 15 days of age had cheesy yellow exudate within the conjunctival sac of one eye and small (1 mm diameter) white nodular lesions in lungs and on thoracic air-sac membranes. Histopathologic examination of the eyes revealed septate fungal hyphae and inflammatory cells in the anterior chamber, cornea, and conjunctival sac. Similar fungal hyphae were present within lung granulomas. **Aspergillus fumigatus** was isolated from the eyes. Eye infections were the only health problem reported for several consecutive flocks on this farm. Elimination of moldy feed from the diet and environment and proper management of sawdust litter have prevented fungal **ophthalmitis** in subsequent flocks.

Richard and DeBey (1995) gave **turkey poults** either of two different dosages of two different gliotoxin-producing strains of Aspergillus fumigatus. Infected lung tissue was examined postmortem for the presence of gliotoxin. Gliotoxin was found in lung tissue of ten poults infected with one strain and in seven of ten poults infected with the other strain. Concentrations of gliotoxin in the tissue exceeded 6 ppm in some of the infected tissues. The concentration of gliotoxin found in infected tissue did not appear to be correlated with the dosage of organism given. Considering the pathologic changes observed in turkey poults with aspergillosis and the production of gliotoxin during the pathogenic state in turkey poults, gliotoxin is considered likely to be involved in avian aspergillosis.

Kunkle and Rimler (1996) examined pathologic changes after intra-air sac inoculation of 9- and 19-wk-old turkeys with ***Aspergillus fumigatus*** conidia. Turkeys were euthanatized and examined at 24, 48, 72, and 96 hr postinoculation (PI). Lesions were largely confined to **air sac membrane and lung tissues** and were similar between the two age groups. There was progressive severity of gross lesions in both organs and of microscopic lesions in lung tissue. The character and severity of histologic lesions in air sac membranes were roughly similar at 24 through 96 hr PI, but there was an increasing amount and consolidation of exudate adherent to the epithelial surface. Lesions in air sac membranes included edema, heterophil and macrophage infiltrates, granulomas, lymphohistiocytic perivasculitis, necrosis, epithelial loss, and surface exudate. Discreet granulomas containing multinucleate giant cells were present at 24 hr PI and thereafter. Lung lesions progressed from mild interstitial pneumonia to extensive effacement by multifocal coalescing granulomas, necrosis, and hemorrhage. Severe pleuritis with local extension into lung parenchyma was seen at all time points. Numbers of intralesional fungal elements seen histologically were similar between age groups and appeared to decrease in air sac membranes and increase in lung tissues from 24 to 96 hr PI. Lung tissue of the 19-wk-old turkeys contained fewer colony-forming units per gram at time points after 24 hr PI.

Richard *et al.* (1996) cultured 13 samples of infected **turkey lung** tissue from cases of **'airsacculitis'** collected either at the processing plant or from a local turkey farm and performed gliotoxin analysis. **Aspergillus fumigatus** was isolated from 6 of the 13 samples; all isolates were determined to be gliotoxin producers when grown in laboratory culture and assayed by HPLC procedures. Gliotoxin was isolated from 5 of the 13 tissue but was not isolated from all tissues that were infected with A. fumigatus. Gliotoxin was isolated from which no A. fumigatus was isolated and it was not detected in three tissues from which gliotoxin-producing isolates of A. fumigatus were obtained. The ability of this pathogenic fungs to produce this immunomodulating compound in naturally infected turkeys provides further evidence that gliotoxin may be involved in the pathogenesis of the disease, aspergillosis of turkeys.

Graczyk *et al.* (1997) evaluated the applicability of **ELISA** detection of circulating **Aspergillus spp**. antigen (Ag) and systemic antibody (Ab) of IgG class, and the blood parameter values for diagnosis of invasive aspergillosis in Aspergillus spp.-challenged Peking ducks (Anas platyrhynchos). The protective role of Aspergillus spp. IgG was evaluated in Cape shelducks (Tadorna cana) immunized with Aspergillus spp. Ag. Challenged but non-immunized A. platyrhynchos developed invasive aspergillosis on day 21 as demonstrated histopathologically by the presence of fungal micro-granuloma in air sacs and lung tissue, with serum antigenemia fluctuating from 65 to 270 ng of 55-kD basic protein Ag per ml. Immunized A. platyrhynchos did not demonstrate Aspergillus spp. serum antigenemia but did show rare histopathological changes in some air sacs associated with fungal inflammation. Although the differences between immunized and non-immunized T. cana in blood evaluation parameters did not differ significantly, immunized birds mounted high Aspergillus spp.-specific IgG titer. There was no correlation between the blood parameter values and post-immunization timepoints in T. cana and in A. Platyrhynchos. Intramuscular immunization with Aspergillus spp. mycelial phase cultures Ag provided protection against the pathogens. The lack of relations between blood parameter values and increasing Aspergillus spp. IgG titers (in T. cana and A. platyrhynchos) indicate low applicability of these parameters in evaluation of a bird Aspergillus spp. status. Detection of circulating 55-kDa Aspergillus spp. Ag has high early predictive values for invasive aspergillosis in birds.

Jensen *et al.* (1997) described the **immunohistochemistry** for the diagnosis of aspergillosis in **turkey poults** From each of two flocks (A and B) of poults comprising 14,100 females and 11,300 males, respectively, 15 poults were examined pathologically. Poults of flock A had signs of neurological disturbances whereas birds from flock B showed respiratory symptoms. Gross lesions were observed only in two poults from flock A in which minute circular areas of cerebral malaria were seen. Histopathologically, the brain lesions contained fungal elements, and so did some of the pulmonary granulomas detected in three and six poults out of four and six birds examined from flock A and B, respectively. Mycological cultivation was attempted from the brains and lungs of five poults from flock A. However, only from the brain of a single bird a fungus, identified as ***Aspergillus fumigatus***, was grown. Immunohistochemistry was applied because the histomorphology of fungal elements within some lesions did not offer any characteristics allowing an assessment of the identity of the infective fungi. Moreover, as fungi could not be detected within all lesions, immunohistochemistry accomplished the screening of tissues. For immunostaining of tissues a panel of monoclonal and polyclonal antibodies

identifying agents of aspergillosis, candidosis, fusariosis, scedosporiosis, and zygomycosis, was used. Due to a strong and uniform reactivity of all fungal elements with immunoreagents to Aspergillus spp. an unequivocal diagnosis of aspergillosis was established in all mycotic lesions. Apart from the establishment of an aetiological diagnosis, the application of immunohistochemistry also disclosed fungal fragments in granulomas which could not be identified with conventional histochemical stains.

Graczyk *et al.* (1998) evaluated the applicability of **ELISA** detection of circulating Aspergillus spp. antigen (Ag) and systemic antibody (Ab) of IgG class, and the blood parameter values for diagnosis of invasive aspergillosis in Aspergillusspp.- challenged **Peking ducks** (Anas platyrhynchos). The protective role of **Aspergillus spp**. IgG was evaluated in Cape shelducks (Tadorna cana) immunized with Aspergillusspp. Ag. Challenged but non-immunized A. platyrhynchos developed invasive aspergillosis on day 21 as demonstrated histopathologically by the presence of fungal microgranuloma in air sacs and lung tissue, with serum antigenemia fluctuating from 65 to 270 ng of 55-kD basic protein Ag per ml. Immunized A. platyrhynchosdid not demonstrate Aspergillus spp. serum antigenemia but did show rare histopathological changes in some air sacs associated with fungal inflammation. Although the differences between immunized and non-immunized T. cana in blood evaluation parameters did not differ significantly, immunized birds mounted high Aspergillus spp.-specific IgG titer. There was no correlation between the blood parameter values and post-immunization timepoints in T. cana and in A. platyrhynchos. Intramuscular immunization with Aspergillus spp. mycelial phase cultures Ag provided protection against the pathogens. The lack of relations between blood parameter values and increasing Aspergillus spp. IgG titers (in T. cana and A. platyrhynchos) indicate low applicability of these parameters in evaluation of a bird Aspergillus spp. status. Detection of circulating 55-kDa Aspergillusspp. Ag has high early predictive values for invasive aspergillosis in birds.

Kunkle and Rimler (1998a) **assessed the potential of lipopolysaccharide (LPS) purified from Pasteurella multocida to cause pulmonary pathology or exacerbate lesions produced by gamma-irradiated nonviable** *Aspergillus fumigatus* **conidia when** administered via the intra-air sac route in turkeys**. LPS provoked suppurative airsacculitis, pleuritis, and pneumonia. Nonviable conidia produced airsacculitis and transient pneumonitis but did not elicit multinucleate giant cells, which are a feature of the inflammatory process in A. fumigatus infection. LPS in combination with A. fumigatus conidia resulted in accelerated pulmonary inflammation and apparently delayed clearance of conidia from pulmonary tissues. This study presents a model of aseptic airsacculitis and pneumonia with clinical relevance**

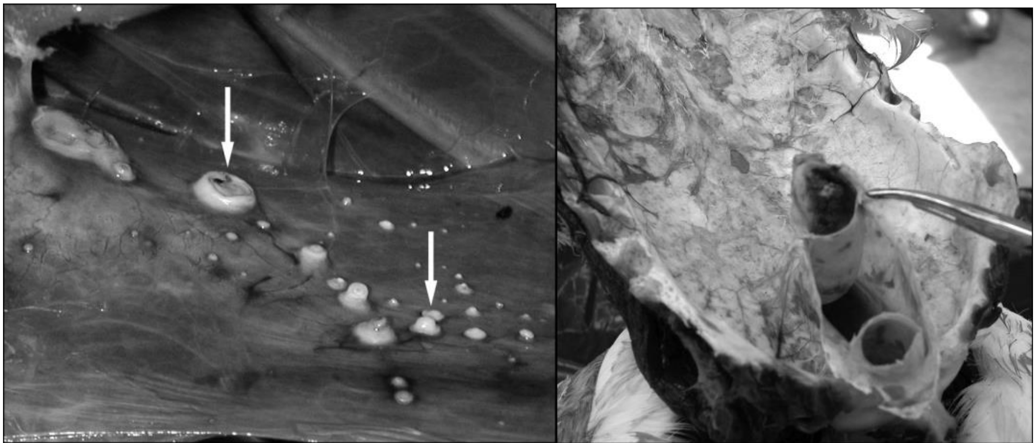
Kunkle and Sacco (1998b) assessed **pulmonary lesions** resulting from ***Aspergillus fumigatus* inoculation** in convalescent **turkeys** and compared them with those in previously noninoculated (control) turkeys. In addition, lesions observed in small Beltsville white (SBW) turkeys were compared with those in broad-breasted white (BBW) turkeys challenged with the same inoculum. Turkeys were challenged by unilateral posterior thoracic air sac (PTAS) inoculation, rechallenged via the contralateral air sac after 5 wk, and then necropsied 1 wk later. Pulmonary lesions induced by the initial challenge had resolved in 6 of 10 SBW and 9 of 10 BBW turkeys. However, convalescence did not protect against pulmonary aspergillosis subsequent to rechallenge; 10 of 10 SBW and 9 of 10 BBW developed granulomatous pulmonary lesions on the side of reexposure. A greater

proportion of control SBW turkeys developed pneumonia and airsacculitis following challenge as compared with the BBW breed. Lesions were limited to the lower respiratory tract in all turkeys and were confined to the ipsilateral lung and PTAS in the singly inoculated control turkeys. This study demonstrates that convalescence from pulmonary aspergillosis does not confer protection against rechallenge but may, instead, decrease resistance to subsequent infection.

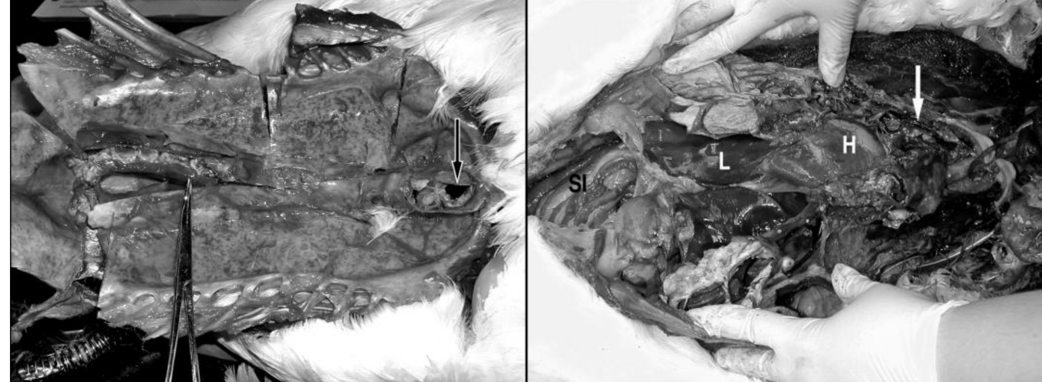
TÜRKÜTANIT (1999) examined pulmonary aspergillosis in a total of 22 geese (2-13 week-old), brought from A Geese Production Station in Kars and geese breeders. The lesions were seen as a hard nodules in all cases in the lungs, and also in 3 cases in the air sacs. They were multifocalty localized in each lobules, and 1-4 mm. in diameter and yellow in color. The lesions were characterized by granulomatous pneumonie and airsacculitis. The characteristic hyphae of Aspergillus spp. were demonstrated in granuloma in histopathological sections stained by periodic acid Schiff stain.

Ivey (2000) used **serologic assays and protein electrophoresis** to aid diagnosis of aspergillosis in several species of captive birds, but sensitivities of these tests have not been established in psittacine birds. In 7 psittacine birds with respiratory tract aspergillosis confirmed by cytologic or histopathologic analysis, 1 bird had a positive ***Aspergillus*** **antibody enzyme-linked immunosorbent assay (ELISA)** titer, and 3 birds had positive *Aspergillus* antigen ELISA titers. In 3 birds, plasma protein electrophoretograms showed moderately to markedly increased β-globulin concentrations. Six birds had moderately to markedly decreased plasma albumin to globulin ratios. On the basis of this information, the antibody and antigen ELISA tests used in this study do not appear to be highly sensitive screening tests for aspergillosis in psittacine birds. The changes in plasma protein electrophoretograms were the more consistent findings in birds with aspergillosis, but results could also be normal in affected birds.

Souza *et al.* (2000) reported **swans** with gross pathologic lesions consistent with **aspergillosis** (trumpeter swan, n 5 62; tundra swan, n 5 2). Mild fungal infections consisted of small, non-obstructive lesions in the air sacs, trachea, or lungs, with lesions isolated to 1 area. Severe fungal infections consisted of large, extensive infiltrating lesions in the respiratory tract, with 68% of lesions in more than 1 area (lungs, air sacs, or trachea) and many with complete fibrinous casts within air sacs or trachea. Male swans were twice as likely as females to have fungal lesions, although there was no difference in the ratio of males and females with mild or severe infections. Juvenile swans (,1 year of age) were twice as likely as adults and sub-adults to have fungal lesions and were also more likely to develop severe versus mild aspergillosis infections (n 5 19, n 5 1, respectively). Although the number of swans with concurrent lead poisoning and aspergillosis was nearly 30% higher than that of swans with aspergillosis only, when compared with the total number of swans submitted for necropsy, swans with lead poisoning were 75% less likely to have fungal lesions than non-lead–poisoned swans, possibly because of rapid death from lead poisoning.



A wild trumpeter swan that died with lesions consistent with aspergillosis. Small, gray-green, umbilicated fungal granulomas are located throughout the left caudal thoracic air sac (arrows). A trumpeter swan that died with lesions consistent with aspergillosis. The trachea is cut open, and a gray, nonobstructive fungal granuloma is located in the dorsal tracheal loop (see tip of the hemostat). **Souza *et al.* (2000)**



The dorsal surface of the keel in a trumpeter swan that died with lesions consistent with aspergillosis, showing both the caudal and dorsal loops of the trachea. A gray-green, fibrinous, obstructive fungal lesion fills the caudal loop of the trachea (portions of the keel and trachea have been cut away; see tip of hemostat). A smaller, gray fungal granuloma fills the dorsal loop of the trachea (trachea cut open; arrow). An adult trumpeter swan that died with lesions consistent with aspergillosis. Bilateral yellow, fibrinous casts fill the cranial and caudal thoracic air sacs (between both thumbs and fingers). Fungal lesions are also present in the intraclavicular air sac (arrow). The heart (H), liver (L), and small intestines (SI) are visible. **Souza *et al.* (2000)**

Ramisz *et al.* (2001) used 2 imidazol preparations--5 per cent Miconazole powder and 2 **Clotrimazole** solution in the treatment of two geese farms with a total number of 11.143 - 4 weeks old birds. Miconazole was applied as feed additive for 200 with aspergillosis infected geese, in a dosis of 10 mg of active substance on one kg of body weight. Clotrimazole was administered in a form of inhalation in a dose of 1,5 1 of 2 per cent solution per geese house of 3000 m3. Spraying was performed using gas-pipes of steam ganerator joined to the air compressor of the type 3 JW - 60 (6hp). In this way 5 - 10 microm partiches were obtained. The preparation was sprayed twice ad 2 - 4 days intervals. After Miconazole administration the recovery of sick birds and inhibition of the disease in geese were observed.

German *et al.* (2002) developed an indirect **ELISA** for the detection of ***Aspergillus fumigatus***-specific immunoglobulin in penguins. The results were calculated quantitatively as ELISA units, derived by polynomial regression analysis, and semi-quantitatively as end titres. Serum samples from 61 captive penguins were tested with

the assay, and the results were compared with those obtained by counterimmunoelectrophoresis (CIE). The ELISA results correlated with the CIE results only when end titres were reported (R(s) = -0.676, P < 0.002). Fifty-seven of the penguins (93 per cent) were seropositive, but the detection of immunoglobulin did not correlate with clinical disease. At Whipsnade Wild Animal Park, Humboldt's penguins (Spheniscus humboldti) demonstrated higher seropositivity than king penguins (Aptenodyptes patagonicapatagonicus) (P = 0.022), but Humboldt's penguins at Fota Wildlife Park had a significantly higher seropositivity than Humboldt's penguins at Whipsnade (P = 0.035).

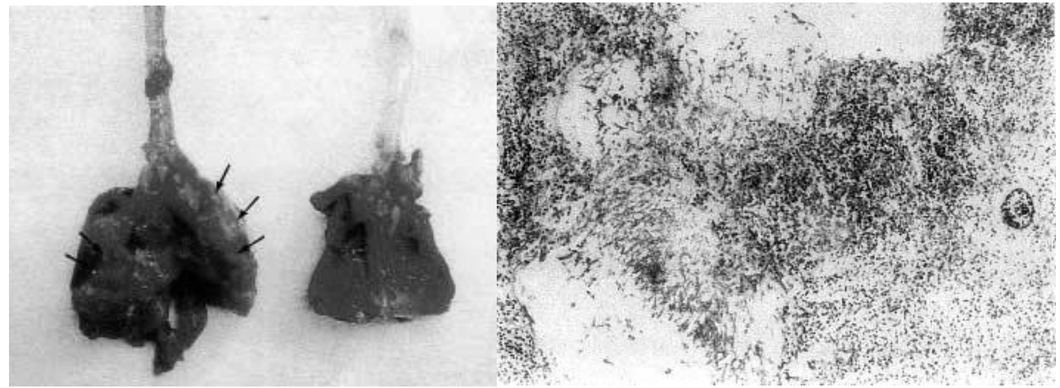
Akan *et al.* (2003) documented a case of **aspergillosis in a broiler breeder flock** having respiratory and nervous system problems caused by ***Aspergillus fumigatus*** and ***Aspergillus niger***. Dyspnea, hyperpnea, blindness, torticollis, lack of equilibrium, and stunting were observed clinically. On postmortem examination of the affected birds, white to yellow caseous nodules were observed on lungs, thoracic air sacs, eyes, and cerebellum. Histopathologic examination of lungs and cerebellum revealed classic granulomatous inflammation and cerebellar lesions, necrotic meningoencephalitis, respectively. No lesions were noted in the cerebrum histopathologically. Aspergillus hyphae were observed in stained sections prepared from lesioned organs. Fungal spores and branched septate hyphae were observed in direct microscopy. Aspergillus fumigatus and A. niger were isolated from the inoculations prepared from the suspensions of organs showing lesions.

Bhattacharya (2003) recorded an **outbreak** of ***A. fumigatus*** infection in **Khaki Campbell ducks** and **ducklings** in a farm in Tripura, India in 2002. Affected birds died within 24-72 h of onset of clinical signs, and 30.04% mortality was recorded in the ducklings. Postmortem examination showed gross lesions, and culture samples yielded mycelial growth within 24 h.

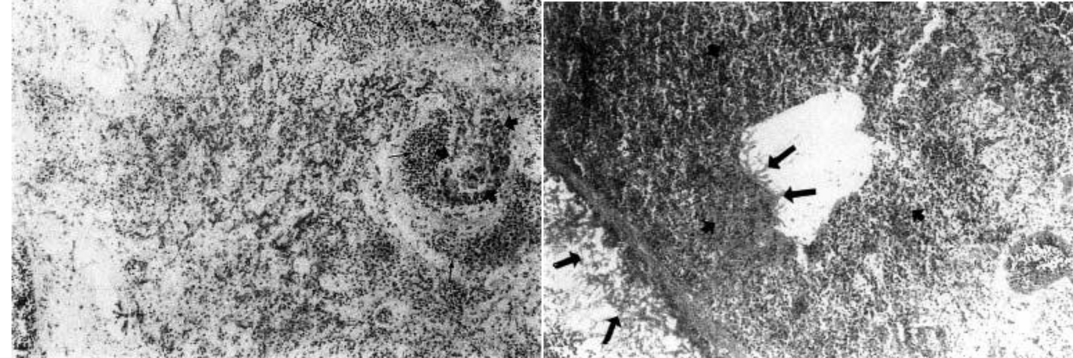
Lair-Fulleringe *et al.* (2003) genotyped 114 Aspergillus fumigatus isolates from sacrificed **turkeys** and 134 ***A. fumigatus*** isolates from air samples were collected and genotyped by microsatellite polymorphism marker analysis. Air sampling confirmed the huge diversity of A. fumigatus populations. Whereas older animals harbored several combinations of genotypes, 1-day-old chicks carried a unique genotype, suggesting a unique source of contamination.

Throne Steinlage *et al.* (2003) presented an 8-wk-old **layer cockerels** and **pullets** with a history of increased mortality, ruffled feathers, lameness, and recent vaccination. At necropsy, the birds had large multifocal granulomas in multiple tissues. Only light bacterial growth was seen on culture. On histopathology, a mixed population of fungi was seen within the granulomas including zygomycetes and **Aspergillus**, with the zygomycetes being the predominant organism. Because of the coinfection with Aspergillus and Penicillium, obtaining the zygomycetes in pure culture was unsuccessful. The source of the zygomycete fungi remains unknown; however, zygomycetes are known to be ubiquitous. Serology was performed to evaluate the flock's immune status. There was no evidence of immunosuppression caused by chicken anemia virus or bursal disease infections. No flock treatment was initiated.

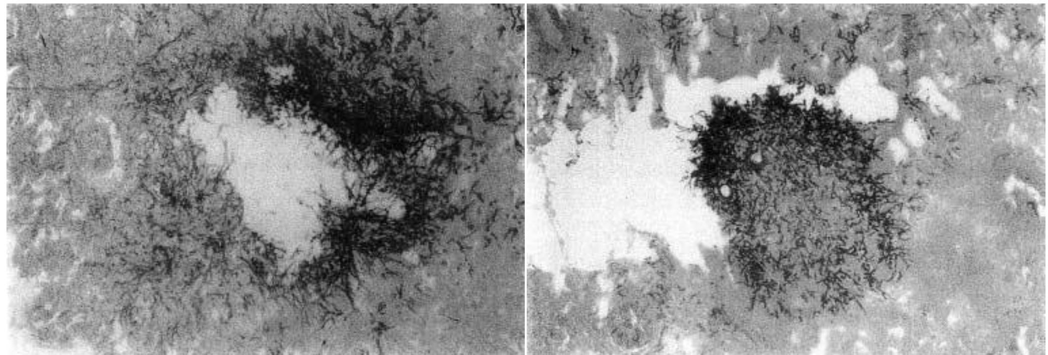
Atasever and Gümüşsoy (2004) described the pathological, clinical and mycological findings in **experimental aspergillosis** infections of 24 **wild starlings** (*Sturnus vulgaris*), in undefined age categories but at least post-pubertal. Six starlings were kept as a control group and 18 starlings served as the infection group. The starlings in the infection group were infected with inoculums of 1.35×10^6/0.2 ml ***Aspergillus fumigatus*** via intratracheal route whereas the control group was administered only placebo in the same way. Six, four, two, four and two birds died on 2, 3, 4, 5 and 6 days post inoculation respectively. At the necropsy of the dead birds, caseous foci were determined in the lungs, on the air sacks, myocardium, thoracic wall and abdominal serosa. In the histopathological examination of the white-yellowish caseous foci ranging from pinhead to chick pean in size, necrotic granulomatous foci consisting of macrophages, heterophil leukocytes and gigant cells encapsulated with a fibrous tissue were observed. Hyphae and spores of ***A. fumigatus*** were determined in these foci using the Gridley staining method.



Yellowish caseous foci in lung tissue on the left (arrows); the appearance of intact lung tissue on the right. Necrosis, inflammatory cells and fungal hyphae in tissue. Lung, **Atasever and Gümüşsoy (2004)**



Invasion of tissue with fungal hyphae, thrombosis (bold arrows) and haemorrhage (thin arrows) in blood vessels. Lung, Invasion of tissue with fungal hyphae originating from parabronchioles (thin arrows), inflammatory cell infiltration (bold arrows). Lung, H · E, 200. **Atasever and Gümüşsoy (2004)**



Fungal hyphae and conidiophores in tissue. Lung, Gridley Invasion of tissue with fungal hyphae and spores originating from the lumina of para-bronchioles. Lung, Gridley, **Atasever and Gümüşsoy (2004)**

.

Beytut *et al.* (2004) studied 90 **goslings** with **pulmonary and systemic aspergillosis** . The lungs and air sacs were the main sites affected by the disease, and were generally characterised by diffuse yellowish-white granulomas. In 7 cases with pulmonary and air-sac involvement the granulomas were scattered to the serosal linings of the gastrointestinal and upper respiratory tracts, to the liver, spleen and kidneys, and in two cases also to the bursa of Fabricius, musculus (m.) longus colli and adventitia of aorta. The granulomas were often characterised by a necrotic centre surrounded by heterophils, macrophages, lymphocyte and plasma cells, and in late granulomas by multinucleated foreign-body giant cells, and again by an outer thin fibrous capsule. Numerous fungal hyphae were found within the necrotic debris of the granulomas by Gridley and PAS staining techniques. Immunohistochemistry reliably confirmed aspergillosis in all of the cases. Fungal elements in the lungs of goslings severely affected by the disease stained heavily within the centre of the granulomas, whereas few antigens reacted in the chronic cases. Fungal fragments, which were not discernible using routine fungal stains, reacted clearly in the cytoplasm of macrophages and giant cells. Thus, although fungal elements within the granulomas were histologically indicative of aspergillosis, immunohistochemistry also had to be applied to obtain a definitive diagnosis of the disease and to differentiate it from many of the filamentous fungi.

Copetti *et al.* (2004) reported a **pulmonary aspergillosis outbreak** that occurred in **great rhea** (Rhea americana) in the south of Brazil. About 50 birds aged 30 to 60 days died suddenly and one of them was submitted to autopsy which revealed the presence of white caseous nodules 0.5 mm in diameter occupying 95% of the lung area. One lung was sent to the Federal University of Santa Maria for histopathological and mycological analyses. Histopathological analysis revealed multifocal areas with necrosis and inflammatory infiltrates and the presence of fungal hyphae, giant cells and fibrous tissue proliferation at the periphery. Aspergillus fumigatus was recovered as pure culture from all culture media. This appears to be the first report of aspergillosis among great rhea in Brazil and the second in the world.

Ozmen and Dorrestein (2004) evaluated different staining methods for studying aspergillosis of the central nervous system (CNS). **The pathological changes** and fungal elements in cerebrum and cerebellum of 17 turkey poults with aspergillosis were examined and described. Tissue sections were stained with hematoxylin-eosin (HE), Kluver-Barrera's and Grocott's methods, and periodic acid-

Schiff (PAS). Focal granulomatous reactions with central necrosis were observed in the HE stained slides. Fungal hyphae were easily demonstrated using Grocott's method and PAS. These two methods, however, were not suitable for describing detailed histopathological changes. The Kluver-Barrera method was used to demonstrate the neural tissue reaction. Neurons were found to be sensitive to aspergillosis, in contrast to glial cells that showed fewer pathological changes. The fungal elements were clearly visible with the Kluver-Barrera method, resulting in better information about the interactions of neural tissue, the inflammatory response, and the fungus. The use of the Kluver-Barrera method for this purpose has not been documented previously.

Yokota *et al.* (2004) reported an 11-month-old female **ostrich** (Struthio camelus) that had become gradually emaciated over a 2-week period and subsequently died. Necropsy revealed white to green mold growth on the walls of caseous thickened air sac membranes and multiple white necrotic foci in the lungs and liver. Histologically, the multiple exudative, necrotic and granulomatous lesions were compatible with mycotic infection in the air sacs and lungs, and hyphae positively reacted with a monoclonal antibody (Mab-WF-AF-1) to ***Aspergillus fumigatus*** wall fractions. Multifocal hepatic necrosis was also found, and several spores were observed in the blood vessels. Fungal culture of these lesions yielded pure growth of A. fumigatus. This is an established case of fatal A. fumigatus infection in an ostrich reared in Japan.

Balseiro *et al.* (2005) examined 2,465 **seabirds, mainly common murres** (Uria aalge), **razorbills** (Alca torda), and **puffins** (Fratercula arctica) that beached in the northwestern part of Spain after the "Prestige" oil spill in 2002 by pathological methods. Birds were divided into three groups: dead birds with the body covered (group 1) or uncovered (group 2) by oil and birds recovered alive but which died after being treated at a rescue center (group 3). The main gross lesions were severe dehydration and emaciation. Microscopically, hemosiderin deposits, related to cachexia and/or hemolytic anemia, were observed in those birds harboring oil in the intestine. Severe aspergillosis and ulcers in the ventriculus were found only in group 3 birds, probably because of stress associated with attempted rehabilitation at the rescue center. The mild character of the pathological changes suggests that petroleum oil toxicosis causes multiple sublethal changes that have an effect on the ability of the birds to survive at sea, especially weak and young, inexperienced animals. Dehydration and exhaustion seem to be the most likely cause of death.

Cortes *et al.* (2005) diagnosed omphalitis associated with aspergillosis in four cases of commercial **turkey poults** ranging in age from 3 to 9 days old. In two cases, the mycotic agent present in the yolk sac was isolated and identified as Aspergillus fumigatus. In the other two cases, the fungi were identified as Aspergillus sp. on the basis of morphologic characteristics of the fungi in tissue sections. The fungi present were further confirmed to be of the genus Aspergillus by immunohistochemistry. Omphalitis by ***A. fumigatus*** infection has not been documented before.

Low *et al.* (2005) reported a 3-year-old female **North Island robin** (Petroica longipes), which was found dead on Tiritiri Matangi Island during the breeding season. The bird was in poor condition, and there was a 13 x 8 mm granulomatous mass in the thoracic cavity causing displacement of the heart and left lung. Histologically, the mass was a large granuloma infiltrated with fungal hyphae, and the

liver contained multifocal aggregates of inflammatory cells. The case was diagnosed as thoracic aspergillosis and multifocal hepatitis.

Arca-Ruibal *et al.* (2006) tested a commercial sandwich **ELISA** (Platelia Aspergillus EIA; Bio-Rad) developed for the detection of galactomannan, a major cell wall constituent of Aspergillus species for its efficacy in the diagnosis of aspergillosis in falcons. Ninety serum samples from 50 aspergillosis-positive falcons and 182 samples from 142 aspergillosis-negative falcons were tested. The sensitivity of the test was only 12 per cent and its specificity was 95 percent. The test was therefore unsatisfactory for detecting galactomannan in the serum samples and cannot be used as a screening test for aspergillosis in **falcons**.

Mukaratirwa (2006) diagnosed disseminated zygomycosis and concomitant **pulmonary aspergillosis** in breeder layer cockerels. Five- to 9-week-old breeder layer cockerels with a history of an increased mortality rate were presented to a diagnostic laboratory for examination. On necropsy, large, multifocal, firm, tan, nodules were observed in the lungs, air sacs, peritoneum, livers, spleens and kidneys. On histopathology, mixed populations of zygomycetes and Aspergillus hyphae were observed in the granulomas in the lungs, and zygomycete hyphae were observed in the granulomas in the air sacs, peritoneum, livers, spleens and kidneys. No bacteria were isolated from any of the lesions. Aspergillus fumigatus was isolated from the lung lesions only and hyphae that were consistent with those of a Rhizopus spp. were isolated from the lesions in several organs. Pullets, which were kept together with the cockerels from the day they were hatched, were not affected. The absence of infection in the pullets, which were kept together with the cockerels, suggests that the cockerels were either infected during incubation, with the fungi penetrating the egg shell, or that they were infected during hatching before they were mixed with the pullets.

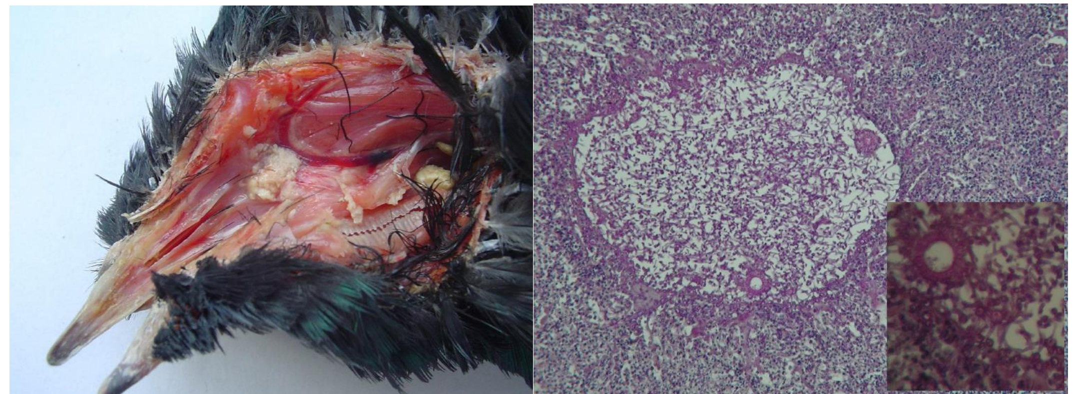
Tell *et al.* (2006) described two studies using **mallard ducks** (Anas platyrhynchos). The first study evaluated in vivo release of ITZ from **subcutaneously injected** controlled-release gel formulations and the second study compared pharmacokinetic parameters for two ITZ oral suspensions. ITZ-A suspension was prepared by mixing contents of commercially available capsules with hydrochloric acid and orange juice. ITZ-B suspension was prepared by dispersing the complex of the drug with hydroxypropyl-beta-cyclodextrin in water. Concentrations of ITZ and its active metabolite, hydroxyitraconazole (OH-ITZ), in plasma and tissue samples were measured using high-performance liquid chromatography. In the second study, drug concentrations in plasma samples were also analyzed using a bioassay. After administration of two ITZ controlled-release formulations, plasma and tissue concentrations of ITZ and OH-ITZ were either very low (< or = 52 ng/mL) or undetectable. Exceptions included skin, subcutaneous fat, and muscle adjacent to the injection site. The drug from ITZ-A and ITZ-B suspensions was absorbed after oral administration. ITZ pharmacokinetic parameters for both suspensions in mallard ducks were similar and the bioassay successfully measured ITZ equivalents in plasma samples from ducks.

Femenia *et al.* (2007) induced **experimental aspergillosis** in 1-day-old **turkeys** by intra-air-sac inoculation of a spore suspension of a 3-day-old ***Aspergillus fumigatus*** culture (CBS 144.89) containing 10(7) spores. Ten additional poults were used as controls. Infected and non-infected animals were closely observed at least twice a day for the appearance of clinical signs and were sequentially sacrificed at days 1, 2, 3, 5 and 7 post-inoculation. In the infected group, most lung tissues and air sac swabs were

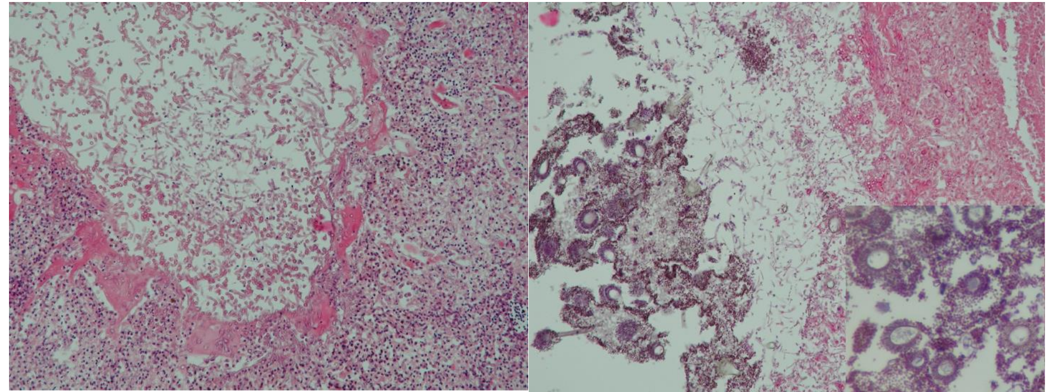
culture positive from day 1 to day 5. At 1 day post-inoculation, air sac membranes were multifocally and moderately to severely thickened by an oedema and covered by an exudate. A small number of germinating conidia were present in the superficial exudate, already giving rise to small radiating hyphae. Lung lesions were mild, dominated by a diffuse congestion and a mild heterophilic infiltration. From 2 to 3 days post-inoculation, air sac membranes were more severely affected and several granulomas were observed. Both granulomas and exudates were rich in germinated conidia and hyphae. Pulmonary lesions consisted in a diffuse pneumonia. Five days post-inoculation, air sac membrane lesions progressed to a severe, multifocal, heterophilic and granulomatous inflammation. Seven days post-inoculation, a reduction of the severity of the diffuse pneumonia was detected. Concomitantly, the fungal elements were mainly observed as fragmented tubules in the cytoplasm of multinucleate giant cells. The present study demonstrated that healthy turkey poults might be able to withstand exposure to 10(7) A. fumigatus spores.

Martin *et al.* (2007) reported increased morbidity and mortality in a 5-wk-old **broiler breeder replacement pullet flock**. The affected broiler pullet flock was housed on the first floor of a two-story confinement building. Mortality increased to 0.1%/day compared to the flock on the second floor, which had mortality levels of less than 0.01%/day. Clinical signs in the affected chickens included inactivity, decreased response to stimuli, and anorexia. No respiratory or neurologic signs were observed. On necropsy, affected pullets were dehydrated and emaciated and had disseminated variably sized single or multiple heterophilic granulomas that contained intralesional septate and branching fungal hyphae. Lesions were extensive around the base of the heart in the thoracic inlet and in the kidneys. Other affected organs included eyelid, muscle, proventriculus, ventriculus, intestine, liver, spleen, lung, and heart. ***Aspergillus flavus*** was cultured from the visceral granulomas. The source of flock exposure to the organism was not determined.

Tokarzewski *et al.* (2007) described an **aspergillosis outbreak** in a flock of near 350 **pigeons** clinically, microbiologically, and histopathologically. The pigeons showed dyspnoea, depression, rattling, and dyskinesia, and numerous cases of death were noted. Five young moribund pigeons, their bedding, and fodder were examined. The examinations were conducted according to the generally accepted methodologies and recommendations for mycological diagnostics. Paraffin sections of the lungs, trachea, pharynx, and thoracic air sacs were stained with haematoxylin and eosin and periodic acidSchiff method. The mycological examinations demonstrated the presence of Aspergillus fumigatus cells in swabs from the beak cavity of living birds, and in the lungs and air sacks examined post mortem. The presence of Candida albicans and single isolates of Penicillium sp. and Scopulariopsis sp. were also detected in the beak cavity. The mycological examinations of bedding (coniferous shavings) showed its evident contamination. The dominant presence of A. fumigatus and some colonies of Mucor sp., Acremonium sp., and Trichoderma sp. were recorded. The feed supplied, regardless of its kind, did not contain any ***A. fumigatus*** cells. Macroscopically, white-yellowish nodules observed in the lung and air sacs corresponded to acute aspergillosis lesions. Histopathological analysis of the affected organs displayed multifocal areas of necrosis, inflammatory infiltration, and the presence of fungal hyphae, giant cells, and fibrous tissue proliferation at the periphery of the nodules were noted.



White-yellowish caseous mass in beak cavity. The fungal colony in the lung. PAS staining, 100x.
Tokarzewski *et al.* (2007)



Branching septate hyphae of *A. fumigatus* in the lung. Inflammatory reaction at the periphery. HE, 200x. Hyphae, conidiophores, and many conidia in the lung. HE, 200x. **Tokarzewski *et al.* (2007)**

Xavier *et al.* (2007) described the epidemiology, macroscopic and histological lesions as well as the isolation of *Aspergillus flavus* and *A. fumigatus* from Magellanic penguins (*Spheniscus magellanicus*) during recovery in the Center for Recovery of Marine Animals (CRAM - 32ºS/52ºW), over a period of two years. From January 2004 to December 2005 the Center received 52 Magellanic penguins, and 23% (12/52) died. Necropsies were performed and tissue samples were collected for histological and microbiological examination. From 12 dead animals, aspergillosis was confirmed in five animals, corresponding to 42% of the mortality. Granulomatous nodules were observed mainly on air sacs and lungs. Histologically, septate and branching hyphae, measuring 3-5 µm and PAS positive were found. Two of these cases were caused by *A. fumigatus*, two other by *A. flavus*, and in one the diagnostic was established by macroscopic lesions observed in the necropsy without sample collection for fungal isolation and identification. The five aspergillosis cases occurred in the first year of the study, when a disinfection program was not yet established in the CRAM. This paper points out the importance of aspergillosis in the rehabilitation process of captive penguins, and emphasize the necessity of an environmental disinfection on the aspergillosis prevention, mycosis that caused a high rate of mortality of the seabirds found on the Brazilian coast and admitted in the CRAM.

Abou-Rawash *et al.* (2008) reported on disseminated aspergillosis in a **Whooper Swan** (Cygnus Cygnus) in Japan. A Whooper Swan which usually migrates from Siberia to the north of Japan in the winter season was found in Kume Island at the south of Japan in a state of pronounced illness. Blood and serum analysis revealed

hypoalbuminaemia elevated serum level of uric acid (UA) and creatinin phosphokinase (CPK). The bird succumbed after unsuccessful attempts for treatment. At necropsy, the bird had multiple focal and coalescent caseated granulomatous nodules of whitish greenish color on the thoracic and abdominal air sacs, the lungs, and the serosal surfaces of the spleen, liver and kidneys. At the inner side of the air sacs hyphal growth with dark greenish color were scattered on most of the surface. Histopathologically, the present case had a sever degree of chronic disseminated aspergillosis. The granulomas had a central necrotic areas consisted of necrotic cell debris and hyphae with the microscopical features of Aspergillus. ***A. fimigatus*** was identified in tissues by PAS, GMS, and immunohistochemically. PCR was very successful to identify the causative fungus like other infectious agent.

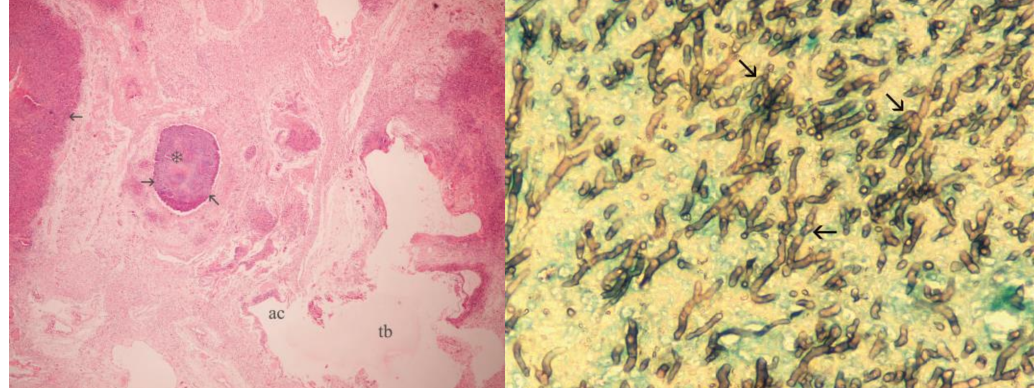
Beernaert *et al.* (2008) examined the impact of the use of different **inoculation routes** and immunosuppression on the course of an infection with Aspergillus fumigatus in racing pigeons (Columba livia domestica). ***A. fumigatus*** conidia were inoculated in the thoracic air sac, lung or trachea in immunocompetent or immunosuppressed pigeon squabs. Immunosuppression was induced by three dexamethasone injections before inoculation. Mortality in the A. fumigatus-inoculated groups varied between 1/4 and 4/4. The highest and more acute mortality was seen in immunocompetent pigeons inoculated in the thoracic air sac and in pigeons inoculated in the thoracic air sac or lung after immunosuppression. Pigeons inoculated in the lung or inoculated intratracheally after immunosuppression developed an aspergillosis infection with a slower course of disease and more prominent clinical symptoms. Using microsatellite length polymorphism, it was confirmed that all mycoses were caused by the inoculated strain except for one isolate in a dexamethasone-treated pigeon. In conclusion, inoculation in the lung is selected as the preferred model for chronic aspergillosis in pigeons, and inoculation in the thoracic air sac as the preferred model for acute aspergillosis. The use of immunosuppressed birds seems to be contra-indicated due to the risk of opportunistic infections.

Khosravi *et al.* (2008) described clinical, mycological and histopathological findings in **black neck ostriches** affected with severe aspergillosis in a flock including 80 birds, near Tehran, Iran. The signs included anorexia, depression, notable weight loss, diarrhoea, severe respiratory distress and death. Grossly, the lungs showed numerous white to yellow caseous nodules and the walls of the thoracic and abdominal air sacs were thickened with inflammatory exudates containing cellular debris, necrotic masses and green mold colonies. Multiple nodules were observed in the liver, spleen and gastrointestinal tract as well. Histopathologically, there were conidial heads and fungal hyphae in the air sacs and multifocal necrotic and granulomatous lesions with septated and dichotomously branched hyphae in various tissues, which were stained with haematoxylin and eosin and Grocott's methenamine silver nitrate. ***Aspergillus fumigatus*** was isolated in various tissues taken from affected ostriches.

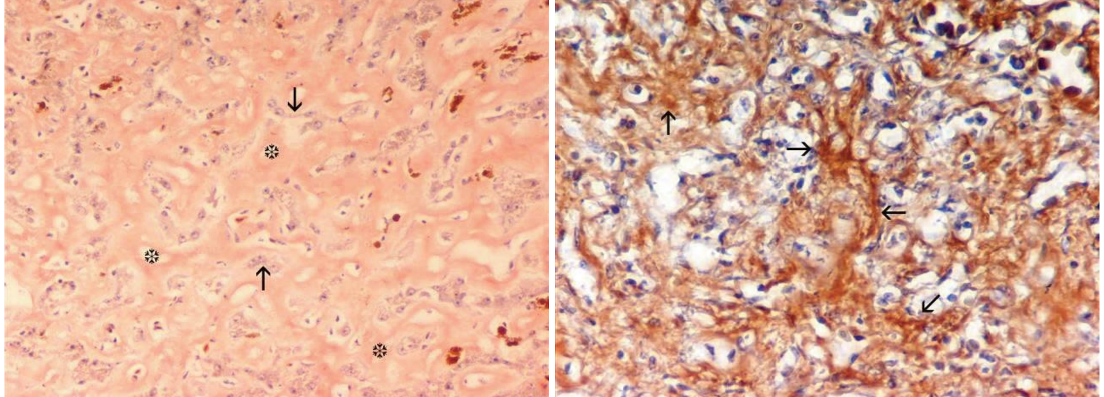
Zafra *et al.* (2008) presented 2 flocks of broiler chickens aged 15 to 30 days with respiratory signs such as dyspnea and up to 25% mortality. These were the only two flocks in the farm where a bed of sunflower shells was used instead of the rice-hull bedding used in other flocks. At necropsy, severe ascites, right heart hypertrophy, pulmonary congestion, and extensive multifocal granulomatous pneumonia were recorded. Histopathologic examination revealed chronic multifocal mycotic granulomatous pneumonia. Aspergillus fumigatus was identified by microbiologic study from pulmonary specimens. After disinfecting the floor and changing the

bedding, no clinical signs were recorded in the farm. Severe chronic granulomatous pneumonia caused by A. fumigatus in the chickens of the present study may have caused hypoxia, leading to pulmonary hypertension, heart failure, and ascites.

Akkoc *et al.* (2009) described fatal pulmonary aspergillosis and AA type amyloid accumulations in the liver and spleen in a female **ostrich** (Struthio camelus). The animal had had respiratory problems and long-term inappetence over an 8-week period. Necropsy revealed that several soft, grayish to white nodules ranging from 1 to 3 mm in diameter were scattered throughout the lungs and thoracic air sacs. Prominent enlargement of the liver and spleen was observed. No gross lesions were found in the other organs studied. The microscopic examination showed severe, necrotizing, granulomatous pneumonia, and air sacculitis. ***Aspergillus fumigatus*** was recovered from the lungs and air sacs as pure culture colonies. Amyloid deposition was demonstrated in the liver and spleen slides by Congo red and immunohistochemistry. To the authors´ knowledge, to date no case of amyloid accumulation in ostrich has been reported. We report, for the first time, diffuse AA amyloidosis in the liver and spleen of an ostrich, probably occurring secondary to aspergillosis.



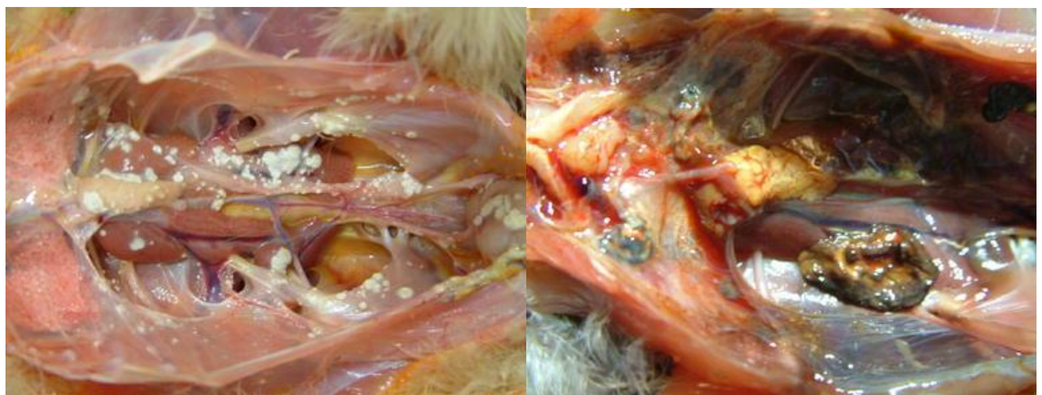
Lung, areas of caseous necrosis (snow flake) and cellular debris (arrows), HE, original magnification □40.Lung, fungal hyphae arranged in a radial pattern (arrows) GMS, original magnification **Akkoc *et al.* (2009)**.



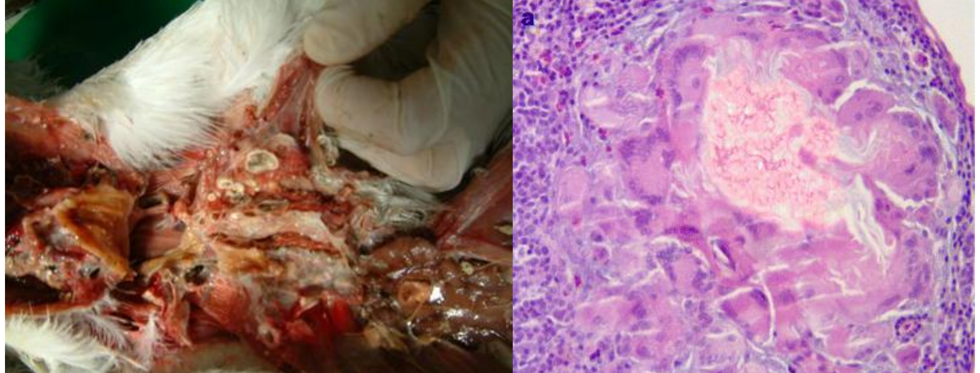
Liver, orange amyloid depositions (snow flakes), atrophic and degenerated hepatocytes (arrows), Congo red, original magnification □400. Spleen, positive staining for AA amyloid (arrows), streptavidin-biotin-peroxidase complex method, DAB with hematoxylin counterstain, original magnification □400 **Akkoc *et al.* (2009)**

Cacciuttolo *et al.* (2009) described anatomopathological aspects resulting from a chronic infection from **Aspergillus spp** in the **chicken** (Gallus domesticus), in the herring gull (Larus cachinnans micaelli) and in **the red-legged partridge** (Alectoris

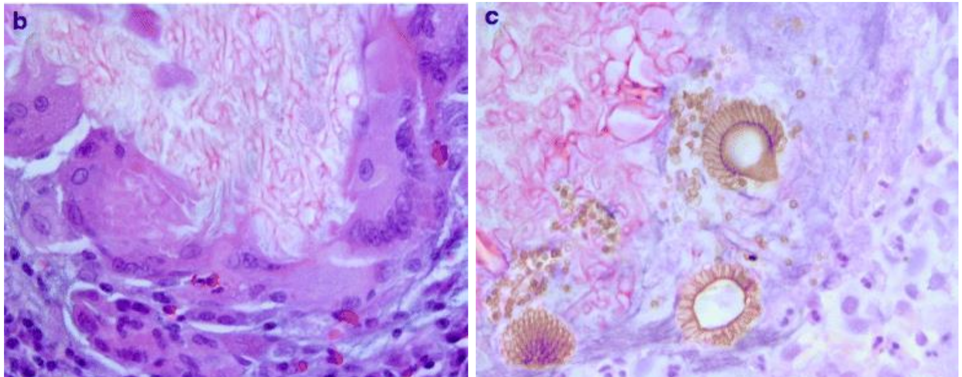
rufa rufa). Microscopically. some histological lesions that are related to the two typical forms of Aspergillosis: a deep nodular form, typical of organs with a non-aerated parenchyma, and a non-encapsulated superficial diffuse form typical of the serosae and the lung were observed. The observed forms of aspergillosis have been found in animals raised in poor hygienic environmental conditions or malnourished animals (chicken); in wild birds from wildlife recovery centres (herring gull), which underwent some forms of stress, such as traumas, detention, starvation, extended antibiotic treatments; in game birds (red-legged partridge) used for restocking natural areas that had been negatively affected by such stressors as captivity in aviaries, containment and transport in cages, release in unsuitable environments and malnutrition. The observed anatomopathological and istopathological aspects can therefore be regarded as the outcome of a number of factors that have reduced the typical resistance of the species and impaired the efficiency of their immune systems.



Cockerels: diffuse lesions in the air sacs and on parietal and visceral serosae, with foci in varying size protruding from the surface of the affected organ, white in colour and dry in texture Red-legged partridge: brownish patches, compact in appearance, with a clear-cut cross section and a necrotic-caseous texture on the surface of the parietal serosae and the pulmonary parenchyma**. Cacciuttolo *et al.* (2009)**



Herring gulls: lesions in the lungs and air sacs covered in a whitish caseous material, with grey-greenish mould on top, suggesting fungal sporulation, a) Histological lesions with necrotic-caseous material surrounded by giant cells, macrophages, heterophiles and lymphocytes (40X);**Cacciuttolo *et al.* (2009)**



b) Fungal hyphas with or without septi are clearly visible inside, using the Periodical Acid Shift dye (PAS) (60X); c) Non-encapsulated diffuse pulmonary lesions, with a massive development of the vegetative forms containing sporangia with a typical morphology (60X), **Cacciuttolo *et al.* (2009)**

Cray *et al.* (2009a) compared **galactomannan results** from plasma samples between birds with histologically confirmed aspergillosis and those that were clinically normal presumptively non-Aspergillus infected birds per submitting practitioners' responses to a questionnaire. It was observed that infected birds demonstrated a 2.6-fold increase in galactomannan over birds without evidence of aspergillosis. With the use of a galactomannan index of 0.5 as a cutoff, the sensitivity and specificity of the test were found to be 67% and 73%, respectively. In addition, plasma samples were analyzed for abnormalities in protein electrophoretic patterns. Infected birds had a higher incidence of increased beta and/or gamma globulin concentrations. Test sensitivity and specificity were 73% and 70%, respectively. If the 2 tests were used as a panel, then the sensitivity was 89% and specificity was 48%. These data indicate that both **galactomannan and protein electrophoresis** may be valuable tools in the diagnosis of avian aspergillosis.

Cray *et al.* (2009b) conducted a multiyear study using an enzyme-linked immunosorbent assay to measure antibody to address the application of the test to the diagnosis of aspergillosis in avian species. In general serostudies (*n* = 1314), four avian groups (psittaciform, raptor, penguin, and zoo) were found to have samples with antibody reactivity. Penguin, raptor, and zoo groups were found to have higher levels of antibody to *Aspergillus* than the psittaciform group. Additional clinical information was collected on 303 cases, which resulted in the definition of presumptive normal, probable, and confirmed infection groups. Although the confirmed group was more likely to have antibody reactivity, the mean antibody index was not found to be significant between presumptive normal and probable or confirmed cases.

Islam *et al.* (2009) investigated pneumomycosis (Aspergillosis, Mycotic pneumonia, Brooder pneumonia) in commercial chickens (Broiler, layer, cockerel) around Hajee Mohammad Danesh Science and Technology University at Dinajpur of Bangladesh from 2007 to 2008 and diagnosed based on pathological and therapeutical findings. The disease was commonly found in commercial poultry farms and caused moderate to severe economic loss to the small scale poultry farmers by a remarkable mortality of the birds and their reduced weight gain. Among the 11 incidences in the commercial chickens, 6 in broiler, 3 in layer and 2 in cockerel flocks were detected during the course of the study. The morbidity and mortality rates were not more than 70% and 9.03%, respectively. Highest mortality rate was found in cockerel (9.03%) followed by broiler (5.48%) and layer (1.92%), respectively. The major clinical signs

were varying degrees of dyspnea, gasping, whitish watery diarrhoea, progressive emaciation, remarkable dehydration and death. Circumscribed granulomatous nodules in the lungs, airsacs, and peritoneal cavity were the striking gross morbid lesions. The lungs were histopathologically characterized as severe necrosis of alveolar epithelia, purple coloured granular mass centrally of the nodules surrounded by zone of inflammation, mononuclear cells infiltration, highly congested blood vessels. Better response to sulphadiazine-trimethoprim combination along with copper sulphate solution was observed.

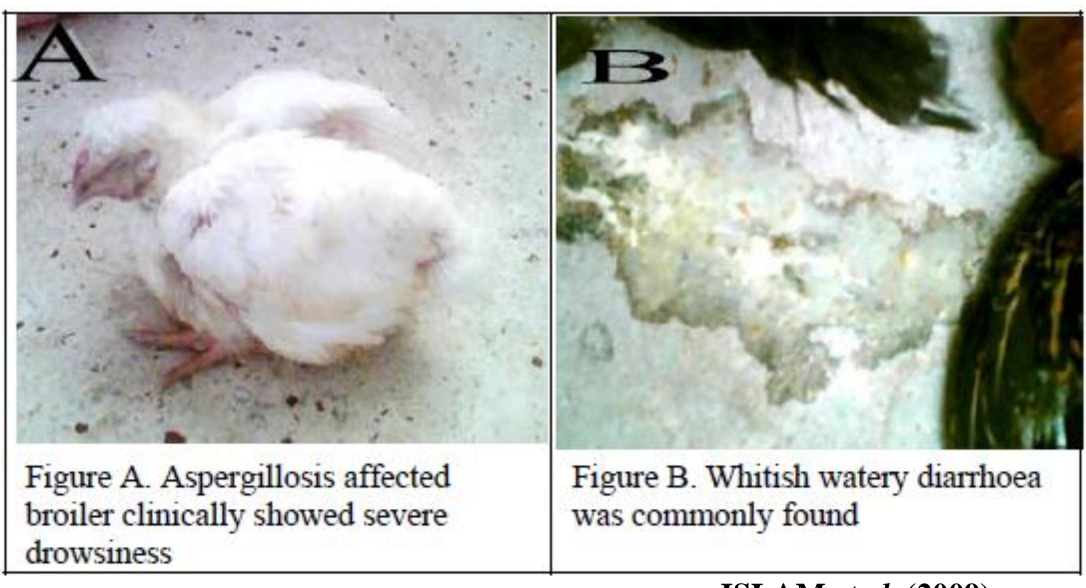
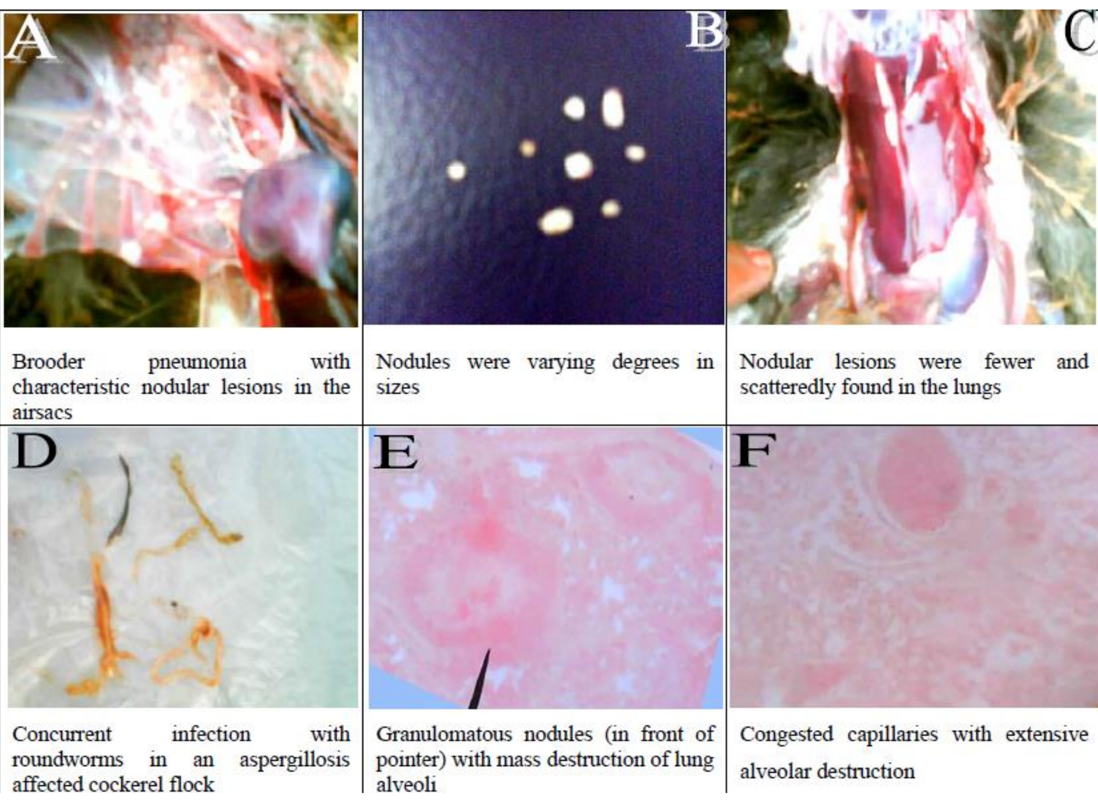


Figure A. Aspergillosis affected broiler clinically showed severe drowsiness

Figure B. Whitish watery diarrhoea was commonly found

Different characteristic clinical features of the aspergillosis affected birds, **ISLAM *et al.* (2009)**



A: Brooder pneumonia with characteristic nodular lesions in the airsacs

B: Nodules were varying degrees in sizes

C: Nodular lesions were fewer and scatteredly found in the lungs

D: Concurrent infection with roundworms in an aspergillosis affected cockerel flock

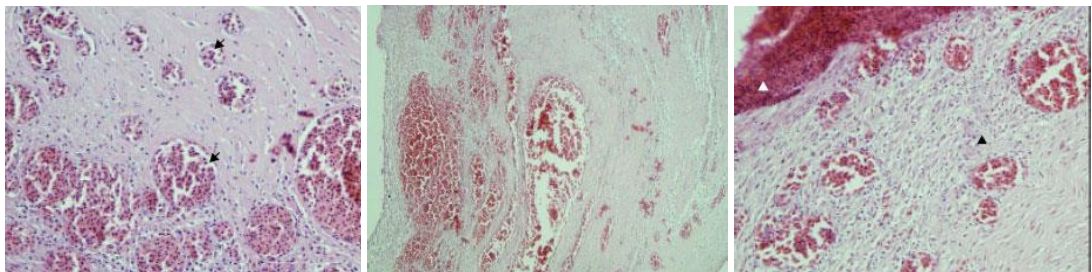
E: Granulomatous nodules (in front of pointer) with mass destruction of lung alveoli

F: Congested capillaries with extensive alveolar destruction

Different characteristic postmortem features of the aspergillosis affected birds **ISLAM *et al.* (2009)**

Jung *et al.* (2009) reported 2 Eurasian black vultures (Aegypius monachus Linnaeus) which were found dead or clinically ill from carbofuran insecticide during the winter of 2004. Carbofuran was detected in the stomach contents by gas chromatograph-mass spectrometry. Gross lesions showed severe granulomatous pneumonia and serofibrinous pleuropneumonia in both birds, with most lesions restricted to the pulmonary system. Histological lesions included pyogranulomatous pneumonia and suppurative parabronchiolitis/pleuritis/air sacculitis with a number of septated fungal hyphae, suggesting severe pulmonary aspergillosis. Fungal isolates from each vulture were identified as Aspergillus fumigatus by both lactophenol cotton blue staining and genetic analysis. This is the first report of pulmonary aspergillosis caused by A. fumigatus in wild Eurasian black vultures and suggests that Aspergillus infection could be an important cause of death in these birds which migrate from Mongolia to Korea during the winter. The incidence of the disease may be related to impaired immunity caused directly or indirectly by carbofuran poisoning.

Shathele *et al.* (2009) carried out a laboratory study to investigate fatal aspergillosis in an ostrich (*Struthio camelus*) predisposed by pulmonary haemangioma in the Kingdom of Saudi Arabia. The examination of Post Mortem (PM) revealed numerous ulcerated (1x0.5 cm) subcutaneous opaque thick masses with turbid materials (exudates) in the cut section together with fibrosis in between air sacs and the thoracic wall. The microscopic appearance indicated the presence of capillary type haemangioma in ostrich. The proliferating cells were highly differentiated, uniform with spindle-shaped nuclei resembling normal vascular endothelia and were arranged in the form of numerous capillaries distended with large amounts of blood erythrocytes and separated by fibrous stroma. However, large vascular spaces lined by a single layer of endothelium were also observed. The superficial parts of the tumour showed hyperkeratosis of the epidermis and diffuse infiltration of lymphocytes in the interstitial areas. In addition, the fibrous stroma was more abundant and dense with more prominent collagen in the peripheral parts of the tumour. On PM, the tiny yellowish white foci were detected on the lung's specimens and yielded ***A. fumigatus*** in pure culture. The histopathologic examination of the lesions showed fungal hyphae, inflammatory and multinucleate giant cells

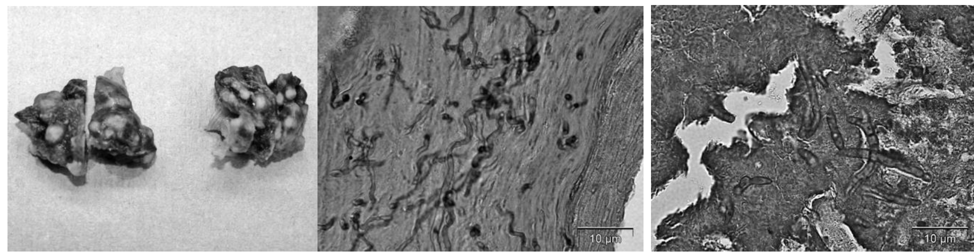


Lung numerous capillaries distended with large amounts of blood erythrocytes and separated by fibrous stroma. Notice the highly differentiated endothelial-like cells with spindle-shaped nuclei (black arrow head). H and E. Regular-shaped but variable in size capillaries. H and E. x ..Lung hyperkeratosis of the epidermis (white arrow head) and diffuse infiltration of lymphocytes (black arrow head) in the interstitial areas. H and E. x **Shathele *et al.* (2009)**

Singh *et al.* (2009) recorded ***Aspergillus fumigatus*** infection in **turkey farm** of 120 brooding poults. Grossly, air sacs were slightly opaque with few scattered miliary white foci. Lungs were moderately firm and had pin point to pin head sized yellowish white caseous nodular growth throughout the lung parenchyma. Histopathologically,

lung granuloma showed necrotic cellular debris, heterophils and long septated fungal hyphae in the center surrounded by macrophages, multinucleated giant cells and aggregates of lymphocytes. Gomori's Methanamine Silver Nitrate staining revealed black coloured fungal hyphae

Stoute *et al.* (2009) reported 5-week-old broad-breasted white commercial meat **turkeys** with the colonization of footpad epidermis and subcutis by fungal hyphae in commercial turkey species. No fungal cultures were undertaken at the time of the necropsy; therefore, paraffin-embedded tissue sections of lung and footpads were used to extract, amplify, and sequence mycotic DNA. The fungi identified from lungs were Aspergillus species, most closely matching ***Aspergillus flavus***. Mycotic pododermatitis in avian species is considered a rare pathologic finding, and few documented reports are available. The on-farm prevalence of footpad lesions was estimated at 3%, and there was no associated increase in the incidence of lameness or weight depression in affected birds. Microscopically, a granulomatous inflammatory reaction associated with fungal hyphae was observed in lung parenchyma. Disruption of keratinized epidermis, encrustations, and acute inflammation were also noted in footpads invaded with fungal hyphae.



Cut sections showing an extensive infiltration of pale, circular nodules throughout the lung parenchyma (formalin-fixed specimens). Periodic acid-Schiff-stained section of affected foot pad showing hyaline fungal hyphae within the dermis. Oval structures suggestive of yeast (arrow). Bar = 10 µm. Periodic acid-Schiff-stained section of lung revealing the morphology of the hyaline fungal hyphae. Bar = 10 µm. **Stoute *et al.* (2009)**

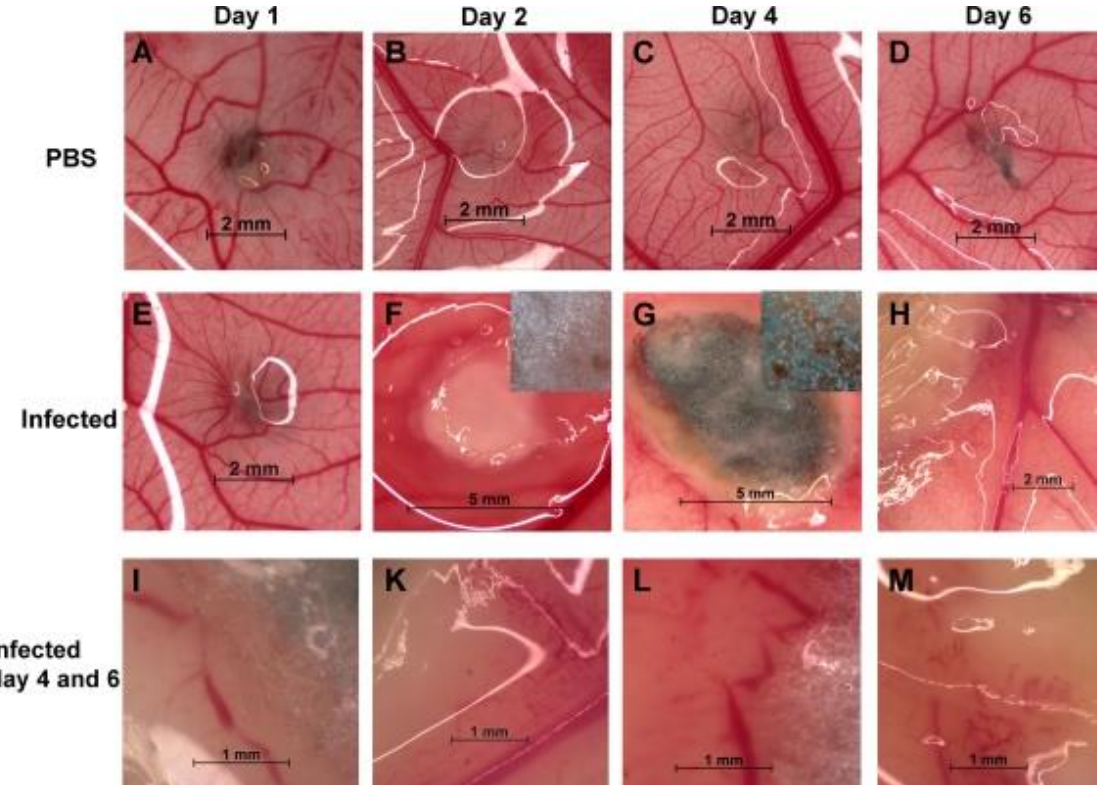
ARAGHI *et al.* (2010) discussed some **outbreaks of Aspergillus infection in ostrich** farms of eastern regions of Iran during 2010-2012. Signs of respiratory involvement, anorexia, depression, progressive emaciation and decreased production were the most commonly reported in affected farms. Morbidity rate was 43% and 54.53% in breeding birds and chickens, respectively. Mortality rate was 31.89% in breeding birds and 44.69% in chickens. Necropsy findings were suggestive of fungal infections in respiratory and alimentary tracts. ***Aspergillus fumigatus*** and ***Aspergillus niger*** were identified in microbiological and pathological examinations. Management reforms and using some supportive treatments were beneficial for controlling the disease.

Alvarez-Perez *et al.* (2010) studied the possible coexistence of different ***A. fumigatus*** genotypes in five clinical cases of invasive aspergillosis in captive penguins. Differences in other relevant characteristics of the isolates, including mating type and invasiveness, were also considered. Alkaline protease and elastase production by the A. fumigatus isolates was evaluated by plate assays. Random amplified polymorphic **DNA, and microsatellite analysis techniques** were used for molecular typing, and mating type (MAT1-1 or MAT1-2) was determined by multiplex PCR. Although all isolates showed protease activity, differences in elastase

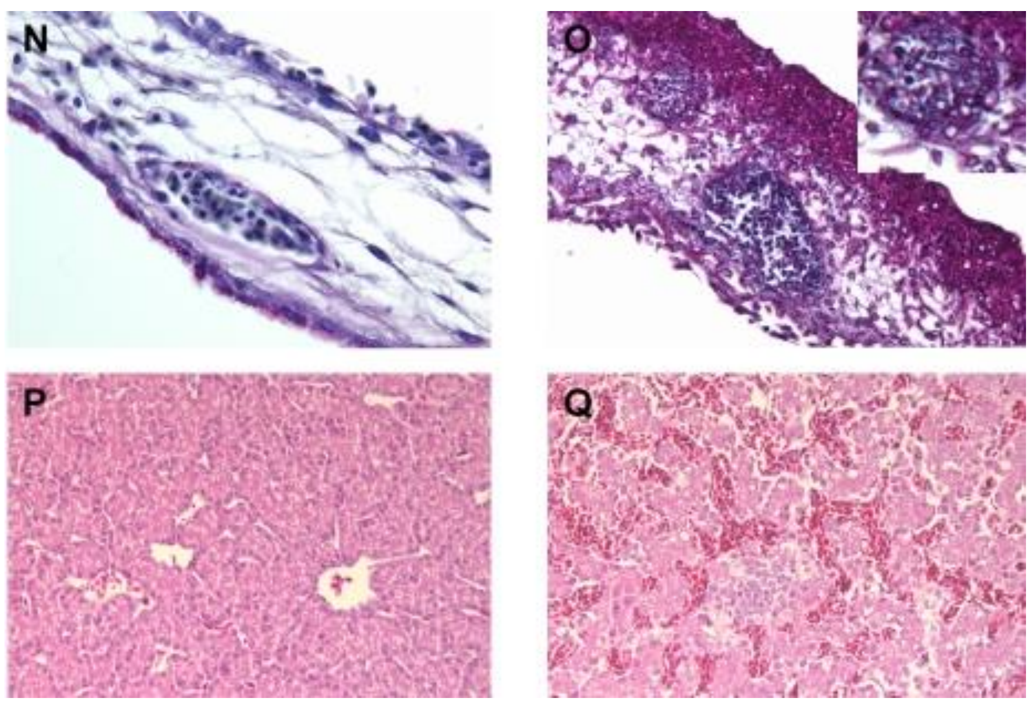
activity were observed. The typing techniques indicated different genotypes in all the penguins, although one genotype was predominant in some cases. Fungal strains of different mating type were found in two different penguins, confirming infection polyclonality. In conclusion, captive penguins are susceptible to infection by multiple strains of A. fumigatus that differ not only in their genotype, but also in mating type and invasiveness. This finding has important consequences for the diagnosis and treatment of avian aspergillosis.

Beernaert *et al.* (2010) published a review with the aim to present the current knowledge on aspergillosis with main emphasis on A. fumigatus infections in captive and free-living birds rather than domestic poultry. The review covered aetiology, epidemiology, pathogenesis, clinical signs and lesions, diagnosis, treatment and prevention. They mentioned that aspergillosis is an infectious, non-contagious fungal disease caused by species in the ubiquitous opportunistic saprophytic genus Aspergillus, in particular Aspergillus fumigatus. This mycosis was described many years ago, but continues to be a major cause of mortality in captive birds and, less frequently, in free-living birds. Although aspergillosis is predominantly a disease of the respiratory tract, all organs can be involved, leading to a variety of manifestations ranging from acute to chronic infections. It is believed that impaired immunity and the inhalation of a considerable amount of spores are important causative factors. The pathogenesis, early diagnostic methods and antifungal treatment schedules need to be further studied in order to control this disease.

Jacobsen *et al.* (2010) developed an alternative, low-cost, and easy-to-**use infection model** for **Aspergillus species** based on embryonated eggs. The outcome of infections in the egg model is dose and age dependent and highly reproducible. We show that the age of the embryos affects the susceptibility to A. fumigatus and that increased resistance coincides with altered chemokine production after infection. The progress of disease in the model can be monitored by using egg survival and histology. Based on pathological analyses, we hypothesize that invasion of embryonic membranes and blood vessels leads to embryonic death. Defined deletion mutant strains previously shown to be fully virulent or partially or strongly attenuated in a mouse model of bronchopulmonary aspergillosis showed comparable degrees of attenuation in the egg model. Addition of nutrients restored the reduced virulence of a mutant lacking a biosynthetic gene, and variations of the infectious route can be used to further analyze the role of distinct genes in our model. Our results suggest that embryonated eggs can be a very useful alternative infection model to study A. fumigatus virulence and pathogenicity.



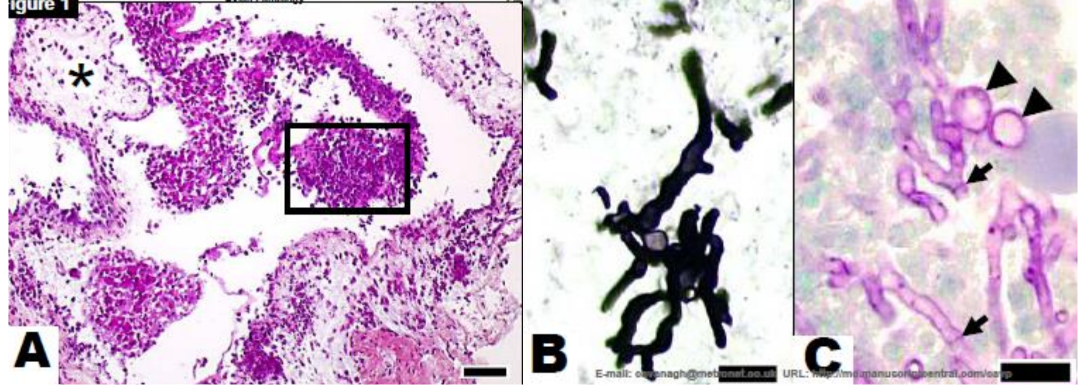
Macroscopic changes on the CAM surrounding the hole drilled for infection (darker area). (A to D) PBS control. The days after infection are indicated at the top. (E to H) Infection with 10^2 *A. fumigatus* CEA17Δ*akuB* conidia/egg. The insets in panels F and G show higher magnifications of mycelia. (I to M) Higher magnifications of eggs infected with *A. fumigatus* on day 4 after infection (I and K) and on day 6 after infection (L and M). **Jacobsen *et al.* (2010)**



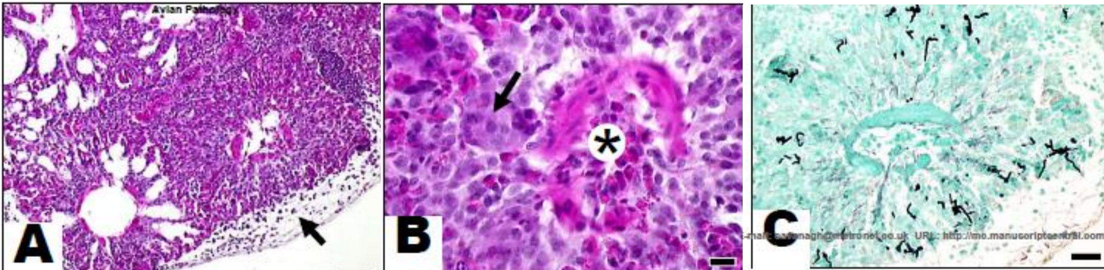
(N to Q) Histological analysis of the CAM (N and O) and livers (P and Q) of infected embryos. (N and O) CAM stained with PAS stain. Magnification, ×63. (N) PBS control. (O) CAM infected with 10^2 *A. fumigatus*CEA17Δ*akuB* conidia/egg (inset, higher magnification of left blood vessel). (P and Q) Liver

stained with H&E. Magnification, ×20. (P) PBS control. (Q) Liver after infection with 10^2 *A. fumigatus* CEA17Δ*akuB*conidia/egg. **Jacobsen *et al.* (2010)**

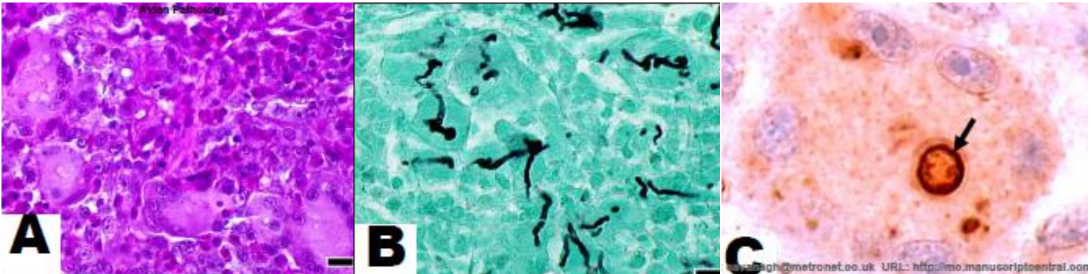
Lisa *et al.* (2010) induced **experimental aspergillosis** in one-day-old **turkeys** by intra-air sac inoculation of a spore suspension of a 3-day-old ***Aspergillus fumigatus*** culture (CBS 144.89) containing 107 spores. Ten additional poults were used as controls. Infected and non-infected animals were closely observed at least twice a day for the appearance of clinical signs and were sequentially sacrificed at days 1, 2, 3, 5 and 7 post-inoculation (pi). In the infected group, most lung tissues and air sac swabs were culture positive from day 1 to day 5. At one-day pi air sac membranes were multifocally and moderately to severely thickened by an oedema and covered by an exudate. A small number of germinating conidia were present in the superficial exudate, already giving rise to small radiating hyphae. Lung lesions were mild, dominated by a diffuse congestion and a mild heterophilic infiltration. From two to 3 days pi air sac membranes were more severely affected and several granulomas were observed. Both granulomas and exudates were rich in germinated conidia and hyphae. Pulmonary lesions consisted in a diffuse pneumonia. Five days pi air sac membrane lesions progressed to a severe, multifocal, heterophilic and granulomatous inflammation. Seven days pi a reduction of the severity of the diffuse pneumonia was detected. Concomitantly, the fungal elements were mainly observed as fragmented tubules in the cytoplasm of multinucleate giant cells. The present study



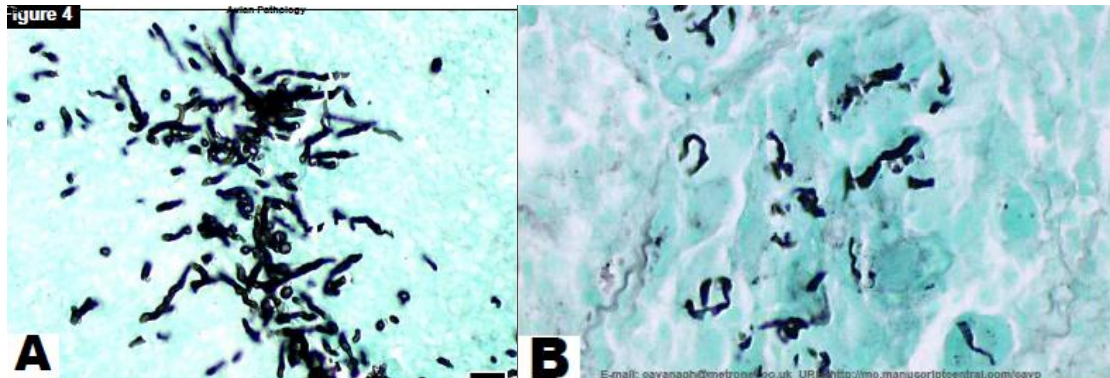
Air sac1-day post-inoculation. (A) Oedema of the air sac membrane (star) and heterophil-rich exudate collected in the lumen (frame). Haematoxylin-Eosin-Safran (HES). Bar = 50μm. (B) Details of the exudate, showing numerous small radiating hyphae strongly stained in black by Methenamine Silver (MS). Bar = 10μm. (C) Same sample stained with Periodic Acid-Schiff (PAS), allowing the observation of septae within the hyphae (arrows); swollen conidia (arrowheads) are present and characterised by a larger diameter than the hyphae. Bar = 10μm **Lisa *et al.* (2010)**



Lung,1 day post-inoculation. (A) Pleural oedema (arrow); diffuse densification of the parenchyma by a congestion and by an inflammatory cellular infiltration. HES. Bar = 50μm. (B) Details showing a parabronchus filled by a heterophil-rich exudate (star) and a parenchymal infiltration by heterophils and mononuclear inflammatory cells (presumptive macrophages); a multinucleate giant cell is already present (arrow). HES. Bar = 10μm. (C) Small hyphae radiating in a pulmonary lobule. MS. Bar = 25μm. **Lisa *et al.* (2010)**



Early evolution of the inflammatory cell population. (A) Pulmonary parenchyma, 3 days post-inoculation. Mononuclear cells are more numerous than at 1 day post-inoculation and heterophils are fewer. HES. Bar = 10µm. (B) Same sample showing hyphae in black within the multinucleated giant cells (cytoplasm stained in green). MS. Bar = 10µm. (C) A swollen conidium, phagocytized by a multinuclear giant cell. Anti-Aspergillus immunolabelling, showing that the fungi stained by MS belong to the species Aspergillus fumigatus. Bar = 5µm. Page 24 of 25 E-mail: cavanagh@metronet.co.uk URL: http://mc.manuscriptcentral.com/cavp Avian Pathology For Peer Review Only **Lisa *et al.* (2010)**



Morphological evolution of fungi. (A) Numerous, well-stained and welldelineated conidia and hyphae in an acute exudative lesion, 2 days post-inoculation. MS. Bar = 10µm. (B) Intra-granulomatous fungi, phagocytised by multinucleate giant cells, 7 days post inoculation. Hyphae are badly delineated and fragmented, attesting their destruction by the inflammatory cells. MS. Bar = 10µm. **Lisa *et al.* (2010)**

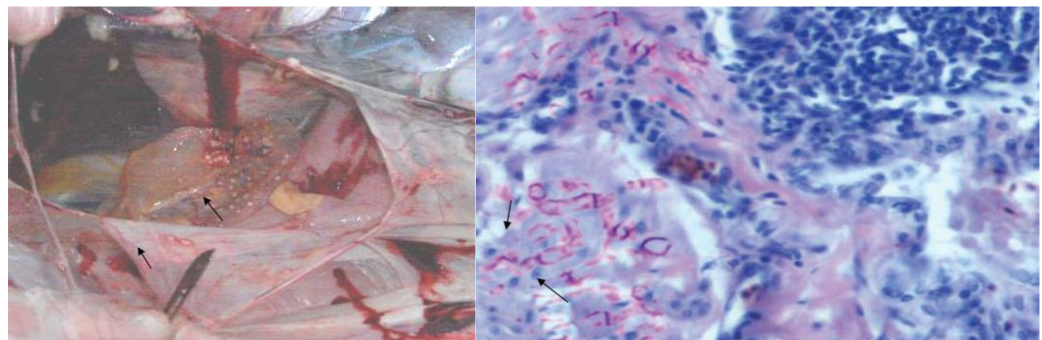
Olias *et al.* (2010) reported an unusual outbreak of articular aspergillosis in a flock of meat turkeys with clinical signs of lameness. Between 7 and 11 weeks of age, turkeys had severe granulomatous osteoarthritis of the hip joints with necrosis of the femur head. Fungal morphology and PCR amplification and sequencing of the first ITS1-5.8S-ITS2 rDNA region identified ***Aspergillus fumigatus*** as the infectious agent. Concurrently, Staphylococcus spp. was isolated from the hip joints, which may have promoted the tropism of the fungus. Mild respiratory tract aspergillosis was observed in only one case. The findings suggest that fungal arthritis may present a specific disease entity in turkeys and should be considered as further cause of lameness in turkeys.

Tijani *et al.* (2010) reported an adult male ostrich (*Struthio camelus*) that had become anorexic and emaciated over a period of 10-days and died subsequently. Necropsy revealed numerous yellowish nodules in the lungs and in markedly thickened, opaque thoracic air sacs. There were also subepicardial and intestinal haemorrhages. The liver and spleen were moderately enlarged and congested. Histologically, the pulmonary lesions were consistent with granulomatous pneumonia due to a mycotic agent. Numerous branching, septate fungal hyphae within pulmonary granulomata were observed histologically. ***Aspergillus flavus*** was

isolated from the pulmonary nodules. This is an established case of fatal *Aspergillus flavus*in fection in an adult male ostrich reared in Nigeria.

Arné e*t al.* (2011) stated that ***Aspergillus fumigatus*** remains a major respiratory pathogen in birds. In poultry, infection by A. fumigatus may induce significant economic losses particularly in turkey production. A. fumigatus develops and sporulates easily in poor quality bedding or contaminated feedstuffs in indoor farm environments. Inadequate ventilation and dusty conditions increase the risk of bird exposure to aerosolized spores. Acute cases are seen in young animals following inhalation of spores, causing high morbidity and mortality. The chronic form affects older birds and looks more sporadic. The respiratory tract is the primary site of A. fumigatus development leading to severe respiratory distress and associated granulomatous airsacculitis and pneumonia. Treatments for infected poultry are nonexistent; therefore, prevention is the only way to protect poultry. Development of avian models of aspergillosis may improve our understanding of its pathogenesis, which remains poorly understood.

İÇEN *et al.* (2011) described the clinical, microbiological and pathological findings, and the results **of Amphoterisin** B and Biostarter for supported treatment, of focal aspergillosis in a flock of **ostriches.** The clinical signs were listlessness, anorexia, diarrhoea, increased respiration, dyspnoea, and mucoid discharge from the nostrils. At post-mortem examination caseous nodules were observed in various organs. Histopathological examination of the lungs, air sacs and the pleural membrane showed in different sizes in different parts of necrosis in the center of the surrounding foreign body giant cells, epitheloid macrophages, lymphocytes and granulomas surrounded by a fibrous connective tissue. In treatment, Amphotericin B and Biostarter was given orally as a supported treatment. There were no sick birds after the treatment. As a conclusion, aspergillosis could be treated with amphotericin B and as a supported treatment Biostarter, especially in the early stages of the disease



Nodules in the chest cavity, lungs (arrows) Numerous hyphae (arrows, PAS stain

Olias *et al.* (2011) reported endemic **outbreaks of invasive aspergillosis at white stork** nesting sites close to human habitation in Germany with significant subsequent breeding losses. Therefore, they hypothesized that A. fumigatus strains with higher virulence in birds may have evolved in this environment and performed the first epidemiological analysis of invasive aspergillosis in free-ranging wild birds. Sixty-one clinical and environmental ***A. fumigatus*** isolates from six affected nesting sites were genotyped by microsatellite analysis using the STRAf-assay. The isolates showed a remarkable high genomic diversity and, contrary to the initial hypothesis, clinical and environmental isolates did not cluster significantly. Interestingly, storks

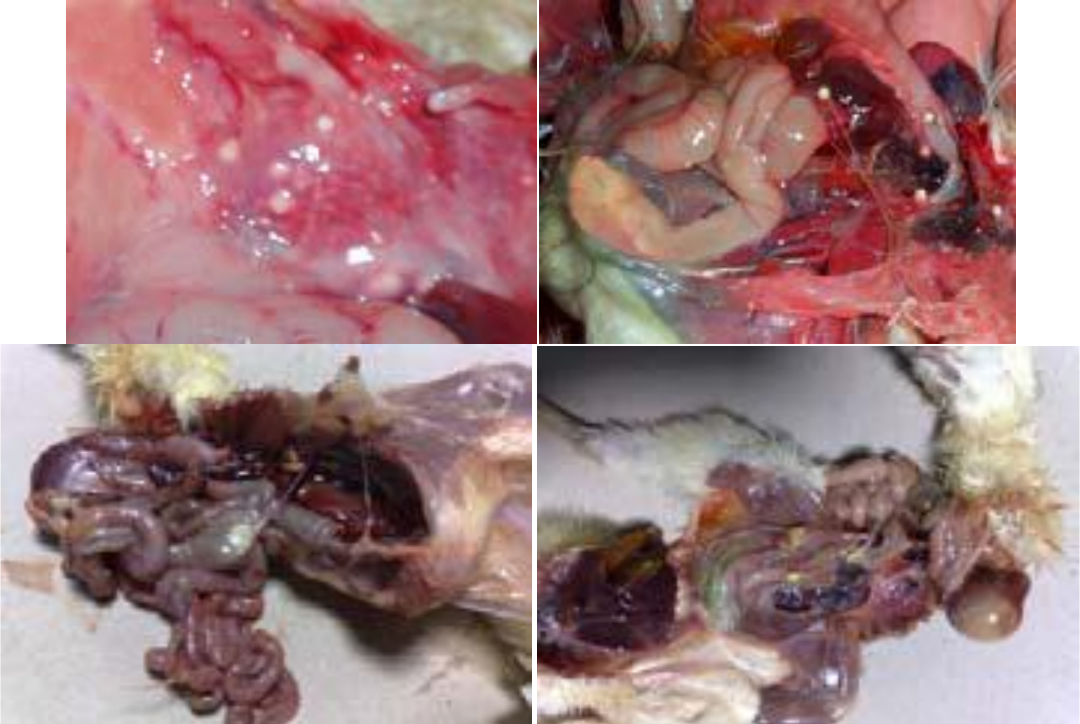
were infected with two to four different genotypes and in most cases both mating types MAT-1.1 and MAT-1.2 were present within the same specimen. The majority of selected clinical and environmental strains exhibited similar virulence in an in vivo infection model using embryonated chicken eggs. Noteworthy, virulence was not associated with one distinct fungal mating type. These results further support the assumption that the majority of A. fumigatus strains have the potential to cause disease in susceptible hosts. In white storks, immaturity of the immune system during the first three weeks of age may enhance susceptibility to invasive aspergillosis.

Van Waeyenberghe *et al.* (2011) used. **microsatellite typing** to analyse 65 clinical avian isolates and 23 environmental isolates of ***A. fumigatus***. The 78 genotypes that were obtained were compared with a database containing genotypes of 2514 isolates from human clinical samples and from the environment. There appeared to be no specific association between the observed genotypes and the origin of the isolates (environment, human or bird). Eight genotypes obtained from isolates of diseased birds were also found in human clinical samples. These results indicate that avian isolates of A. fumigatus may cause infection in humans.

M i l o š et *al.* (2011) analyzed the occurrence of Aspergillus sp. in poultry according to the clinical and laboratory investigations performed during the two selected years, 2000 and 2010. Widespread aspergillosis was noted in poultry flocks of different age, both in young and adult birds. During the years 2000 and 2010, acute aspergillosis was found in 12 and 16 commercial flocks of chickens and turkeys, respectively. Ocular infection with Aspergillus was determined in 10 day old broilers from two flocks. **Aspergillus sp.** was isolated from unhatched eggs (6.86%), litter (23.07%), environmental (36.17%) and hatchery swabs (3.85%). Besides the appropriate antifungal therapy, enforcement of proper sanitary-hygiene measures on poultry farms and hatcheries, as well as microbiological control of feed are considered essential for an efficient control of infection and its spreading.

Besides the symptoms previously mentioned, the investigation of mass pneumomycoses discovered one distinctive finding which was grupping of the chickens toward the source of fresh air ("hunger for oxygen"; Figures 1 and 2). **M i l o š et *al.* (2011)**

In that case protruding eye lids are observed because of formation of yellowish-cheesy small pellets around the membrane nicticans with central ulceration (Figures 3 and 4). **M i l o š et *al.* (2011)**



Postmortem findings included nodules – aspergillus granuloma, oval or round shaped, single or in conglomerate, size of a pin head to pea, located on air sacs, lungs and on visceral serosae of abdominal cavity, liver and intestines (Figures 5, 6, 7 and 8).

Ceolin *et al*. (2012) observed in an intensive poultry farm a mortality rate exceeding 20%, hoarseness and difficulty breathing in male ***Gallus gallus*** of approximately two weeks of age. The batch was treated with Terramycin (oxytetracyclinehydrochloride) in the first week and Trissulfin (sulfamethoxazole, trimethoprim and bromhexine hydrochloride) in the second week. Necropsy was performed in three affected birds and pulmonary aspergillosis was suspected due to local **pulmonary and disseminated** injuries in the coelomic cavity, associated with the clinical signs. In birds assessed by necropsy, nodules were commonly seen in the internal cavity and lungs, as well as caseous masses in the air sacs, little pigmentation on the feet and beaks, and fragile bones. Portions of lungs and granulomas were examined for isolation and identification of fungi. The result of mycological examination showed ***Aspergillus fumigatus*** to be the agent. The histopathological lesions observed in the lung were consistent with aspergillosis, characterized by multifocal granulomas

associated with intra-lesional dichotomously branched fungal hyphae, morphologically compatible with *Aspergillus* sp.

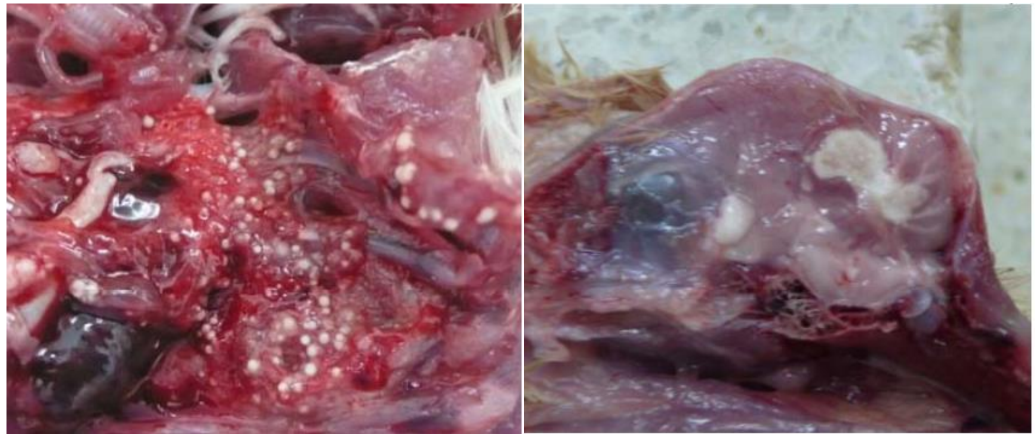
Burco *et al.* (2012) evaluated the utility of measuring (1-->3)-**beta-D-glucan (BG)** concentrations in avian plasma samples to aid in the diagnosis of aspergillosis. They tested a commercially available BG assay (Fungitell, Beacon Diagnostics) using 178 plasma samples from naturally infected, experimentally infected, and aspergillosis-free birds. Although there was variation in BG concentration, as reflected by high standard deviations, seabirds with confirmed aspergillosis had the highest mean BG concentrations (M = 3098.7 pg/dl, SD = 5022.6, n = 22) followed by companion avian species and raptors with confirmed aspergillosis (M = 1033.8 pg/dl, SD = 1531.6, n = 19) and experimentally infected Japanese quail (Coturnix japonica; M = 1066.5 pg/dl, SD = 1348.2, n = 17). Variation in severity of disease, differences among species of birds with and without disease, and also different levels in environmental exposure likely contribute to the differences among avian groups. The overall sensitivity and specificity of the BG test for diagnosis of aspergillosis in birds was 60.0 and 92.7%, respectively, with an overall optimized avian cut-off value of > or = 461 pg/dl for positive disease. Our findings suggest that, although BG concentrations are highly variable between and within different avian groups, it could serve as a useful adjunctive diagnostic test for aspergillosis that is applicable to multiple avian species in some settings, particularly as a negative predictor of infection.

França *et al.* (2012) tested serum samples from commercial broiler chickens and turkeys diagnosed with respiratory and disseminated aspergillosis for the presence of **antigen and antibody** to Aspergillus. Antigen detection consisted of testing for two cell-wall components, beta-glucan and galactomannan, which have been used extensively in human medicine. There were significantly higher levels of galactomannan in all broiler chicken submissions (100%) and antibody to Aspergillus in 6 out of 9 submissions (66.6%) vs. control birds. Beta-glucan analyses did not show any differences among levels in the broiler chicken groups. There were significantly higher levels of galactomannan antigen in 4 out of 7 submissions (57.1%) of aspergillosis in commercial turkeys, while only 2 out of 7 submissions (28.5%) had higher levels of antibody to Aspergillus vs. the control group. This study shows that diagnosis of respiratory and disseminated aspergillosis may be performed by detection of galactomannan antigenemia and antibodies in broiler chickens and to an extent in turkeys.

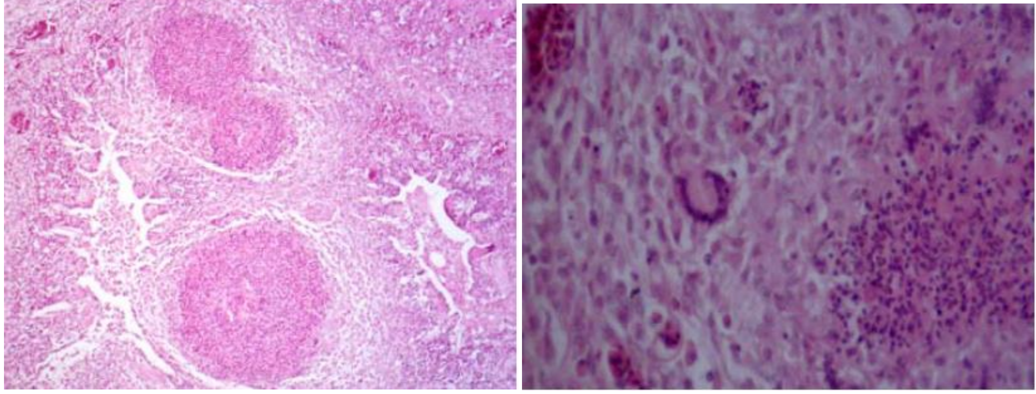
Kummrow *et al.* (2012) performed serum protein **electrophoresis** by using high-resolution agarose gels on blood samples collected from 105 falcons, including **peregrine falcons** (*Falco peregrinus*), gyrfalcons (*Falco rusticolus*), saker falcons (*Falco cherrug*), red-naped shaheens (*Falco pelegrinoides babylonicus*), and hybrid falcons, that were submitted to the Dubai Falcon Hospital (Dubai, United Arab Emirates) between 2003 and 2006. Reference values were established in clinically healthy birds and compared with values from falcons infected with *Aspergillus* species (n = 32). Falcons with confirmed aspergillosis showed significantly lower prealbumin values, which is a novel finding. Prealbumin has been documented in many avian species, but further investigation is required to illuminate the diagnostic significance of this negative acute-phase protein.

Kureljušić *et al.* (2012) examined a flock of turkey poults, 21 days old, at one farm in Serbia. Clinical signs of central nervous system in the form of ataxia, torticollis,

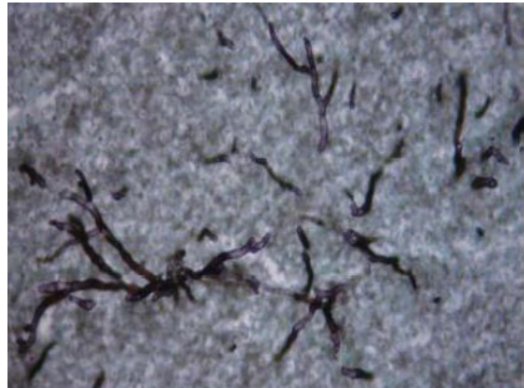
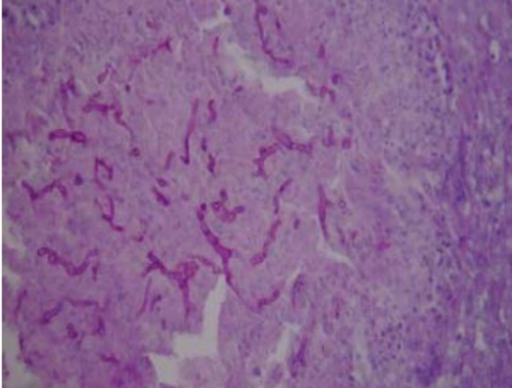
paresis and paralysis of legs and wings were observed. The mortality rate in the flock was 7,2 %. In ten out of twelve necropsied turkey poults multiple yellowish-white granulomas, one to three millimeters in diameter on lungs were found. In nine out of twelve necropsied turkey poults solitary yellowish-white granuloma, three to five millimeters in diameter on sagital section of the cerebrum or cerebellum were found. Mycological finding revealed fungi ***Aspergillus fumigatus***. For the evaluation of histopathological changes in lung and brain and demonstration of fungal hyphae, three stain methods were used: haematoxylin-eosin (HE), Grocott methenamine silver and periodic acid Schiff (PAS) method. Microscopic examination of lung and brain has revealed the presence of granulomatous foci and caseous necrosis with surrounding region of proliferation including giant cells, macrophages, heterophils and lymphocytes and outer capsule of connective tissue. The fungal hyphae were hardly or not visible in HE stained sections, while septed and arborized hyphae were easily demonstrated by Grocott and PAS stain predominantly in central parts of granuloma. For diagnostic of mycotic infection is necessary to use different histochemical methods for evaluation of histopathological changes and detection of etiological agent, including isolation to obtain etiological diagnosis



Lung of turkey poult, granulomatous pneumonia Brain of turkey poult, granuloma on sagital section of cerebellum Kureljušić *et al.* (2012)

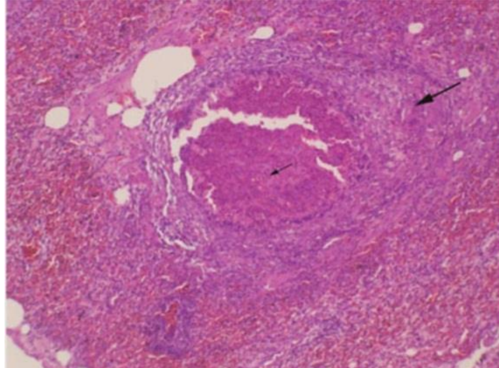
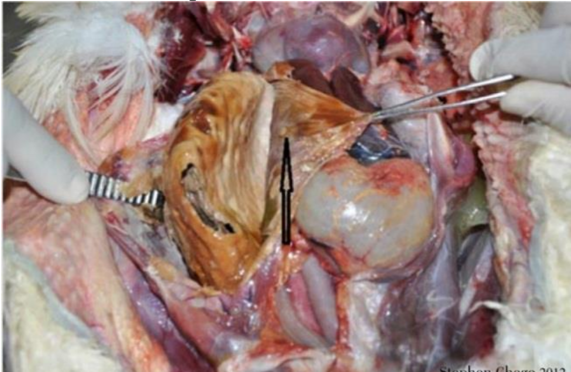


Lung, turkey poult, multifocal granulomatous pneumonia, HE, X200 Lung, turkey poult, infiltrate of granuloma consist of giant cells, macrophages, heterophils and lymphocytes, HE, Kureljušić *et al.* (2012)

Brain, turkey poult, arborized and septed hyphae, Grocott, X400 Lung, turkey poult, arborized and septed hyphae, PAS, Kureljušić *et al.* (2012)

Yellowish granuloma in the caudal abdominal airsacs of a dead Cape vulture at Al Ain Zoo.

Necrotic center of the granuloma with few fungal structures visible in the centre and some giant cells (arrows) at the periphery (HE-staining). Granuloma (arrow) in the caudal abdominal airsacs of a dead Cape vulture at Al Ain Zoo

Hadrich *et al.* (2013a) used **microsatellite markers** to type seven clinical avian isolates and 22 environmental isolates of ***A. flavus***. A. flavus was the only species (28 % prevalence) detected in the avian clinical isolates, whereas this species ranked third (19 %) after members of the genera Penicillium (39 %) and Cladosporium (21 %) in the environmental samples. Upon microsatellite analysis, five to eight distinct alleles were detected for each marker. The marker with the highest discriminatory power had

eight alleles and a 0.852 D value. The combination of all six markers yielded a 0.991 D value with 25 distinct genotypes. One clinical avian isolate (lung biopsy) and one environmental isolate (egg) shared the same genotype. Microsatellite typing of A. flavus grown from avian and environmental samples displayed an excellent discriminatory power and 100 % reproducibility. This study showed a clustering of clinical and environmental isolates, which were clearly separated. Based upon these results, aspergillosis in birds may be induced by a great diversity of

Hadrich *et al.* (2013b) used **microsatellite typing** to analyze 29 ***A. flavus*** clinical and environmental avian isolates and 63 human clinical isolates collected from patients with a variety of aspergillosis diseases. The combination of all six markers yielded 77 different genotypes with a 0.98 D value. A. flavus genotypes obtained from avian isolates were compared with those obtained from human clinical and environmental samples. The standardized indices of association I (A) and rBarD were significantly different from zero ($p < 0.01$), suggesting a prevailing clonal reproduction. There was high genetic diversity between the hospital and poultry environments of A. flavus isolates. The human environmental population was significantly differentiated from environmental and clinical avian populations (F (st) > 0.25). The avian clinical subpopulation exchanged few strains with the environmental human (N (m) = 7.24) and avian (N (m) = 6.60) populations. The minimum spanning tree analysis identified three A. flavus genotype clusters that were highly structured according to the isolation source (p < 10(-4)).

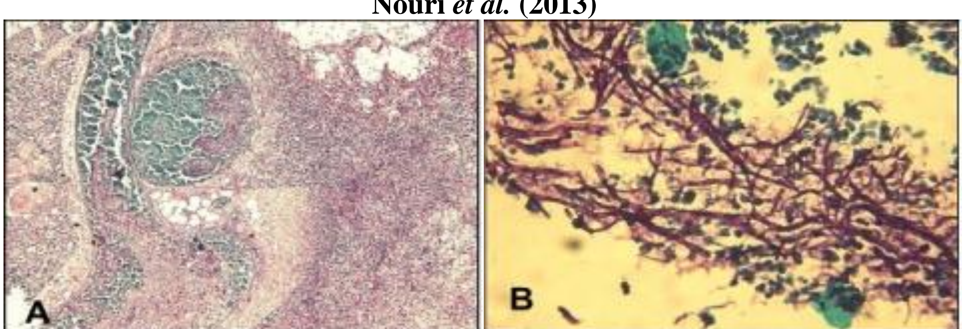
Korniłłowicz-Kowalska and Kitowski (2013) performed a study on the numbers and species diversity of thermophilic fungi (growing at 45 °C in vitro) in 38 nests of 9 species of wetland birds, taking into account the physicochemical properties of the nests and the bird species. It was found that in nests with the maximum weight (nests of Mute Swan), the number and diversity of thermophilic fungi were significantly greater than in other nests, with lower weight. The diversity of the thermophilic biota was positively correlated with the individual mass of bird and with the level of phosphorus in the nests. The dominant species within the mycobiota under study was ***Aspergillus fumigatus*** which inhabited 95% of the nests under study, with average frequency of ca. 650 cfu g(-1) of dry mass of the nest material. In a majority of the nests studied (nests of 7 bird species), the share of A. fumigatus exceeded 50% of the total fungi growing at 45 °C. Significantly higher frequencies of the fungal species were characteristic of the nests of small and medium-sized piscivorous species, compared with the other bird species. The number of A. fumigatus increased with increase in the moisture level of the nests, whereas the frequency of occurrence of that opportunistic pathogen, opposite to the general frequency of thermophilic mycobiota, was negatively correlated with the level of phosphorus in the nest material, and with the body mass and length of the birds. The authors indicate the causes of varied growth of thermophilic fungi in nests of wetland birds and, in particular, present a discussion of the causes of accumulation of A. fumigatus, the related threats to the birds, and its role as a source of transmission in the epidemiological chain of aspergillosis.

Nouri *et al.* (2013) documented a case of **acute aspergillosis** and bacillosis in a canary in which the bacillus and fungal colonization was confined to the trachea and lung. A male canary was presented to clinic with a history of the presence of watery diarrhea and swelling in the left intertarsal joint without weight bearing. Supportive

care was attempted but the bird did not improve and 48 h after beginning of respiratory signs, the bird died. At necropsy, symmetric necrotic zone were seen in the junction of syrinx to lung. The trachea was edematous and congested. The intestine was edematous and dilated. Histopathologic examination of lungs and trachea revealed infiltrative reactions of aspergillosis. Fungal hyphae with dichotomous divisions characteristic of aspergilli were clearly demonstrated in the trachea and lung by PAS and H&E stains. The pinkish amorphous material in the spleen was demonstrated to be amyloid with Congo red stain

Symmetric necrotic zone were seen in the junction of syrinx to lung (yellow arrow) in canary

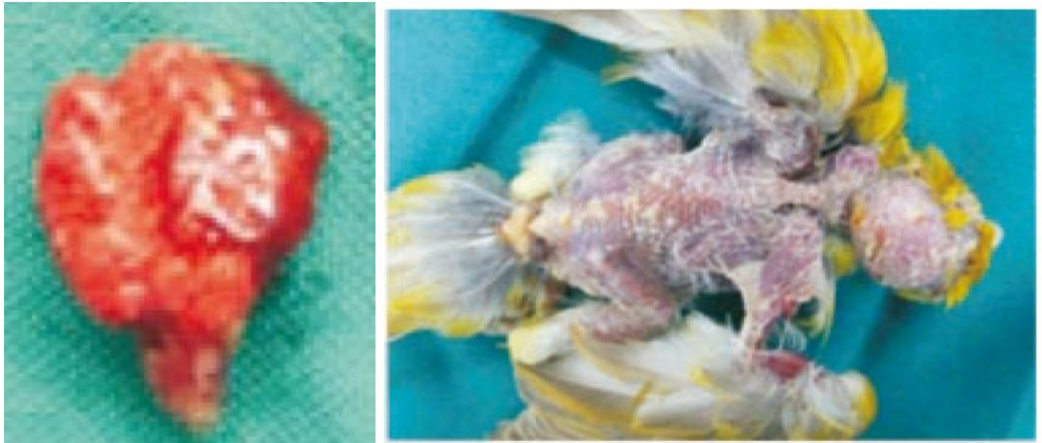
Nouri *et al.* (2013)



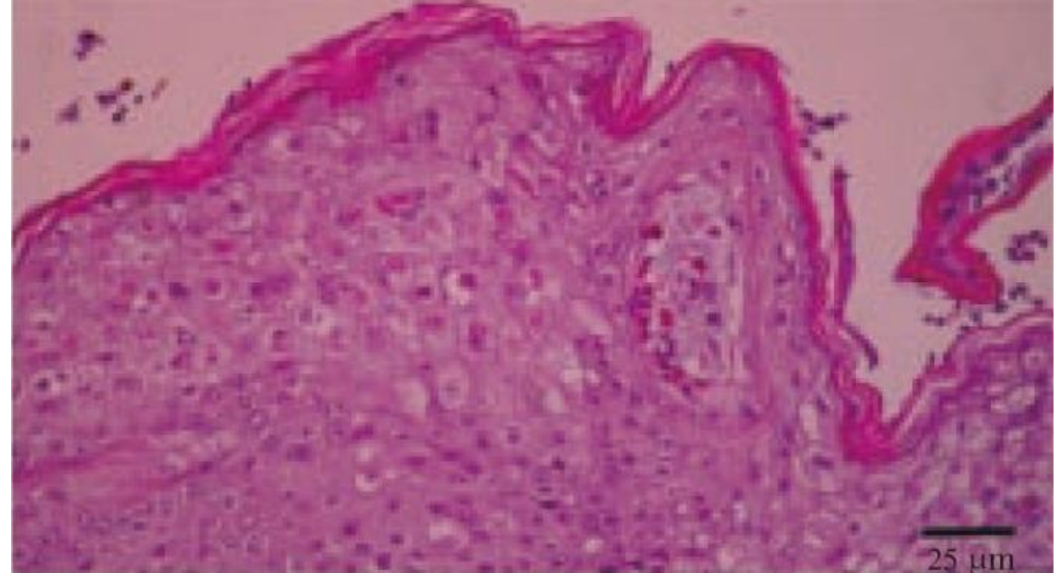
A. Aspergillus spe. Hyphae in the trachea.PAS, Aspergillus sp. Hyphae in the lung. Lesikons contained necrosis, numerous branched septate hyphae radiating and clumps ofof bacilli in the centre **Nouri *et al.* (2013)**

Reza et *al.* (2013) described the clinical, pathological and mycological findings in **canaries**, in which pox lesions and ***Aspergillus fumigatus*** (*A. fumigatus*) infection were observed simultaneously. This study was performed on a breeding colony (about 100 canaries) affected by fatal wasting disease. Necropsy was undertaken on 10 severely affected canaries, and gross lesions were recorded. Samples from internal organs displaying lesions were obtained for histopathological evaluation. Tracheal swap samples of internal organs of the all infected animals with lesions at necropsy were cultured in Sabouraud Dextrose Agar for mycological examination. At necropsy,

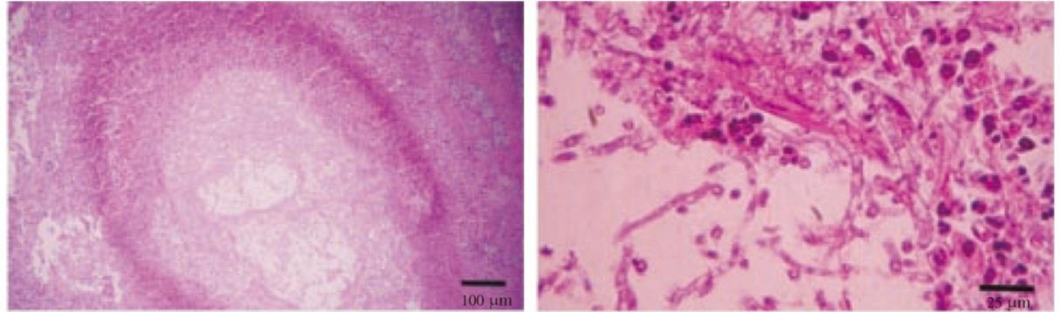
caseous foci were determined in the lungs, on the air sacs, liver, spleen, heart. Swelling of the eyelids, diffuse hemorrhages in the subcutaneous tissue with small papular lesions of the skin were other typical necropsy findings. Histopathologically, pathognomonic eosinophilic intracytoplasmic inclusion bodies, which called Bollinger bodies, in both skin cells and vacuolated air way epithelial cells confirmed canary pox infection. Moreover, histopathological examination of the white-yellowish caseous foci revealed necrotic granulomatous reaction consisting of macrophages, heterophil leukocytes and giant cells encapsulated with a fibrous tissue. After the culture of the tissue samples, the formation of bluish green colonies confirmed *A. fumigatus* infection.



Prominent consolidation of the lungs. **Multiple papular lesions on the skin of the head and back. Reza et *al.* (2013)**

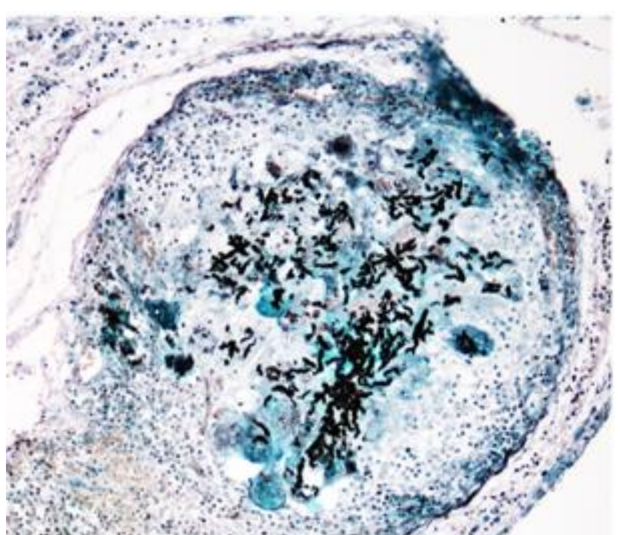


Hyperkeratosis, acanthosis, ballooning degeneration of keratinocytes, and Bollinger bodies of the skin (H&E). **Reza et *al.* (2013)**



Numerous fungal hyphae surrounded by granulomatous reaction in the lung (H&E). Short and thin septate fungal hyphae within granulomatous reaction in the lung (H&E). **Reza et *al.* (2013)**

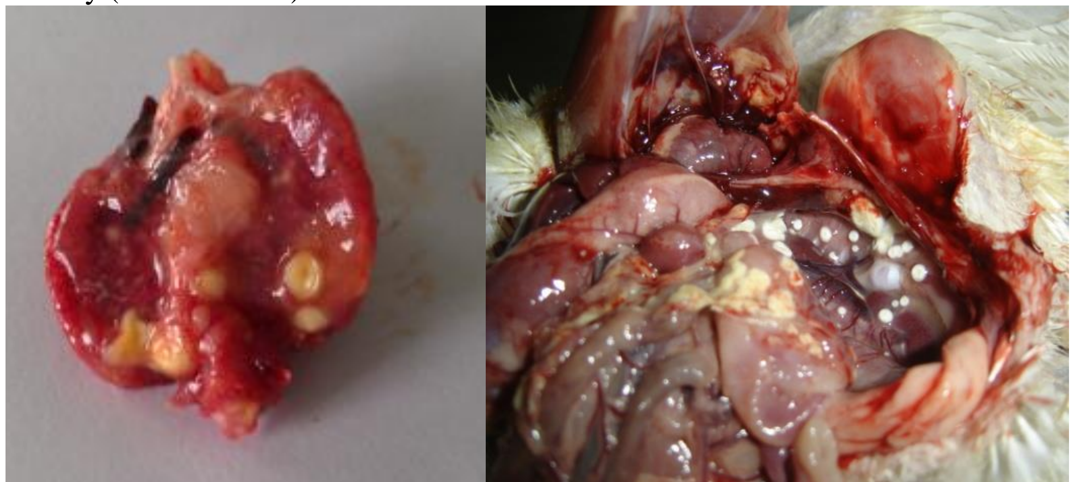
Spanamberg *et al.* (2013) performed a study to assess the occurrence of aspergillosis caused by ***Aspergillus fumigatus*** in **commercial poultry**, through mycological and histopathological diagnosis, and to verify the causal association between the aspergillosis diagnosis criteria and condemnation due to airsacculitis in broilers through a case-control study. The study was carried out with 380 samples. Lungs were collected from broilers that were condemned (95) or not condemned (285) due to airsacculitis directly from the slaughter line. Forty-six (12%) lung samples were positive for *A. fumigatus* in mycological culture. Among all samples, 177 (46.6%) presented histopathological alterations, with necrotic, fibrinous, heterophilic pneumonia; heterophilic pneumonia and lymphoid hyperplasia being the most frequent. Out of the 380 lungs analyzed, 65.2% (30) showed histopathological alterations and isolation of fungi. The statistical analysis (McNemar's chi-square test) indicated a significant association between the presence of histopathological lesions and the isolation of *A. fumigatus.* Mycological cultivation and histopathological diagnosis increase the probability of detecting pulmonary alterations in birds condemned by the Final Inspection System, which suggests that such diagnostic criteria can improve the assessment and condemnation of birds affected by airsacculitis.



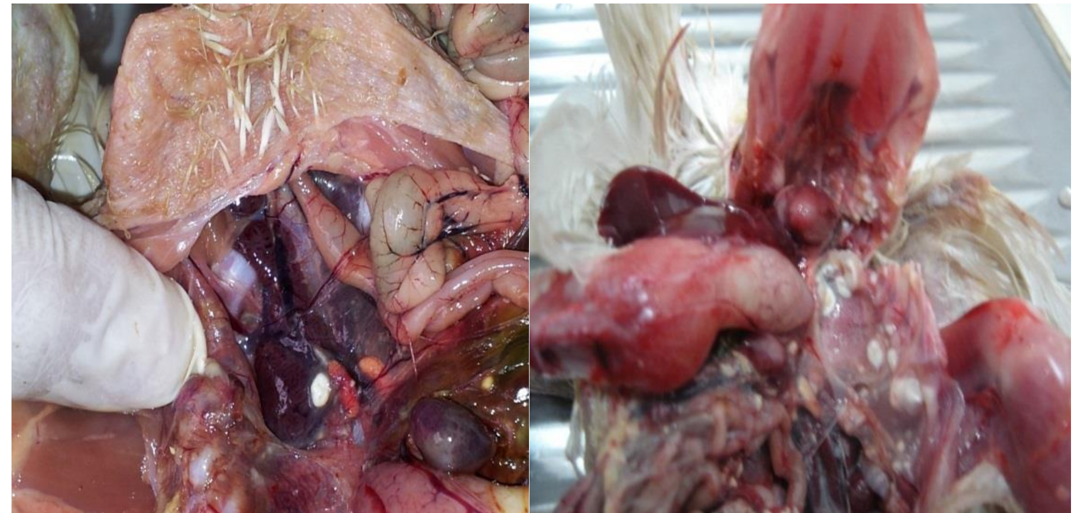
Hyaline, branching septate hyphae of ***A. fumigatus***, Grocott staining, **Spanamberg *et al.* (2013)**

Thierry *et al.* (2013) performed a study to assess the **pathogenicity *of A. fumigatus*** in two lineages of **chicken** (Gallus gallus): **SPF White Leghorn** PA12 layers and **conventional JA657 broilers**. Four-day-old birds were experimentally infected in an inhalation chamber in order to reproduce a "natural" contamination and to obtain a large repartition of conidia into the respiratory tract. Half of the chicks were injected subcutaneously with dexamethasone for 4 days before the infective challenge. At days 0 and 7, the effects of chicken lineage and immunosuppressive treatment on pulmonary fungal burden were analyzed using two linear mixed models. The pathogenicity of A. fumigatus varied according to the lineage: no clinical signs and no mortality were observed in layer chickens whereas more than 50% of mortality occurred in broilers. The effect of immunosuppressive treatment was also demonstrated, notably on birds weight but also on mortality.

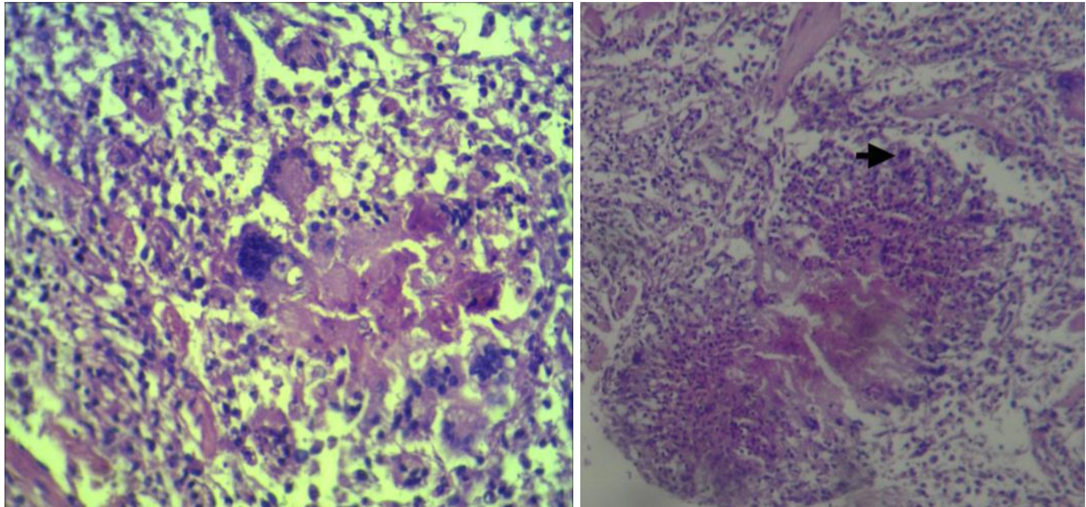
Abdulrahman *et al.* (2014) observed **outbreaks of aspergillosis in broiler chicks** (4-15 days old) from January to July, 2011 in five broiler farms. The disease was detected on the basis of clinical signs, gross, histopathological and cultural findings. Infected chicks showed signs of ruffled feathers, green watery diarrhea, anorexia, gasping and dyspnea. On examination, numerous small white yellowish nodules were seen in the lungs, air sacs, kidneys, thoracic wall and abdominal serosa. Microscopically the lungs revealed granulomas with central areas of caseation surrounded by heterophils and giant cells. ***Aspergillus fumigatus*** could be isolated in Sabouraud's dextrose agar from the lesions. Higher morbidity (76 to 100%) and mortality (62.5 to 100%) rates were recorded in the five farms.



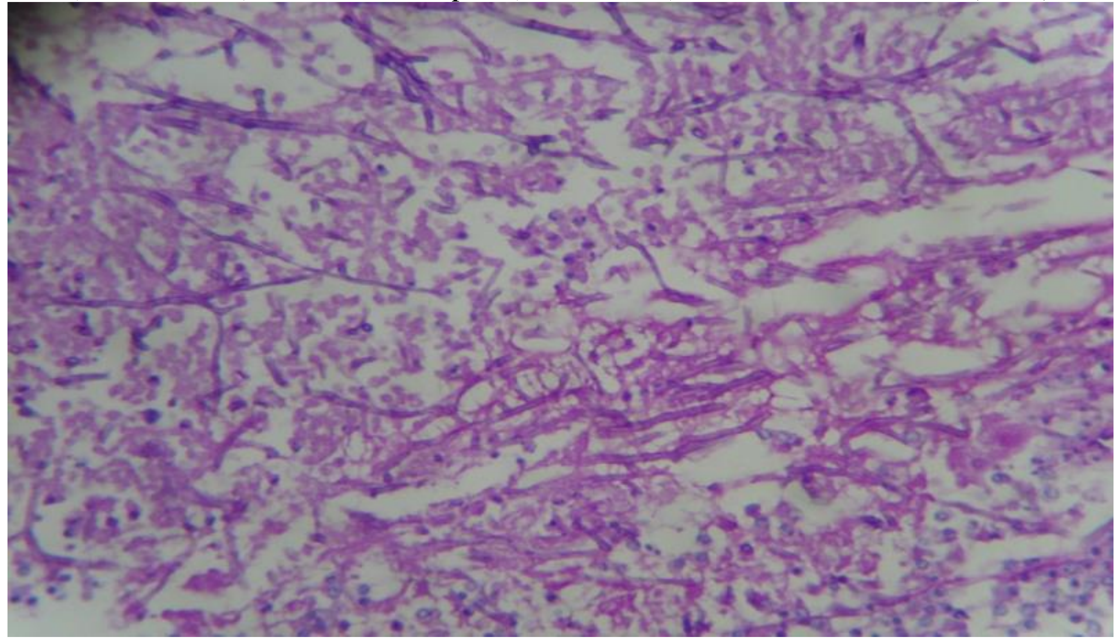
white-yellowish caseous nodules in the lung, white-yellowish caseous nodules on the air sacs, **Abdulrahman *et al.* (2014)**



white-yellowish caseous nodules in the kidney, white-yellowish caseous nodules on the thoracic wall and abdominal serosa**, Abdulrahman *et al.* (2014)**

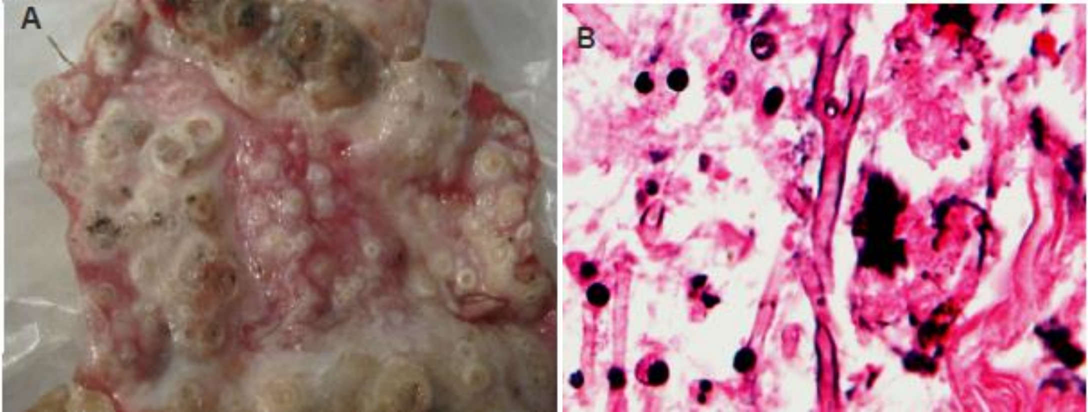


Aspergillus granuloma with a central eosinophilic necrosis surrounded by foreign-body giant cells (a=H&E 100X), scattered heterophils(b=H&E 40X). **Abdulrahman *et al.* (2014)**



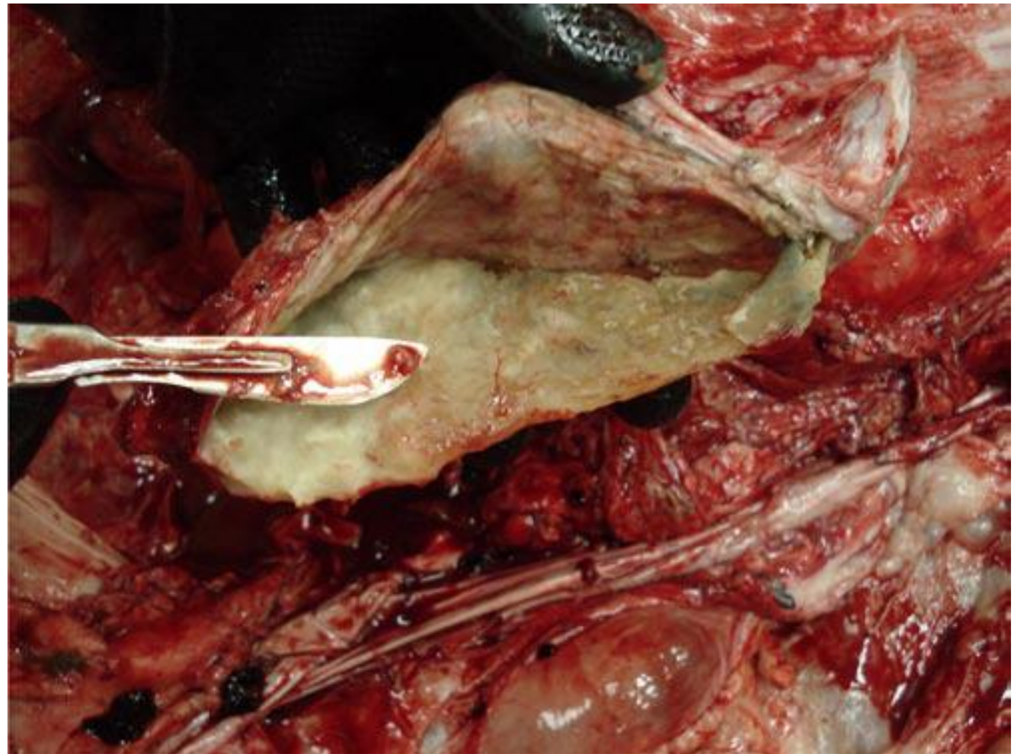
Fungal spores and grown Hyphae could be observed among the inflammatory necrotic masses (PAS, 100X). **Abdulrahman *et al.* (2014)**

Araghi *et al.* (2014) discussed some outbreaks of Aspergillus infection in **ostrich** farms of eastern regions of Iran during 2010-2012. Signs of respiratory involvement, anorexia, depression, progressive emaciation and decreased production were the most commonly reported in affected farms. Morbidity rate was 43% and 54.53% in breeding birds and chickens, respectively. Mortality rate was 31.89% in breeding birds and 44.69% in chickens. Necropsy findings were suggestive of fungal infections in respiratory and alimentary tracts. ***Aspergillus fumigatus*** and ***Aspergillus niger*** were identified in microbiological and pathological examinations. Management reforms and using some supportive treatments were beneficial for controlling the disease.



Aspergillus infection. A. Postmortem changes in air sac and lung; B. Histopathologic slide of infected air sac examined microscopically (×100) showing *Aspergillus* hyphae. **Araghi *et al.* (2014)**

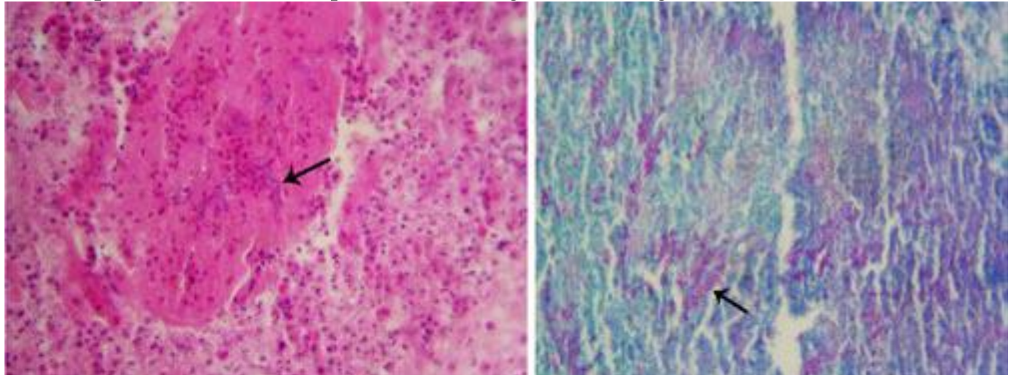
Azizi *et al.* (2014) described concurrent occurrence of aspergillosis and proventricular impaction in a 4-year-old male ostrich. The bird had respiratory problems, coughing and anorexia. Postmortem examination revealed numerous greenish-white caseous foci, 0.5 to 1 cm in diameter distributed on the surfaces of the air sacs and throughout the lungs. In histopathological study, multifocal areas of caseous necrosis that surrounded by inflammatory cells including heterophils, lymphocytes and macrophages were present. Long branching septated hyphae were visible in the necrotic areas with hematoxylin and eosin and Periodic acid-Schiff staining. Thrombi were present in the blood vessels. The proventriculus was full of gravel.



In the inner surface of thoracic air sac, massive caseous materials arecumulated. **Azizi *et al.* (2014)**

Numerous greenish-white caseous foci, 0.5 to 1 cm in diameter distributed on the surfaces of the air sacs (a) and throughout the lung (b). **Azizi *et al.* (2014)**

The proventriculus was impacted with a large amount of gravel. **Azizi *et al.* (2014)**



Lung: caseous necrotic area contains fungal hyphae (arrow) staining with HE (a) and PAS (b) (× 400)
Azizi *et al.* (2014)

Burco *et al.* (2014) compared fungal, particularly *Aspergillus* spp., burdens potentially encountered by seabirds in natural and rehabilitation environments. Differences among the various microenvironments in the rehabilitation facility were evaluated to determine the risk of infection when seabirds are experiencing high stress and poor immune function. *Aspergillus* spp. counts were quantified in three wildlife rehabilitation centers and five natural seabird loafing and roosting sites in northern California using a handheld impact air sampler and a water filtration system. Wildlife rehabilitation centers demonstrated an increase in numbers of conidia of *Aspergillus* spp. and ***Aspergillus fumigatus*** in air and water samples from select aquatic bird rehabilitation centers compared with natural seabird environments in northern California. Various microenvironments in the rehabilitation facility were identified as having higher numbers of conidia of *Aspergillus* spp. These results suggest that periodic monitoring of multiple local areas, where the birds spend time in a rehabilitation facility, should be done to identify "high risk" sites, where birds should spend minimal time, or sites that should be cleaned more frequently or have

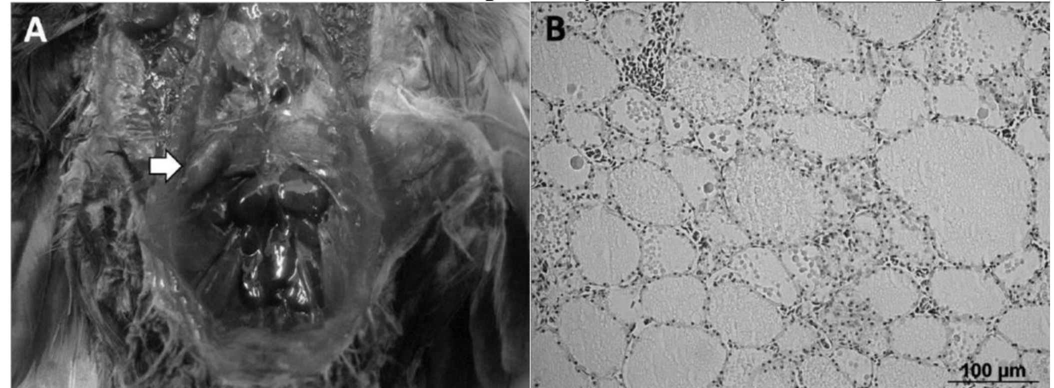
improved air flow to reduce exposure to fungal conidia. Overall, these results suggest that seabirds may be more likely to encounter *Aspergillus* spp. in various microenvironments in captivity, compared with their native habitats, which could increase their risk of developing disease when in a debilitated state.

Cafarchia *et al.* (2014) collected and cultured 57 air samples from 19 sheds (Group I), 69 from faeces (Group II), 19 from poultry feedstuffs (Group III) and 60 from three anatomical sites (i.e. nostrils, pharynx, ears) of 20 farm workers (Group IV). The Aspergillus spp. prevalence in samples ranged from 31.6% (Group III) to 55.5% (Group IV), whereas the highest conidia concentration was retrieved in Group II (1.2 × 10(4) c.f.u. g(-1)) and in Group III (1.9 × 10(3) c.f.u. g(-1)). The mean concentration of airborne Aspergillus spp. conidia was 70 c.f.u. m(-3) with ***Aspergillus fumigatus*** (27.3%) being the most frequently detected species, followed by ***Aspergillus flavus*** (6.3%). These Aspergillus spp. were also isolated from human nostrils (40%) and ears (35%) ($P<0.05$) (Group IV). No clinical aspergillosis was diagnosed in hens. The results demonstrate a relationship between the environmental contamination in hen farms and presence of Aspergillus spp. on animals and humans. Even if the concentration of airborne Aspergillus spp. conidia (i.e. 70 c.f.u. m(-3)) herein detected does not trigger clinical disease in hens, it causes human colonization. Correct management of hen farms is necessary to control environmental contamination by Aspergillus spp., and could lead to a significant reduction of animal and human colonization.

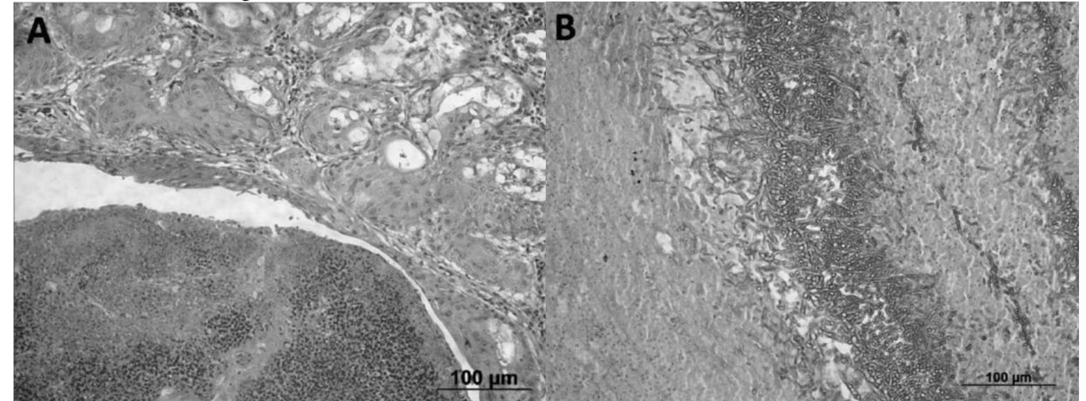
Glare *et al.* (2014) carried out a study to survey nocturnal **kiwi** houses in New Zealand and to assess the levels of Aspergillus currently present in leaf litter. Samples were received from 11 nocturnal kiwi houses from throughout New Zealand, with one site supplying multiple samples over time. Aspergillus was isolated and quantified by colony counts from litter samples using selective media and incubation temperatures. Isolates were identified to the species level by amplification and sequencing of ITS regions of the ribosomal. Aspergillus spp. were recovered from almost every sample; however, the levels in most kiwi houses were below 1000 colony-forming units (CFU)/g of wet material. The predominant species was Aspergillus fumigatus, with rare occurrences of **Aspergillus niger, Aspergillus nidulans, and Aspergillus parasiticus.** Only one site had no detectable Aspergillus. The limit of detection was around 50 CFU/g wet material. One site was repeatedly sampled as it had a high loading of A. fumigatus at the start of the survey and had two recent clinical cases of aspergillosis diagnosed in resident kiwi. Environmental loading at this site with Aspergillus spp. reduced but was not eliminated despite changes of the litter. The key finding of our study is that the background levels of Aspergillus spores in kiwi nocturnal houses in New Zealand are low, but occasional exceptions occur and are associated with the onset of aspergillosis in otherwise healthy birds. The predominant Aspergillus species present in the leaf litter was A. fumigatus, but other species were also present. Further research is needed to confirm the optimal management of leaf litter to minimize Aspergillus spore counts. However, in the interim, our recommendations are that leaf litter should be freshly collected from areas of undisturbed forest areas and spread immediately after collection, without interim storage.

Guilherme *et al.* (2014) described a case of aspergillosis and colloidal goiter in a male Black-masked **lovebird** (Agapornis personata) diagnosed by post mortem exam. The bird was presented for examination due to severe respiratory signs. An initial

palliative treatment was performed in order to relieve the symptoms. Despite this, the patient came to die without performing additional ancillary tests. On gross exam, a pulmonary nodule was observed from which we were able to isolate Aspergillus fumigatus on microbial culture. Histological assessment revealed pulmonary aspergilosis and colloid goiter. Based on histopathological and microbiological assessments we conclude that infection probably was secondary to colloid goiter.



.A firm and raised nodule in the ventro-distal pleural surface. B) Thyroid. Follicles are larger in size and with flattened lining cells and abundant colloid material **Guilherme *et al.* (2014)**



A) Pleural granuloma. Note caseous necrosis and several inflammatory cells in the periphery (HE, 200x). 3B) Pleural granuloma. Note several hyphae in the central portion (PAS, 200x).

Kwanashie *et al.* (2014) carried out a study to determine the prevalence of *Aspergillus* species in **dead-in-shell chick embryos**. Materials and Methods: A total of three thousand dead-in-shell embryonated chicken eggs were collected from the four hatcheries over a period of six months. The content of 10 eggs were pooled after decontamination of the egg surface and swab of pooled contents inoculated onto the entire surface of Sabourauds Dextrose Agar (SDA) and Corn Meal Agar (CMA) slants and growths. Results: Out of the 300 groups of pooled eggs a total of 122 (40.67%) isolates of fungi belonging to 4 species of the Genera *Aspergillus* viz ***A. fumigatus*, *A. niger*, *A. flavus* and *A. terreus*** made up 48.40% (59), 22.10% (27), 22.30% (26) and 5.70% (70) of the 122 *Aspergillus* respectively were isolated. Conclusions: The presence of these *Aspergillus* species indicates that they may have been primary or secondary contributors to the embryonic mortality. Decontamination of hatcheries at regular intervals is recommended for control of these fungi.

Melloul *et al.* (2014) challenged different groups of few-day-old **turkeys** via intratracheal aerosolisation with increasing concentrations (10(5) up to 10(8)) of conidia using a MicroSprayer(®) device. The fungal burden was assessed by real-time PCR, galactomannan dosage, CFU counting and histopathological evaluation in order

to provide a comparison of these results within each inoculum groups. Significant mortality, occurring in the first 96h after inoculation, was only observed at the highest inoculum dose. Culture counts, GM index and qPCR results on the one hand and inoculum size on the other hand appeared to be clearly correlated. The mean fungal burden detected by qPCR was 1.3log10 units higher than the mean values obtained by CFU measurement. The new model and the markers will be used to evaluate the efficacy of antifungal treatments that could be used in poultry farms.

Singh *et al.* (2014) described a case of **mycotic tracheitis in an adult Rhode Island Red** bird of about 20 weeks of age. The bird had a history of dyspnoea, gasping and was dull prior to death. On postmortem examination lungs showed multiple circumscribed granulomatous nodules in the lungs and the trachea was occluded with caseous plugs. Microscopically there were fungal hyphae penetrating the tracheal mucosa together with a caseative plug having central necrotic mass adhering to the tracheal wall, foci of severe congestion and hemorrhage, fungal granuloma surrounded by mononuclear cell infiltration, giant cell, fungal hyphae and fibrous tissues were recorded. The fungal hyphae were also demonstrated by Grocott's methanamine silver stain.

Ziołkowska *et al.* (2014) carried out a study to determine the in vitro susceptibility of 85 Aspergillus fumigatus strains isolated from domestic geese and from their environment **to amphotericin B, clotrimazole, voriconazole, itraconazole, enilconazole, miconazole, ketoconazole, and tioconazole**. Samples were collected from clinically healthy birds (oral cavity) and from birds with aspergillosis (lungs and air sacs). The study was carried out using the disk diffusion method according to the Clinical Laboratory and Standards Institute (CLSI) M44-A2 procedure in parallel with the microdilution broth method according to CLSI M38-A2. The disk diffusion method showed that the all of the strains, irrespective of source, were resistant to miconazole. Resistance to the remaining azoles and amphotericin B ranged from 90.6 to 70.6%. Complete susceptibility was noted for voriconazole and enilconazole. Determination of the minimum inhibitory concentration (MIC) confirmed the high resistance of the strains tested to clotrimazole (MIC90 = 16 μg•mL(-1)), amphotericin B (MIC90 = 16 μg•mL(-1)), varied susceptibility to itraconazole (MIC 0.5-8 μg•mL(-1)), and 100% susceptibility to enilconazole and voriconazole. A correlation was noted between the susceptibility of the strains and their source. The highest percentage of resistant strains was noted in isolates from the lungs (100% for amphotericin B and clotrimazole and 35.7% for itraconazole). To the best of our knowledge, this is the first monitoring conducted in Poland in this area of research.

Fischer and Lierz. (2015) reported that antemortem diagnosis of avian aspergillosis is very challenging. Diagnostic assays using blood samples would aid in an early and more definitive diagnosis. In the current study, detection of anti ***Aspergillus* antibodies**, *Aspergillus* antigen, and *Aspergillus* toxin (fumigaclavine A), protein electrophoresis and measurement of acute-phase protein concentrations were performed on serum of 18 adult and plasma of 21 juvenile gyr–saker hybrid **falcons** (*Falco rusticolus* × *Falco cherrug*). Adult (n = 15) and juvenile (n = 18) falcons were experimentally inoculated with different dosages of the same strain of *Aspergillus fumigatus* and an additional three falcons from each age group were used as uninfected control animals. Blood samples were collected prior to inoculation and at

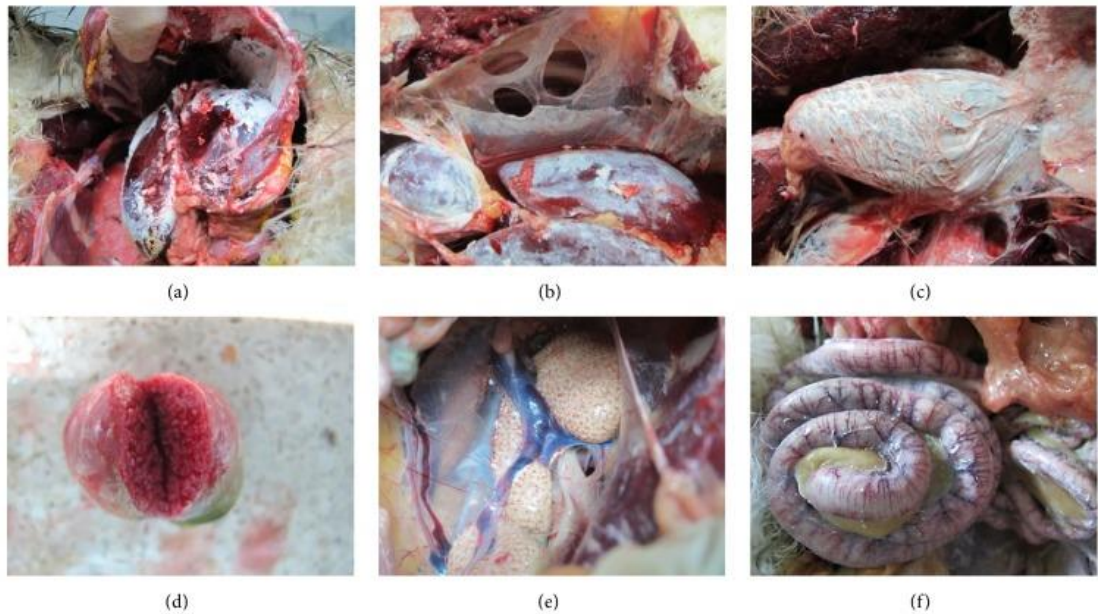
28 days postinoculation. Of the 33 inoculated falcons, 16 demonstrated clinical signs (vomiting, greenish urates, dyspnea, ruffled feathers) commonly associated with aspergillosis and in 14 falcons necropsy revealed aspergillosis granulomas confirmed by mycology and histopathology. Positive galactomannan results were rare, with only 3/15 positive samples from adult falcons and none in the juvenile birds. Most of the inoculated falcons showed an increase of serum amyloid A (66.7%) and haptoglobin (70.4%), but fumigaclavine A was not detected in the blood from any of the experimental animals. Elevated antibody indices were detected in 96.7% of the inoculated birds, but also in 66.7% of the controls. Significant decreases in albumin:globulin ratio were obvious in 81.5% of the inoculated birds, including 100% of the birds with granulomas. Blood from falcons with granulomas demonstrated significantly increased concentration values of alpha 2 and β globulins, decreased percentages of prealbumin and albumin, and increased percentages of alpha 2 and β globulins compared to inoculated falcons without granulomas. In conclusion, acute-phase proteins and the electrophoretic profile of birds challenged with ***A. fumigatus*** show significant alterations, which in combination with other diagnostic procedures, assist in the early diagnosis of avian aspergillosis.

Hausmann *et al.* (2015) collected serum samples from **wild adult cranes** (n = 22) at Aransas National Wildlife Refuge, Texas, USA during winter. Wild juvenile cranes (n = 26) were sampled at Wood Buffalo National Park, Northwest Territories, Canada, in midsummer. All captive crane samples were acquired from the International Crane Foundation, Baraboo, WI, USA. Captive adult cranes (n = 30) were sampled during annual examinations, and archived serum samples from captive juvenile cranes (n = 19) were selected to match the estimated age of wild juveniles. Wild juveniles had significantly lower concentrations of all protein fractions than wild adults, except for prealbumin and γ globulins. All protein fraction concentrations for wild juveniles were significantly lower compared with captive juveniles, except for prealbumin and γ globulins, which were higher. Wild adults had significantly greater γ globulin concentrations than captive adults. Captive juveniles had significantly lower prealbumin and albumin concentrations and albumin : globulin ratios than captive adults. The higher γ globulin concentrations in wild versus captive cranes were likely because of increased antigenic exposure and immune stimulation. Protein fraction concentrations varied significantly with age and natural history in this species.

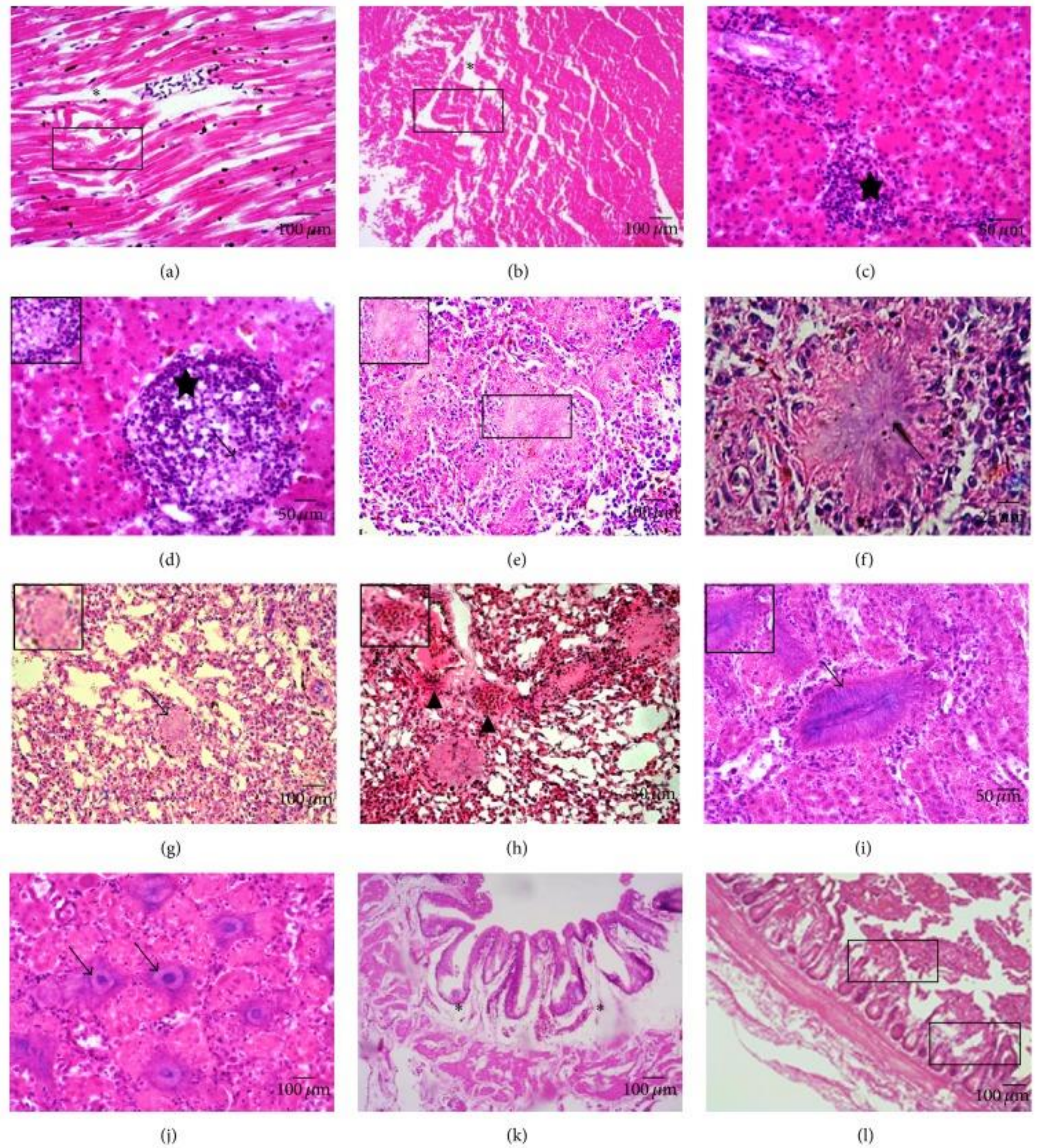
Hurley-Sanders *et al.* (2015) described **ducklings** (Bucephala albeola) with pelvic limb paresis. On postmortem examination, the duckling had intralesional fungal hyphae consistent with Aspergillus sp. in the spinal vertebrae and within pulmonary granulomas.

Li *et al.* (2015) reported a death which occurred in four **Himalayan griffons** housed in Beijing zoo, China. Based on pathogen identification and the pathological changes observed, the fungi and Hepatitis E virus (HEV) in four dead Himalayan griffons were characterized. Pathological changes were severe. Membranous-like material was observed on the surface of the internal organs. Spleen was necrotic. Focal lymphocyte infiltration in the liver and many sunflower-like fungi nodules were evident in the tissues, especially in the kidney. **PCR** was used to identify the pathogen. Based on the 18SrRNA genomic sequence of known fungi, the results confirmed that all four dead Himalayan griffons were infected with *Aspergillus*. At the same time the detection of HEV also showed positive results. To the best of our knowledge, this work appears to

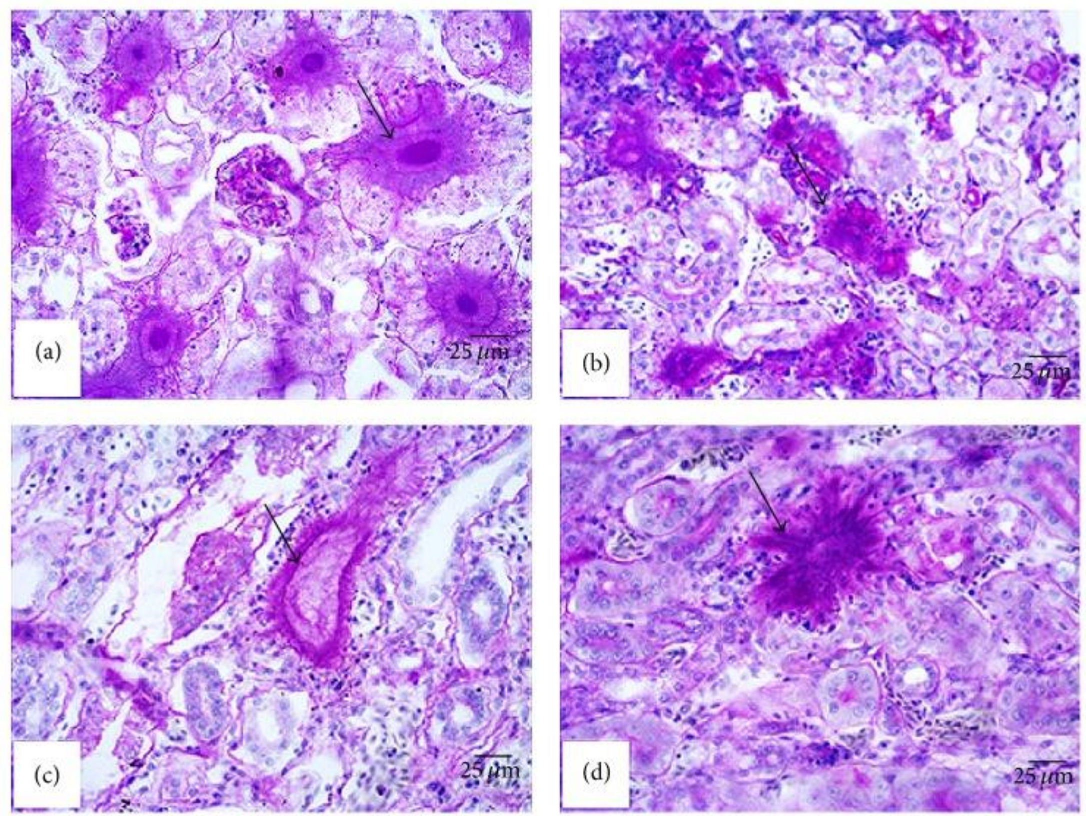
be the first report of concurrent presence of Aspergillosis and Hepatitis E virus in rare avian species.



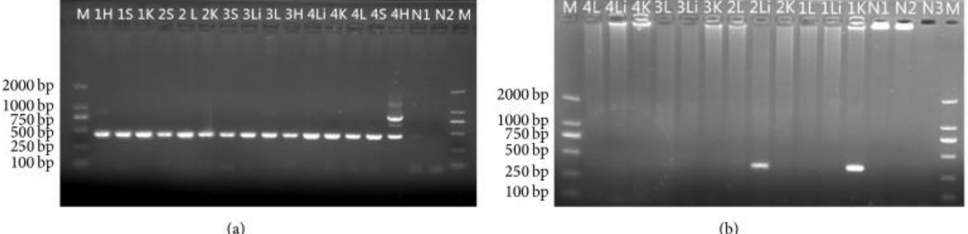
Necropsy observations. (a) Internal organs. (b) White liver and air sac. (c) Thick pericardium. (d) Spotted spleen. (e) Enlarged kidney. (f) Congestive vessel of the intestine. **Li *et al.* (2015)**



Histological lesions in multiple organs. Pathological changes were characterized by degeneration, edema, inflammatory infiltration, necrosis, and appearance of the flower-like fungi nodes. Heart (a, b): necrosis, edema, and wave-like degeneration in the cardiac muscle fiber. Liver (c, d): liver exhibiting hepatic necrosis and lymphocyte infiltration. Spleen (e, f): necrosis and fungi nodes in the spleen. Lung (g, h): lung with hemorrhage and fungi nodes. Kidney (i, j): many sunflower-like fungi nodes in the kidney. Intestine (k, l): edema and abruption of intestinal villi. **Li *et al.* (2015)**



Detection of fungi in the kidneys of the four dead Himalayan griffons. (a) Positive fungi signal in the kidney in number 1 griffon. (b) Positive fungi signal in the kidney in number 2 griffon. (c) Positive fungi signal in the kidney in number 3 griffon. (d) Positive fungi signal in the kidney in number 4 griffon. **Li *et al.* (2015)**

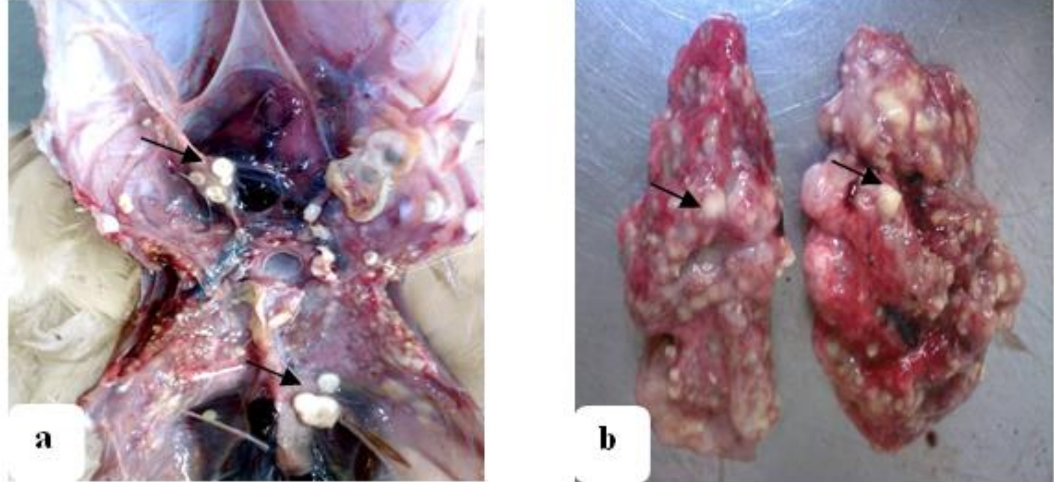


PCR assays of tissues with primers specific for fungi and HEV. (a) Fungi: lane M, DL2000 marker; 1, griffon 1 heart; 2, griffon 1 spleen; 3, griffon 1 kidney; 4, griffon 2 spleen; 5, griffon 2 lung; 6, griffon 2 kidney; 7, griffon 3 spleen; 8, griffon 3 liver; 9, griffon 3 lung; 10, griffon 3 heart; 11, griffon 4 liver; 12, griffon 4 kidney; 13, griffon 4 lung; 14, griffon 4 spleen; 15, griffon 4 heart; 16, negative control; 17, negative control; 18, lane M, DL2000 marker. The fungi amplicon was 425 bp. (b) HEV: positive for griffon 2 liver and griffon 1 kidney. The HEV amplicon was 348 bp, **Li *et al.* (2015)**

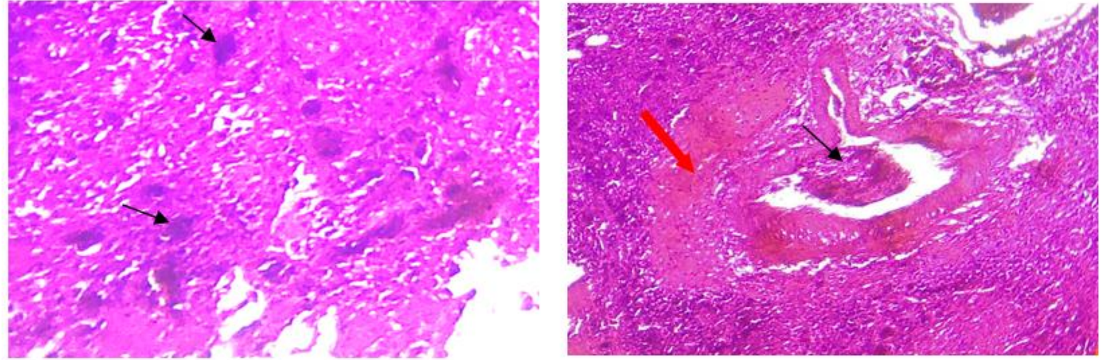
Sultana *et al.* (2015) carried out a study to investigate the pathology of avian aspergillosis in **commercial broiler chickens** at Chittagong district. A total of 912 sick and dead chickens were collected from 20 commercial broiler farms and diagnosed for avian aspergillosis on the basis of clinical signs, symptoms and postmortem findings. The suspected birds were collected for necropsy examination and mycological culture. Gross lesions of multiple hard creamy to yellow colored, circumscribe plaques throughout the lungs surface and consolidated lung with necrotic areas were observed. Microscopically, the typical nodules consisted of caseous necrotic center were present. The overall incidence of avian aspergillosis was found 6.14%. Among five Upazilla, significantly ($p<0.007$) higher and lower

incidence was found in Patenga and Sitakunda that were 9.25% and 3.43% respectively. It was observed that highest incidence (8.22%) in rainy and lowest (3.16%) in winter but moderate (5.16%) in summer season. The disease was significantly ($p<0.050$) higher (8.27%) in age between 6-10 days and lower (4.11%) in age between 0-5 days. It was also found that incidence of avian aspergillosis was significantly ($p<0.042$) higher in flocks reared on sawdust litter (7.69%) as compared to rice husk litter (3. 46%).

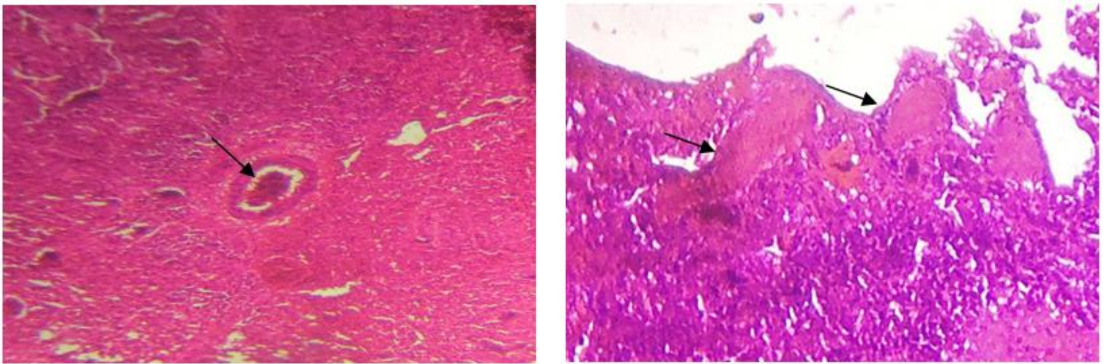
Bird showing gasping and b. Birds showing depression. **Sultana *et al.* (2015)**



Lung shows the presence of cream color nodules in plural surface, air sacs (arrow). b. Creamy to yellow color nodules shows throughout the lung (arrow) **Sultana *et al.* (2015)**.

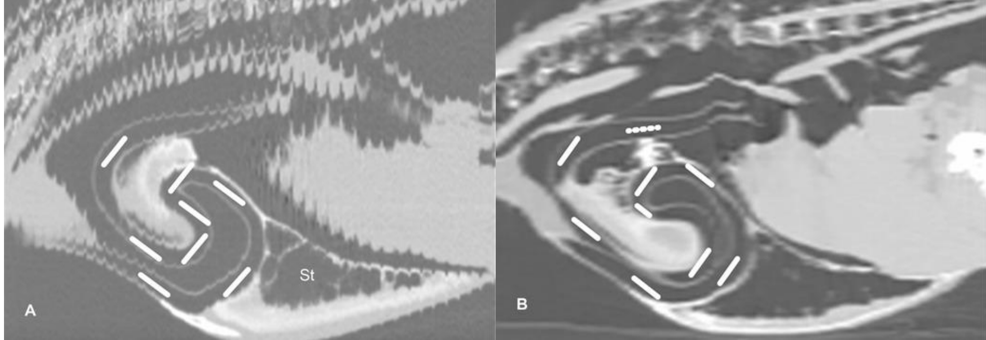


Showing congestion of pulmonary, perialveolar blood vessel and diffuse edema of pulmonary tissues (arrow) in Lung. H&E stain, 10×. Figure 6 . Areas of caseous necrosis (black arrow) and cellular debris (red arrow) in lung. H&E stain, 10×. **Sultana *et al.* (2015)**

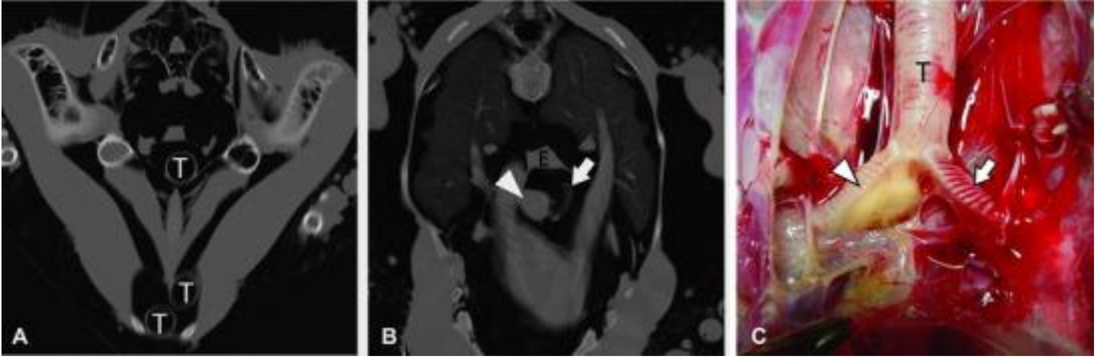


Aspergillosis showing granuloma formation with caseated center (arrow) in lung. H&E stain, 10×.Diffuse densification of the pleural parenchyma by congestion and an inflammatory cellular infiltration (arrows) in lung. H&E stain, 10×. **Sultana *et al.* (2015)**

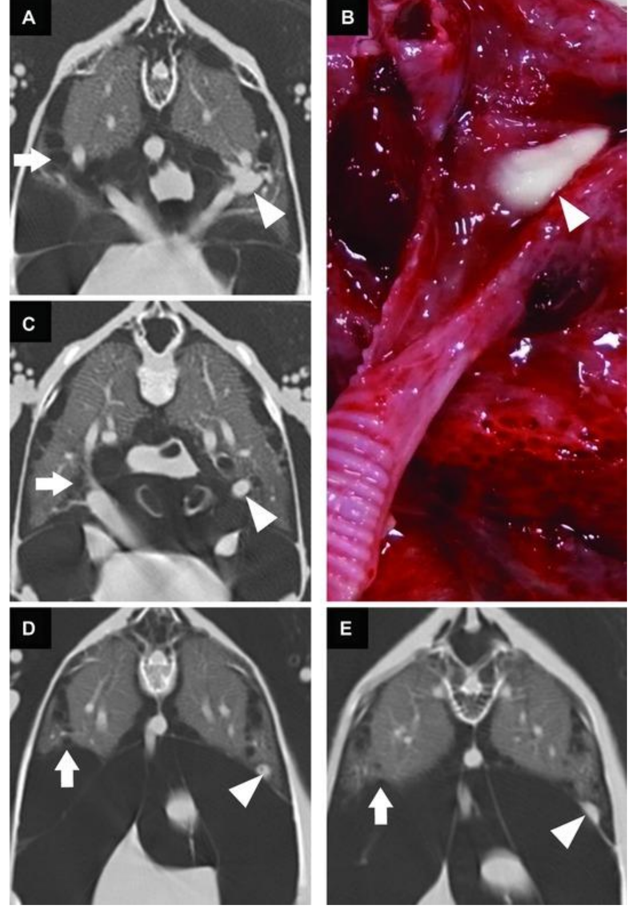
Schwarz *et al.* (2016) performed a retrospective, case series, cross-sectional study to describe computed tomography (CT) respiratory anatomy in a juvenile **whooping crane** without respiratory disease, compare CT characteristics with gross pathologic characteristics in a group of juvenile whooping cranes with respiratory aspergillosis, and test associations between the number of CT tracheal bends and bird sex and age. A total of 10 juvenile whooping cranes (one control, nine affected) were included. Seven affected cranes had CT characteristics of unilateral extrapulmonary bronchial occlusion or wall thickening, and seven cranes had luminal occlusion of the intrapulmonary primary or secondary bronchi. Air sac membrane thickening was observed in three cranes in the cranial and caudal thoracic air sacs, and air sac diverticulum opacification was observed in four cranes. Necropsy lesions consisted of severe, subacute to chronic, focally extensive granulomatous pathology of the trachea, primary bronchi, lungs, or air sacs. No false positive CT scan results were documented. Seven instances of false negative CT scan results occurred; six of these consisted of subtle, mild air sacculitis including membrane opacification or thickening, or the presence of small plaques found at necropsy. The number of CT tracheal bends was associated with bird age but not sex. Findings supported the use of CT as a diagnostic test for avian species with respiratory disease and tracheal coiling or elongated tracheae where endoscopic evaluation is impractical.



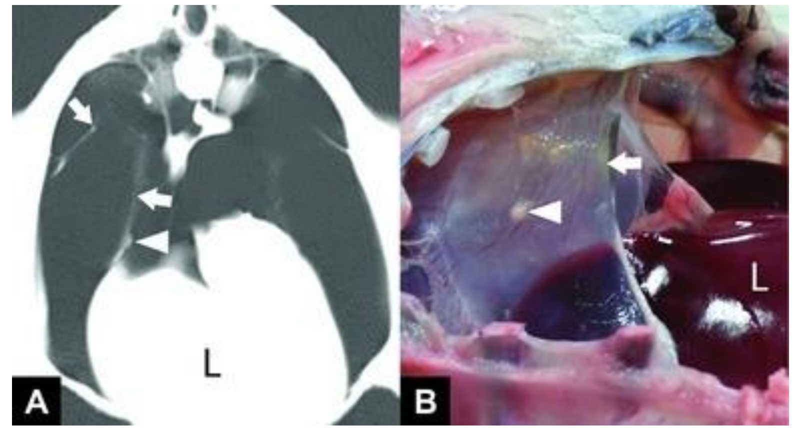
Sagittally reformatted CT images of intrasternal tracheal coiling in two whooping cranes with a total of 7 (A, case 6, 72 days old) and 7.5 bends (B, case 10, 117 days old). The full white bars represent the perpendicular tracheal long axis based on which the number of bends was calculated (dotted bar = half bend). The pneumatized carina of the sternum (St) that is housing the tracheal coils is easily visible. The jagged appearance is due to respiratory motion artifact. Computed tomography (CT) image of the coelom of a 35-day-old whooping crane (case 8) at the level of the caudal scapula (arrowhead). A region of interest tool (green circles) has been placed in the dorsolateral lung for measurement of mean lung density in Hounsfield units. **Schwarz *et al.* (2016)**



Computed tomography (CT) images (A and B) and corresponding necropsy photograph (C) of the trachea and the extrapulmonary portion of the emanating primary bronchi in a 66-day-old whooping crane (case 3). The normal left primary bronchus (arrow) is slit-shaped in cross-section with a thick cartilaginous lateral wall and a thinner medial vocal membrane. The right primary bronchus (arrowhead) is markedly distended and filled with soft tissue material to the level of the lung. The arrowhead is superimposed on the aortic arch. E, esophagus Schwarz et al. (2016)



Computed tomography (CT) images (A, C, and D) and corresponding necropsy photograph (B) of the occluded left lateroventral secondary bronchus (arrowheads) in a 50-day-old whooping crane (case 7) from its origin at the primary bronchus (A and B) via the caudal lung (C and D) to its entrance into the caudal thoracic airsac (E). Notice the similar course of the normal right lateroventral secondary bronchus (arrows). The true tubular shape of the bronchial obstruction is best appreciated in (B), whereas selected transverse CT images in (A, C, and E) give it a nodular appearance. **Schwarz *et al.* (2016)**



Computed tomography image (A) and corresponding necropsy photograph (B) of the right caudal thoracic air sac in a 35-day-old whooping crane (case 8). The air sac membrane (arrows) shows mild diffuse and nodular (arrowheads) thickening. The normally translucent membrane is opaque on necropsy. L, liver. **Schwarz *et al.* (2016)**

Tarello (2016) described parasitological, microbiological, and pathological findings associated with the isolation of **Aspergillus species** in 94 clinically diseased captive falcons from Dubai. Concomitant agents and/or diseases were identified in 64 cases, causing either single or multiple coinfections. Diagnoses found more often in association with aspergillosis were chronic fatigue and immune dysfunction syndrome (CFIDS), Caryospora sp., Serratospiculum seurati infestation, cestodiasis, bumblefoot, trematodosis due to Strigea falconispalumbi, trichomoniasis, Babesia shortti, Mannheimia (Pastorella) haemolytica, interstitial hepatitis, Escherichia coli, and Clostridium perfringens enterotoxemia. Compared with a control group of 2000 diseased falcons without evidence of aspergillosis, the prevalence of Babesia shortti, CFIDS, Mannheimia (Pastorella) haemolytica, Escherichia coli, and falcon herpes virus infection was conspicuously higher in association with aspergillosis. These entities may be considered suitable candidates as predisposing factors for the mycosis.

References:

1. Abdulrahman, N.R.. N.M. Saeed S.F. Muhammad. Clinical and histopathological study of brooder pneumonia in broiler farms. AL-Qadisiya Journal of Vet. Med. Sci. 13 1 2,014
2. Abou-Rawash, A. A., Yanai, T.; Kano R.; Murakami, M.; Masegi, T.; Fukushi, H.; Nagamine, T. and Hasegawa, A. Disseminated aspergillosis in a Whooper Swan (Cygnus Cygnus). Egypt. J. Comp. Path. & Clinic. Path. Vol. 21 No. 2 (April) 2008; 161- 174
3. Adrian, W. .J. T. R. Spraker, and R. B. Davies, "Epornitics of aspergillosis in mallards (Anas platyrhynchos) in North Central Colorado," Journal of Wildlife Diseases, vol. 14, no. 2, pp. 212–217, 1978.
4. Ainsworth, G.C.; Rewell, R.E. (1949). The incidence of aspergillosis in captive wild birds. *J. Comp. Pathol. Ther.*, 59, 213-224..
5. Akan M, Haziroğlu R, Ilhan Z, Sareyyüpoğlu B, Tunca R. A case of aspergillosis in a broiler breeder flock. Avian Dis. 2002 Apr-Jun;46(2):497-501.
6. Akkoc. A., Yilmaz, R., Cangul, I. T., Ozyigit, O. M. (2009) Pulmonary Aspergillosis and Amyloid Accumulation in an Ostrich (*Struthio camelus*). *Turk. J. Vet. Anim. Sci.* 33(2): 157-160.

7. Alvarez-Perez S., Mateos A., Dominguez L., Martinez-Nevado E., Blanco J. L., Garcia M. E. (2010).Polyclonal *Aspergillus fumigatus* infection in captive penguins. Vet Microbiol 144, 444–449
8. Araghi, M., A. Ghaniei & T. Heidari, 2014. Aspergillosis outbreaks in ostrich flocks of eastern Iran during 2010–2012. Bulg. J. Vet. Med., 17, No 4, 325–330.
9. Arca-Ruibal, B., U. Wernery, R. Zachariah, T. A. Bailey, A. Di Somma, C. Silvanose, and P. McKinney.Assessment of a commercial sandwich ELISA in the diagnosis of aspergillosis in falcons. *Vet. Rec.*158:442–444. 2006.
10. Arné P, Thierry S, Wang D, Deville M, Le Loc'h G, Desoutter A, Féménia F, Nieguitsila A, Huang W, Chermette R, Guillot J. Aspergillus fumigatus in Poultry. Int J Microbiol. 2011;2011:746356.
11. Atasever. A. and K. S. Gümüşsoy, "Pathological, clinical and mycological findings in experimental aspergillosis infections of starlings," Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medical, vol. 51, no. 1, pp. 19–22, 2004.
12. Azizi, S., Seyed Razi Ghalebi , Reza Kheirandish , Neda Ghasemi , Fateme Akrami Mohajeri. Aspergillosis and proventricular impaction in an ostrich (Struthio camelus). Journal of Coastal Life Medicine 2014; 2(8): 662-664
13. Balseiro, A., A. Espí, I. Márquez et al., "Pathological features in marine birds affected by the prestige's oil spill in the North of Spain," Journal of Wildlife Diseases, vol. 41, no. 2, pp. 371–378, 2005
14. Barton JT, Daft BM, Read DH, Kinde H, Bickford AA. Tracheal aspergillosis in 6 1/2-week-old chickens caused by Aspergillus flavus. Avian Dis. 1992 Oct-Dec;36(4):1081-5.
15. Bassiyoni, A., Saad, F., **Refai, M**. and El Batrawi, A. : Efficacy of serological and allergic diagnosis of **A. fumigatus** infection in chickens. J. Egypt. Vet. Med. Ass. 41, 161-171 (1981)
16. Beckman BJ, Howe CW, Trampel DW, DeBey MC, Richard JL, Niyo Y. Aspergillus fumigatus keratitis with intraocular invasion in 15-day-old chicks. Avian Dis. 1994 Jul-Sep;38(3):660-5.
17. **Beer, J.V. The incidence of *Aspergillus fumigatus* in the throats of wild geese and gulls. J. Med. Vet. Mycol. 2,4,238-247, 1963**
18. Beernaert L. A., Pasmans F., Haesebrouck F., Martel A. (2008). Modelling *Aspergillus fumigatus*infections in racing pigeons (Columba livia domestica). Avian Pathol 37, 545–549
19. Beernaert LA, Pasmans F, Van Waeyenberghe L, Haesebrouck F, Martel A. Aspergillus infections in birds: a review. Avian Pathol. 2010 Oct;39(5):325-31
20. Beytut E, Ozcan K, Erginsoy S. Immunohistochemical detection of fungal elements in the tissues of goslings with pulmonary and systemicaspergillosis. Acta Vet Hung. 2004;52(1):71-84.
21. Bhattacharya, A. (2003) *Aspergillus fumigatus* infection in Khaki Campbell ducks in an organized duck farm in Tripura. *Indian Vet. J.* 80(11): 1178-1179.
22. Burco JD, Ziccardi MH, Clemons KV and Tell LA. 2012. Evaluation of plasma (1→3) β-D-glucan concentrations in birds naturally and experimentally infected with Aspergillus fumigatus. Avian Diseases, 56(1): 183-191.
23. *Burco, J.D., J. Gregory Massey, Barbara A. Byrne, Lisa Tell, Karl V. Clemons, .and Michael H. Ziccardi, MONITORING OF FUNGAL LOADS IN SEABIRD REHABILITATION CENTERS WITH COMPARISONS TO NATURAL SEABIRD ENVIRONMENTS IN NORTHERN CALIFORNIA. Journal of Zoo and Wildlife Medicine 45(1):29-40. 2014*
24. Cacciuttolo E, Rossi G, Nardoni S, Legrottaglie R, Mani P. Anatomopathological aspects of avian aspergillosis. Vet Res Commun. 2009 Aug;33(6):521-7.

25. Cafarchia C, Camarda A, Iatta R, Danesi P, Favuzzi V, Di Paola G, Pugliese N, Caroli A, Montagna MT, Otranto D. Environmental contamination by Aspergillus spp. in laying hen farms and associated health risks for farm workers. J Med Microbiol. 2014 Mar;63(Pt 3):464-70.
26. Ceolin L.V., Flores F., Oliveira I.M.C., Lovato M., Galiza G.J.N., Kommers G.D., Risso N. & Santurio J.M. 2012. Macroscopic and Microscopic Diagnosis of Aspergillosis in Poultry. Acta Scient. Vet. 40(3):1061.
27. Chaudhary, S. K., J. R. Sadana & A. K. Pruthi. Sequential pathological studies in Japanese quails infected experimentally with Aspergillus fumigatus. Mycopathologia 103:157-166 (1988)
28. Chege S, Howlett J, Al Qassimi M, Toosy A, Kinne J, Obanda V. Opportunistic infection of *Aspergillus* and bacteria in captive Cape vultures (*Gyps coprotheres*). *Asian Pacific Journal of Tropical Biomedicine*. 2013;3(5):401-406.
29. Copetti, M. V., S. D. Segabinazi, M. L. Flores, S. H. Alves, and J. M. Santurio, “Pulmonary aspergillosis outbreak in Rhea americana in Southern Brazil,” Mycopathologia, vol. 157, no. 3, pp. 269–271, 2004.
30. Corkish,J.D. “Mycotic tracheitis in chickens,” Avian Pathology, vol. 11, pp. 627–629, 1982.
31. Cortes PL, Shivaprasad HL, Kiupel M, Sentíes-Cué G. Omphalitis associated with Aspergillus fumigatus in poults. Avian Dis. 2005 Jun;49(2):304-8.
32. Cray, C., T. Watson, M. Rodriguez, and K. L. Arheart, “Application of galactomannan analysis and protein electrophoresis in the diagnosis of aspergillosis in avian species,” Journal of Zoo and Wildlife Medicine, vol. 40, no. 1, pp. 64–70, 2009
33. *Cray, C. Toshiba Watson, and Kristopher L. Arheart. Serosurvey and Diagnostic Application of Antibody Titers to Aspergillus in Avian Species.* Avian Diseases 53(4):491-494. 2009
34. de Wit JJ, van Cutsem J, Schoenmaker GJ, Braunius WW, van den Bergh JP. A severe Aspergillus flavus infection in slaughtering chicks and the effect of disinfection with enilconazole: a case study. Tijdschr Diergeneeskd. 1993 Aug 15;118(16):511-3.
35. Dyar, P.M.,O. J. Fletcher, and R. K. Page, “Aspergillosis in turkeys associated with use of contaminated litter,” Avian Diseases, vol. 28, no. 1, pp. 250–255, 1984.
36. Edris, G., **Refai, M**., Amin, A., Oraby, F., El-Shater, S., Hegazi, A., Azzam, A. and Abdel-Aziz, A. : Studies on the efficiency of antifungal drugs in treatment of experimentally infected turkey poults with A. flavus. Proc. 2d Sci. Conf. Egypt. Vet. Poultry Ass. 101-109 (1990)
37. Eggert, M.J. and Barnhart, J.V. (1953). A case of egg-borne aspergillosis. *Journal of American Veterinary Medical Association.*122:225.
38. Femenia F, Fontaine JJ, Lair-Fulleringer S, Berkova N, Huet D, Towanou N, Rakotovao F, Granet OI, Le Loc'h G, Arné P, Guillot J. Clinical, mycological and pathological findings in turkeys experimentally infected by Aspergillus fumigatus. Avian Pathol. 2007 Jun;36(3):213-9.
39. Fischer, D. and Michael Lierz. (2015) Diagnostic Procedures and Available Techniques for the Diagnosis of Aspergillosis in Birds. *Journal of Exotic Pet Medicine* 24, 283-295.
40. Flach, E.J.; Stevenson, M.F.; Henderson, G.M. (1990). Aspergillosis in gentoo penguins (*Pygoscelis papua*) at Edinburgh Zoo, 1964-1988. *Vet. Rec.*, 126(4), 81-85.
41. França M, Cray C, Shivaprasad HL. Serologic testing for aspergillosis in commercial broiler chickens and turkeys. Avian Dis. 2012 Mar;56(1):160-4.

42. German, A. C., G. S. Shankland, J. Edwards, and E. J. Flach. 2002. Development of an indirect ELISA for the detection of serum antibodies to *Aspergillus fumigatus* in captive penguins. Vet. Rec 150:513–518.
43. Glare TR, Gartrell BD, Brookes JJ, Perrott JK. Isolation and identification of Aspergillus spp. from brown kiwi (Apteryx mantelli) nocturnal houses in New Zealand. Avian Dis. 2014 Mar;58(1):16-24.
44. Ghori, H. M. and S. A. Edgar, "Comparative susceptibility of chickens, turkeys and Coturnix quail to aspergillosis," Poultry science, vol. 52, no. 6, pp. 2311–2315, 1973.
45. Graczyk TK, Cranfield MR, Klein PN. Value of antigen and antibody detection, and blood evaluation parameters in diagnosis of avian invasiveaspergillosis. Mycopathologia. 1997-1998;140(3):121-7.
46. Guilherme Augusto Marietto-Gonçalves, Fabrizio Grandi, Noeme Sousa Rocha, Raphael Lucio , Andreatti Filho. SECONDARY Aspergillus fumigatus infection associated with coloidal goiter in a black-masked lovebird (Agapornis personata). Acta Veterinaria Brasilica,.8,.1,.68-73, 2014
47. Hadrich I, Drira I, Neji S, Mahfoud N, Ranque S, Makni F, Ayadi A. Microsatellite typing of Aspergillus flavus from clinical and environmental avian isolates. J Med Microbiol. 2013a Jan;62(Pt 1):121-125.
48. Hadrich I, Amouri I, Neji S, Mahfoud N, Ranque S, Makni F, Ayadi A. Genetic structure of Aspergillus flavus populations in human and avian isolates. Eur J Clin Microbiol Infect Dis. 2013b Feb;32(2):277-82.
49. Hamet N, Seigle-Murandi F, Steiman R. Contribution to the prophylaxis of chicks aspergillosis: study of the contamination of a hatchery by Aspergillus fumigatus. Zentralbl Veterinarmed B. 1991 Sep;38(7):529-37.
50. **Harold C. McDougle and Richard W. Vaught. An Epizootic of Aspergillosis in Canada Geese,*The Journal of Wildlife Management,* 32, 2 (Apr., 1968), 415-417**
51. Hausmann, J. C. Hausmann, Carolyn Cray and Barry K. Hartup (2015) Comparison of Serum Protein Electrophoresis Values in Wild and Captive Whooping Cranes (*Grus americana*). *Journal of Avian Medicine and Surgery* 29:3, 192-199.
52. Hurley-Sanders JL, Larsen RS, Troan B, Loomis M. FUNGAL OSTEOMYELITIS IN TWO BUFFLEHEAD DUCKLINGS (BUCEPHALA ALBEOLA). J Zoo Wildl Med. 2015 Sep;46(3):613-6.
53. İÇEN, Hasan, Nuretin IŞIK, Simten YEŞİLMEN, Mehmet TUZCU, Servet SEKİN. Diagnosis and Treatment of Aspergillosis in An Ostrich Flock. Kafkas Univ Vet Fak Derg. 17 (4): 671-674, 2011
54. ISLAM, M. N. , S. M. H. RASHID1 , M. S. B. JULI2 , U. K. RIMA2 , AND M. KHATUN. PNEUMOMYCOSIS IN CHICKENS: CLINICAL, PATHOLOGICAL AND THERAPEUTICAL INVESTIGATION. Int. J. Sustain. Crop Prod. 4(3):16-21 (May 2009)
55. Ivey, E. S. 2000. Serologic and plasma protein electrophoretic findings in 7 psittacine birds with aspergillosis. J. Av. Med. Surg 14:103–106.
56. Jacobsen ID, Grosse K, Slesiona S, Hube B, Berndt A, Brock M. Embryonated eggs as an alternative infection model to investigate Aspergillus fumigatus virulence. Infect Immun. 2010 Jul;78(7):2995-3006
57. Jensen HE, Christensen JP, Bisgaard M, Nielsen OL. Immunohistochemistry for the diagnosis of aspergillosis in turkey poults. Avian Pathol. 1997;26:5–18
58. Julian, R. J. and M. Goryo, "Pulmonary aspergillosis causing right ventricular failure and ascites in meat-type chickens," Avian Pathology, vol. 19, no. 4, pp. 643–654, 1990

59. Jung K, Kim Y, Lee H, Kim JT. *Aspergillus fumigatus* infection in two wild Eurasian black vultures (*Aegypius monachus* Linnaeus) with carbofuran insecticide poisoning: a case report. Vet J. 2009;179:307–312.
60. Khosravi AR, Shokri H, Ziglari T, Naeini AR, Mousavi Z, Hashemi H. Outbreak of severe disseminated aspergillosis in a flock of ostrich (Struthio camelus). Mycoses. 2008 Nov;51(6):557-9.
61. Korniłłowicz-Kowalska T, Kitowski I. Aspergillus fumigatus and other thermophilic fungi in nests of wetland birds. Mycopathologia. 2013 Feb;175(1-2):43-56
62. Kummrow M, Silvanose C, Di Somma A, et al. Serum protein electrophoresis by using high-resolution agarose gel in clinically healthy and *Aspergillus* species-infected falcons. *J Avian Med Surg*.2012;26(4):213–220.
63. Kunkle RA, Rimler RB. Pathology of acute aspergillosis in turkeys. Avian Dis. 1996 Oct-Dec;40(4):875-86.
64. **Kunkle RA, Rimler RB. Early pulmonary lesions in turkeys produced by nonviable Aspergillus fumigatus and/or Pasteurella multocida lipopolysaccharide. Avian Dis. 1998a Oct-Dec;42(4):770-8**
65. Kunkle RA, Sacco RE. Susceptibility of convalescent turkeys to pulmonary aspergillosis. Avian Dis. 1998b Oct-Dec;42(4):787-90.
66. Kureljušić B., Radanović O., Kureljušić J., Jezdimirović N., Maslić-Strižak D., Prodanović R., Ivetić V. The occurrence of aspergillosis in flock of turkey poults, Biotechnology in Animal Husbandry, 2012 28(1):129-136
67. Kwanashie,C.N., Paul A Abdu, Jarlath U Umoh, Haruna M. Kazeem. Retrospective study of aspergillosis and other fungi in poultry (1980-2008), Int. J. Livest. Res.. 2012; 2(3): 84-88
68. Kwanashie, C. N.; Kazeem, H. M.; Umoh, J. U.; Abdu, P. A.. *Aspergillus* species associated with dead-in-shell chick embryo in some hatcheries in Northwest Nigeria. Eurasian Journal of Veterinary Sciences 2014 Vol. 30 No. 1 pp. 11-13 1309-6958
69. Lair-Fulleringer S, Guillot J, Desterke C, Seguin D, Warin S, Bezille A, Chermette R, Bretagne S. Differentiation between isolates of Aspergillus fumigatus from breeding turkeys and their environment by genotyping with microsatellite markers. J Clin Microbiol. 2003 Apr;41(4):1798-800.
70. Leishangthem, G.D. , N.D. Singh* , R.S. Brar and H.S. Banga Aspergillosis in Avian Species: A Review. Journal of Poultry Science and Technology, 2015,3 , 1 , 01-14
71. Li H, Zhu R, She R, et al. Case Report Associated with Aspergillosis and Hepatitis E Virus Coinfection in Himalayan Griffons. *BioMed Research International*. 2015;2015:287315. doi:10.1155/2015/287315.
72. Lisa A. Tell· Karl V. Clemons, Yvonne Kline, Leslie Woods, Philip H. Kass,Marife Martinez and David A. Stevens. Efficacy of voriconazole in Japanese quail (*Coturnix japonica*) experimentally infected with*Aspergillus fumigatus.* Med Mycol (2010) 48 (2): 234-244.
73. Low, M., Berggren, A., Morgan, K.J., Alley, M.R.: Aspergillosis in a North Island robin (Petroica longipes). N. Z. Vet. J., 2005; 53:462-464.
74. Martin MP, Bouck KP, Helm J, Dykstra MJ, Wages DP, Barnes HJ. Disseminated Aspergillus flavus infection in broiler breeder pullets. Avian Dis. 2007 Jun;51(2):626-31.
75. M i l o š C . K a p e t a n o v , D u b r a v k a V . P o t k o n j a k , Dubravka S. Milanov, Igor M. Stojanov , Milica M. Živkov Baloš, Bojana Z. Prunić. INVESTIGATION OF DISSEMINATION OF ASPERGILLOSIS IN POULTRY AND POSSIBLE CONTROL MEASURES. Proc. Nat. Sci, Matica Srpska Novi Sad, № 120, 267—276, 2011
76. Melloul E, Thierry S, Durand B, Cordonnier N, Desoubeaux G, Chandenier J, Bostvironnois C, Botterel F, Chermette R, Guillot J, Arné P. Assessment of

Aspergillus fumigatus burden in lungs of intratracheally-challenged turkeys (Meleagris gallopavo) by quantitative PCR, galactomannan enzyme immunoassay, and quantitative culture. Comp Immunol Microbiol Infect Dis. 2014 Dec;37(5-6):271-9.

77. **Moore. E. N.. *Aspergillus fumigatus* as a Cause of Ophthalmitis in Turkeys, Poultry Science (1953) 32 (5):796-799.**
78. Mukaratirwa S. Outbreak of disseminated zygomycosis and concomitant pulmonary aspergillosis in breeder layer cockerels. J Vet Med B Infect Dis Vet Public Health. 2006 Feb;53(1):51-3.
79. Nouri, M. , Abbas Tavasoli and Fatemeh Arabkhazaeli. Dual Infection of Respiratory Tract with an *Aspergillus* sp. and Bacilli in a Canary (*Serinus canarius*). Research Journal of Poultry Sciences. 2013 | Volume: 6 | Issue: 2 | Page No.: 33-37
80. Okoye JO, Gugnani HC, Okeke CN. Clinical and pathological features of Aspergillus fumigatus infections in poultry in southern Nigeria. Rev Elev Med Vet Pays Trop. 1989a;42(2):153-4.
81. Okoye JO, Gugnani HC, Okeke CN. Pulmonary infections due to Aspergillus flavus in turkey poults and goslings. Mycoses. 1989b Jul;32(7):336-9.
82. Olias P, Hauck R, Windhaus H, van der Grinten E, Gruber AD, Hafez HM. Articular aspergillosis of hip joints in turkeys. Avian Dis. 2010 Sep;54(3):1098-1101.
83. Olias P., Gruber A. D., Hafez H. M., Lierz M., Slesiona S., Brock M., Jacobsen I. D. (2011).Molecular epidemiology and virulence assessment of *Aspergillus fumigatus* isolates from white stork chicks and their environment. Vet Microbiol 148, 348–355
84. Olson, L. D., “Case report—aspergillosis in Japanese quail,” Avian Diseases, vol. 13, no. 1, pp. 225–227, 1969.
85. O'Meara, D. C. and H. L. Chute, “Aspergillosis experimentally produced in hatching chicks,” Avian Diseases, vol. 3, pp. 404–406, 1959.
86. Ozmen O, Dorrestein MG. Observations of aspergillosis in the brains of turkey poults using different histopathological staining techniques. Biotech Histochem. 2004 Apr;79(2):95-9.
87. Pal M, Prajapati S, Gangopadhyay RM. Aspergillus fumigatus as a cause of mycotic tracheitis in a chicken. Mycoses. 1990 Feb;33(2):70-2.
88. PALYA, V.; BALOGH, T. Cerebral aspergillosis in geese and turkeys. Magyar Allatorvosok Lapja 1971 Vol. 26 No. 6 pp. 307-310
89. Peden WM, Rhoades KR. Pathogenicity differences of multiple isolates of Aspergillus fumigatus in turkeys. Avian Dis. 1992 Jul-Sep;36(3):537-42.
90. **Perelman B, Kuttin ES. Aspergillosis in ostriches. Avian Pathol. 1992;21(1):159-63**
91. Perelman B, Smith B, Bronstein D, Gur-Lavie A, Kuttin ES. Use of azole compounds for the treatment of experimental aspergillosis in turkeys. Avian Pathol. 1992 Dec;21(4):591-9.
92. Ramisz A, Balicka-Ramisz A. Studies on usefullness of imidasol preparations for treatment of pulmonary and air sacks aspergillosis in geese. Wiad Parazytol. 2001;47(4):833-7.
93. REDIG, P. T, M. R. FULLER, D. L. EVANS. PREVALENCE OF Aspergillus fumigatus IN FREE-LIVING GOSHAWKS (Accipiter gentilis atricapillusjJournal of Wildlife Diseases Vol. 16, No. 2, April, 1980 16175
94. Redmann T, Schildger B. Therapeutic use of enilconazole in broiler chicks with aspergillosis, Dtsch Tierarztl Wochenschr. 1989 Jan;96(1):15-7.
95. **Refai, M**. : Ueber das Vorkommen von Schimmelpilzen in der Gefluegelindustrie, Bestimmung der pathogenitaet von Schimmelpilzen in Huenerembryonentest und Disinfektionsversuche. In Grimmer, H. and Rieth, H., Endomykosen und Dermatmykosen. Grosse Verlag, Berlin (1971)

96. **Refai, M**. and Abbasi, K. : A preliminary investigation on an outbreak of **aspergillosis** in turkey chicks. Vet. Med. J. Giza 12, 377-378 (1966)
97. **Refai, M**. and El-Bahay, G. Incidence of **moulds** in poultry feeds in Egypt. Mykosen 11, 459-462 (1968)
98. Refai, M. and Rieth, H. (1966).Seuchenhaftes Auftreten von Lungenaspergillose be Putenkueken in Aegypten. Mycosen, 9.163-165
99. **Refai, M**., Hammad, H., Salem, N., Abdel-Aziz, A., El-Shater, S., Azzam, A. and Edris, G. :Mycotic infections in birds and rabbits and their control. Vet. Med. J. 38, 129-143 (1990)
100. Reza, K., Nasrin, A., & Mahmoud, S. (2013). Clinical and pathological findings of concurrent poxvirus lesions and aspergillosis infection in canaries. *Asian Pacific Journal of Tropical Biomedicine*, *3*(3), 182–185. http://doi.org/10.1016/S2221-1691(13)60046-5
101. Richard J. L., Thurston J. R. (1983). Rapid hematogenous dissemination of *Aspergillus fumigatus* and*A. flavus* spores in turkey poults following aerosol exposure. Avian Dis 27, 1025–1033
102. Richard JL, DeBey MC. Production of gliotoxin during the pathogenic state in turkey poults by Aspergillus fumigatus Fresenius. Mycopathologia. 1995;129(2):111-5.
103. Richard JL, Dvorak TJ, Ross PF. Natural occurrence of gliotoxin in turkeys infected with Aspergillus fumigatus, Fresenius. Mycopathologia. 1996;134(3):167-70.
104. *Saif, A. and **Refai, M**. : The use of thiabendazole to control moulds in poultry farms. Castellania 5, 183-187 (1977)
105. Schwarz, T., Kelley, C., Pinkerton, M. E., & Hartup, B. K. (2016). COMPUTED TOMOGRAPHIC ANATOMY AND CHARACTERISTICS OF RESPIRATORY ASPERGILLOSIS IN JUVENILE WHOOPING CRANES. *Veterinary Radiology & Ultrasound*, *57*(1), 16–23. http://doi.org/10.1111/vru.12306
106. Shathele, M.S., A. Fadlelmula, F.A. Al-Hizab and M.M. Zaki, 2009. Fatal Aspergillosis in an Ostrich (*Struthio camelus*) Predisposed by Pulmonary Haemangioma in the Kingdom of Saudi Arabia. *International Journal of Zoological Research, 5: 80-85.*
107. Singh, S., M. K. Borah, D. K. Sharma, et al., "Aspergillosis in turkey poults," Indian Journal of Veterinary Pathology, vol. 33, no. 2, pp. 220–221, 2009
108. Singh N, Grewal GS, Brar RS, Singh B, Dhaliwal AS, Jand SK. Outbreak of mycotic tracheitis in domestic fowl. Mycoses. 1993 Jan-Feb;36(1-2):65-7.
109. Singh, B. R., Kumar Rahul*, Chogule Amit Ajit, Banga Harmanjit Singh. Pathology of Mycotic Tracheitis in Poultry. Journal of Animal Research, 2014,: 4, 2: (247) Last page : (250)
110. Sinha, B. K., Sharma, T. S., Sinha, P. N. and Jha, G. J. (1978), Aspergillosis in a Brahmini Duck (Tadoroma ferruginea, Pallus) – A Case Report. Mycoses, 21: 307–310
111. Spanamberg A Gustavo Machado ; Renata Assis Casagrande; Gabriela Miller Sales , Cibele Floriano Fraga; Luís Gustavo Corbellini[1]; David Driemeier[1]; Laerte Ferreiro . *Aspergillus fumigatus* from normal and condemned carcasses with airsacculitis in commercial poultry, Pesq. Vet. Bras. 33,9. Rio de Janeiro Sept. 2013
112. Souza, M. J, and Laurel A. Degernes,. Mortality Due to Aspergillosis in Wild Swans in Northwest Washington State, 2000–02. Journal of Avian Medicine and Surgery 19(2):98–106, 2005
113. Stoute ST, Bickford AA, Walker RL, Charlton BR. Mycotic pododermatitis and mycotic pneumonia in commercial turkey poults in northern California. J Vet Diagn Invest. 2009 Jul;21(4):554-7. Infect Immun. 2010 July;78(7):2995-3006.

114. *Sultana,S., S. M. H. Rashid, M. N. Islam, M. H. Ali, M. M. Islam, and M. G. Azam*. Pathological Investigation of Avian Aspergillosis in Commercial Broiler Chicken at Chittagong District. International Journal of Innovation and Applied Studies ISSN 2028-9324 Vol. 10 No. 1 Jan. 2015, pp. 366-376

115. Tarello, Walter. "Etiologic Agents and Diseases Found Associated with Clinical Aspergillosis in Falcons." *International Journal of Microbiology* 2011 (2011): 176963. *PMC*. Web. 20 Apr. 2016.

116. Tell LA, Craigmill AL, Clemons KV, Sun Y, Laizure SC, Clifford A, Ina JH, Nugent-Deal JP, Woods L, Stevens DA. Studies on itraconazole delivery and pharmacokinetics in mallard ducks (Anas platyrhynchos). J Vet Pharmacol Ther. 2005 Jun;28(3):267-74.

117. Tijani, MO, OO Alaka, AO Ogunleye, OO Esan. Pulmonary Aspergillosis in an Adult Male Ostrich (Struthio camelus): A Case Report. Tropical Veterinarian. 30, 3 (2012)

118. Thierry S, Durand B, Melloul E, Tafani JP, Wang DY, Deville M, Cordonnier N, Chermette R, Guillot J, Arné P. Assessment of Aspergillus fumigatus pathogenicity in aerosol-challenged chickens (Gallus gallus) belonging to two lineages. Comp Immunol Microbiol Infect Dis. 2013 Jul;36(4):379-85

119. Tokarzewski , S., G. Ziółkowska, W. Łopuszyński, and Z. Nozdryn-Płotnick, "Aspergillus fumigatusinfection in a pigeon flock," Bulletin of the Veterinary Institute in Pulawy, vol. 51, no. 4, pp. 563–567, 2007

120. Throne Steinlage SJ, Sander JE, Brown TP, Lobsinger CM, Thayer SG, Martinez A. Disseminated mycosis in layer cockerels and pullets. *Avian Diseases*. 2003;47(1):229–233.

121. *TÜRKÜTANIT*,S. S. Pulmonary Aspergillosis in Geese. Turkish Journal of Veterinary and Animal Science.22,1, 1999, 346 167

122. Van Waeyenberghe L., Pasmans F., Beernaert L. A., Haesebrouck F., Vercammen F., Verstappen F., Dorrestein G. M., Klaassen C. H., Martel A. (2011). Microsatellite typing of avian clinical and environmental isolates of *Aspergillus fumigatus*. Avian Pathol 40, 73–77

123. Veselský M, Otcenásek M, Duba J, Ditrich O. Mass occurrence of mycotic tracheitis in chickens, Vet Med (Praha). 1984 May;29(5):301-6.

124. WALKER, J. Aspergillosis in the Ostrich Chick. (Synonyms: Yellow Liver, Chick Fever). Union of South Africa. Dept. of Agric., 3rd & 4th Reports of the Director of Veterinary Research. 1915, Nov pp. 533-574 pp.

125. Xavier, M. O., M. P. Soares, A. R. M. Meinerz et al., "Aspergillosis: a limiting factor during recovery of captive magellanic penguins," Brazilian Journal of Microbiology, vol. 38, no. 3, pp. 480–484, 2007

126. Yamada,S., S. Kamikawa, Y. Uchinuno, et al., "Avian dermatitis caused by Aspergillus fumigatus," Journal of the Japan Veterinary Medical Association, vol. 30, pp. 200–202, 1977

127. Yokota, T., T. Shibahara, Y. Wada, R. Hiraki, Y. Ishikawa and K. Kadota, 2004. *Aspergillus fumigatus* infection in an ostrich (*Struthio camelus*). J. Vet. Med. Sci., 66: 201-204.

128. Zafra R, Pérez J, Pérez-Ecija RA, Borge C, Bustamante R, Carbonero A, Tarradas C. Concurrent aspergillosis and ascites with high mortality in a farm of growing broiler chickens. Avian Dis. 2008 Dec;52(4):711-3.

129. Zinkl JG, Hyland JM, Hurt JJ. "Aspergillosis in common crows in Nebraska, 1974," Journal of Wildlife Diseases, vol. 13, no. 2, pp. 191–193, 1977.

130. Ziołkowska G, Tokarzewski S, Nowakiewicz A. Drug resistance of Aspergillus fumigatus strains isolated from flocks of domestic geese in Poland. Poult Sci. 2014 May;93(5):1106-12.

3.2. Avian mucormycosis

Clinical symptoms and lesions

- Mucormycosis has been reported in domestic and wild birds. Chickens are less susceptible to Mucor infection. The most common types of the disease conditions are oral and cerebral mucormycosis.
- Mucor species have been implicated as an etiologic agent of pneumonic lesions, infection in the eyes and vertebrae. The infection can spread to gastrointestinal tract, skin and other organs.
- The feeding of damp, germinated seed has been implicated in disseminated mucormycosis causing alimentary granulomas in a group of canaries (Serinus canarius) and nephritis in an African grey parrot; glossitis in an African grey parrot; myocarditis in an Australian parakeet (Psittacula sp.); and nasal infection in waterfowl.
- Hyphal invasion of cerebral blood vessels and dissemination of an Absidia sp. in the cerebrum was identified as the cause of progressive neurologic defects culminating in seizures in a chattering lory (Lorius garrulus).
- Other clinical syndromes described include air sacculitis in a pigeon (Columba sp.), pneumonia in a rock hopper penguin (Eudyptes crestatus) and a group of rock ptarmigan (Lagopus mutus), and an osteolytic mass involving the ribs and air sacs of a penguin (Sphenisciformes).

Diagnosis

- Antemortem diagnosis of mucormycosis is difficult because the organisms do not culture well from clinical samples.
- Presumptive diagnosis requires biopsy examination of the affected tissue, while examination of swabs of tissue or discharges is generally untrustworthy.
- Histopathology of biopsy specimens is more reliable in confirming the diagnosis.
- Isolation and identification of the fungus

Treatment and control

- Contaminated litter need to be removed to effectively control the disease.
- No effective treatment of mucormycosis in birds has been reported
- Administration of one table-spoonful of 33% potassium iodide solution in drinking water per nearly 200 birds or antifungal drugs is helpful
- Amphotericin Bb is the single most reliable agent used in humans. Other antifungal medications including nystatinh , 5-fluorcytosinem, clotrimazolee and miconazolel are reported to have no consistent in vivo activity against the Mucorales.

Aetiology:

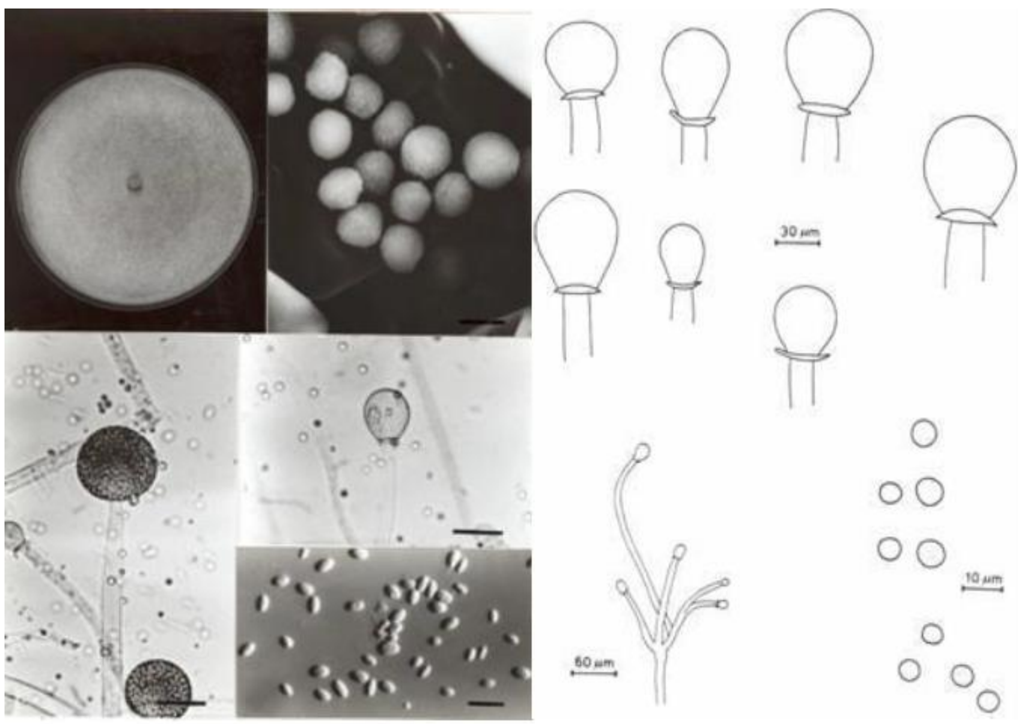
- The order mucorales includes a number of saprophytic fungi that have been implicated as possible avian pathogens.
- Absidia corymbifera is the pathogen most often isolated, although Mucor and Rhizopus spp. also are identified

Description of main aetiological agents of mucormycosis in poultry

1. ***Mucor racemosus*** Fresen., Beiträge zur Mykologie 1: 12 (1850)

Synonyms:

≡Mucor oudemansii Vánová, Ceská Mykologie 45 (1-2): 25 (1991)
=Mucor racemosus f. racemosus
=Mucor racemosus f. brunneus Morini, Malpighia 10: 88 (1896)
=Mucor dimorphosporus Lendn., Matéri la Flore Cryptogam Suisse 3 (1): 93 (1908)
=Mucor christianensis Hagem, Annales Mycologici 8 (3): 268 (1910) [MB#198906]
=Mucor varians Povah, Bulletin of the Torrey Botanical Club 44: 297 (1917)
=Mucor pispekii Naumov, Encyclopédie Mycologique 9: 47 (1939)



Mucor racemosus Mycobank

Colonies (MEA) expanding, pale greyish-brown. Sporangiophores hyaline, up to 20 mm high, 14-17 ?m wide, sympodially and monopodially branched, the short monopodial branches often being recurved. Sporangia brownish, up to 80 (-90) ?m

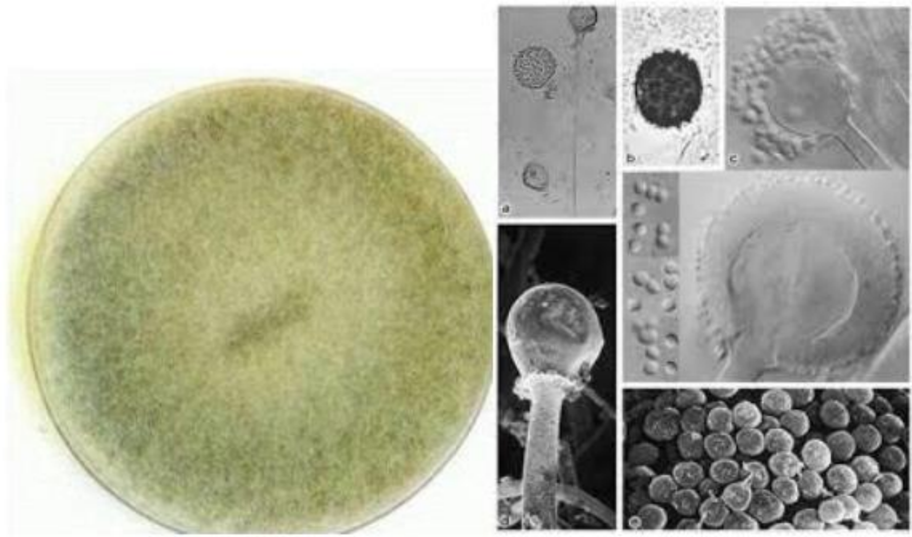
diam; columellae subspherical to pyriform, often with truncate bases, light brown, with collars. Sporangiospores smooth-walled, spherical to broadly ellipsoidal, up to 8-10 ?m diam. Chlamydospores mostly occurring in sporangiophores. Zygospores up to 110 ?m diam, with short spines, brown.

2. Mucor pusillus Lindt, Arch. Exp. Path. Pharmacol.: 272 (1886)

Synonyms:

≡Rhizomucor pusillus (Lindt) Schipper, Studies in Mycology 17: 54 (1978)
=Mucor septatus Bezold, Schimmelmyc. memschl. Ohres: 97 (1889)
=Mucor parasiticus Lucet & Costantin, Compt. Rend. Hebd. Séan Acad. Sci., 1033 (1899)
=Mucor buntingii Lendn., Bulletin de la Société Botanique de Genève 21: 260 (1930)

Colonies on PDA and synthetic Mucor agar (SMA) about 2 mm. High, at first white with unbranched sporangiophores, later brown, slightly smoky with strongly branched brown sporangiophores 5-18 µm diam., always with a septum below the sporangium. Sporangia globose, 50-80 µm diam., bright grey to brown with more or less quickly diffluent margin. Columellae oval or pyriform, bluish-brown, up to 50 x 60 µm, often with a collarette. Sporangiospores globose to subglobose, occasionally oval, 2,5-4 µm, often mixed with crystalline pieces of the sporangial wall. Zygospores homothallic, globose to slightly flattened at the sides, black, 55-75 µm diam., covered with conical warts. Suspensors approximately equal, elongate and conical. Gemmae unknown

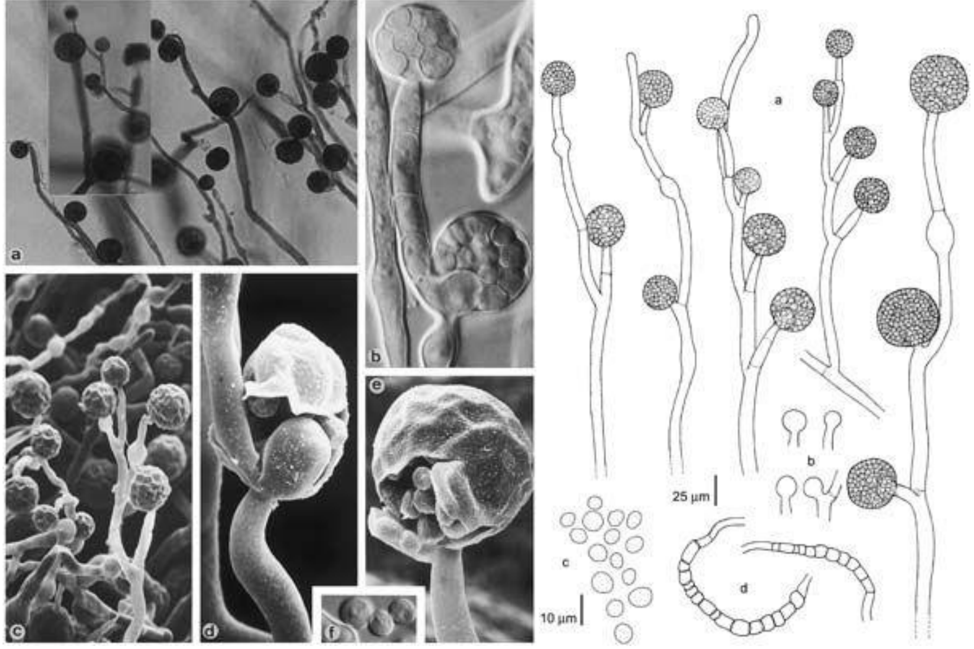


Mucor pusillus archive.bio.ed.ac.uk www.fungalbiodiversitycentre.org

3. *Mucor ramosissimus* Samouts., Mater. Mikol. Fitopat. Ross.: 210 (1927)

Colonies (MEA) restricted, greyish. Sporangiophores hyaline, up to 2 mm high, 18 ?m wide, slightly roughened, tapering towards the apex, repeatedly sympodially branched. Sporangia blackish, spherical to dorsiventrally flattened, up to 80 ?m diam, with persistent walls; columellae applanate, up to 40-50 ?m, absent in small sporangia. Sporangiospores smooth-walled, spherical to broadly ellipsoidal, 5-8 x 4.5-6.0 (-7) ?m. Oidia present in substrate hyphae. Chlamydospores absent.

Mucor ramosissimus colony PDA www.pf.chiba-u.ac.jp

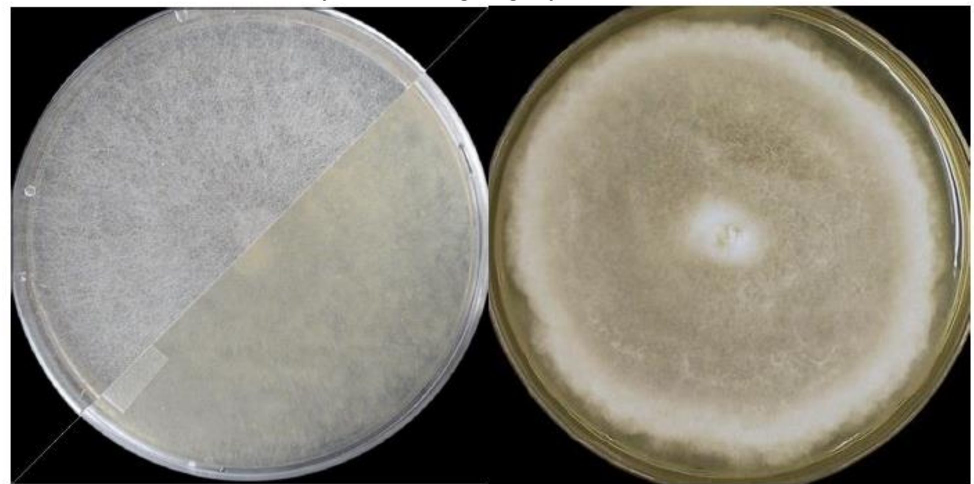


Mucor ramosissimus Mycobank

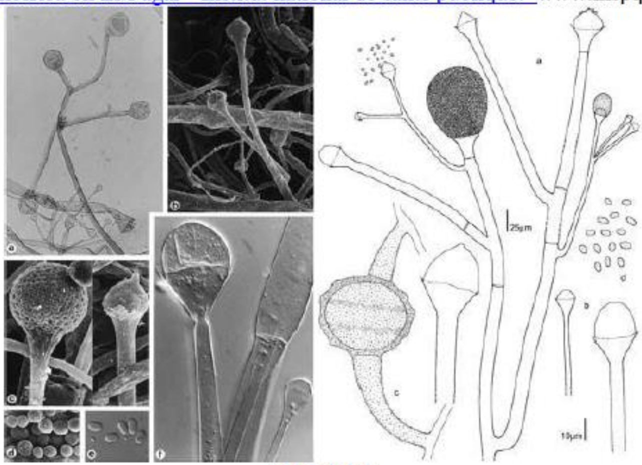
4. Absidia corymbifera (Cohn) Sacc. & Trotter, Sylloge Fungorum 21: 825 (1912)

≡Mucor corymbifer Cohn, Z. Klin. Med.: 147 (1884) ≡Lichtheimia corymbifera (Cohn) Vuill., Bull. de la Société Mycol. de France 19: 126 (1903)
≡Mycocladus corymbiferus (Cohn) J.H. Mirza (1979) [MB#114975] ≡Mycocladus corymbifera (Cohn) J.H. Mirza (1979) [MB#272108]
≡Mycocladus corymbifer (Cohn) J.H. Mirza, Mucorales of Pakistan: 95 (1979) [MB#530483]
≡Mycocladus corymbifer (Cohn) Vánová, Ceská Mykologie 45 (1-2): 26 (1991) [MB#127968]
=Mucor ramosus Lindt, Arch. Exp. Path. Pharmacol.: 269 (1886) [MB#247332]
=Mucor regnieri Lucet & Costantin, Archs Parasit.: 362 (1901) [MB#247578]

Colonies are fast growing, floccose, white at first becoming pale grey with age, and up to 1.5 cm high. Sporangiophores are hyaline to faintly pigmented, simple or sometimes branched arising solitary from the stolons, in groups of three, or in whorls of up to seven. Rhizoids are very sparingly produced and may be difficult to find without the aid of a dissecting microscope to examine the colony on the agar surface. Sporangia are small (10-40 um in diameter) and are typically pyriform in shape with a characteristic conical-shaped columella and pronounced apophysis, often with a short projection at the top. Sporangiospores vary from subglobose to oblong-ellipsoidal (3.0-7.0 x 2.5-4.5 um), are hyaline to light grey and are smooth-walled.



Absidia corymbifera on SAB media after 4 days incubation at 30oC thunderhouse4-yuri.blogspot.com, Absidia corymbifera on EM agar - Institut national de santé publique. www.inspq.qc.ca



Mycobank

Reports:

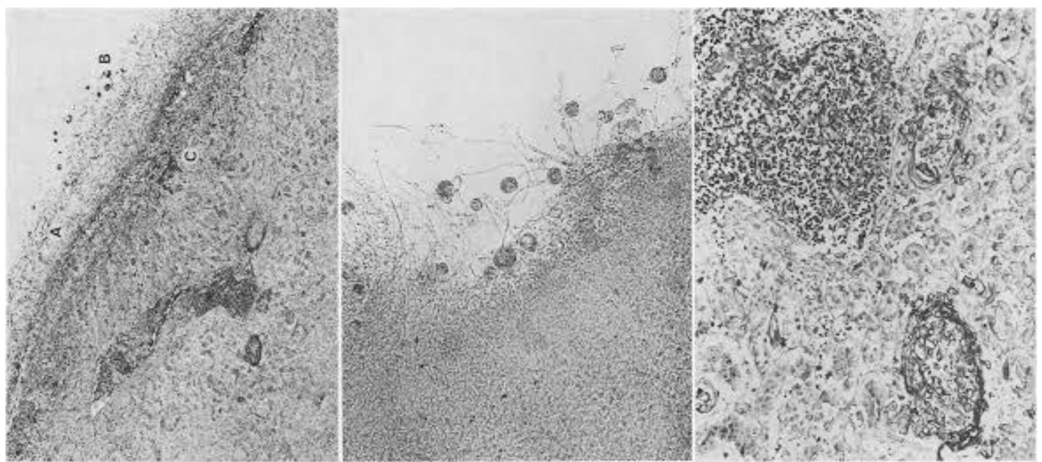
Bigland *et al.* (1961) euthanized, over a 25-mo span from a single zoologic collection, two bufflehead ducklings (*Bucephala albeola*) presented with pelvic limb paresis. On postmortem examination, the first duckling had intralesional fungal hyphae consistent with *Aspergillus* sp. in the spinal vertebrae and within pulmonary granulomas. In the second duckling, evidence of a thoracic spinal lesion was detected antemortem by using thermographic imaging. At postmortem examination, fungal hyphae consistent with *Mucor* sp. were found within the vertebrae. Although fungal infections of the respiratory system are commonly reported in waterfowl, infections that involve the spinal cord and vertebrae are unusual. These cases highlight the importance of consideration of axial skeleton fungal disease in neurologic presentations and the use of thermography for noninvasive diagnostic screening.

Migaki *et al.* (1970) reported pulmonary mucormycosis in a chicken due to a Mucor species

Caretta and Piontelli (1971) described a case pulmonary avian mucormycosis due to ***Mucor racemosus*** in which hundreds of chicks on a farm died from the disease.

Hanssen (1975) reported pulmonary phycomycosis in captive rock ptarmigan (lagopus mutus) and willow ptarmigan (lagopus lagopus) chicks

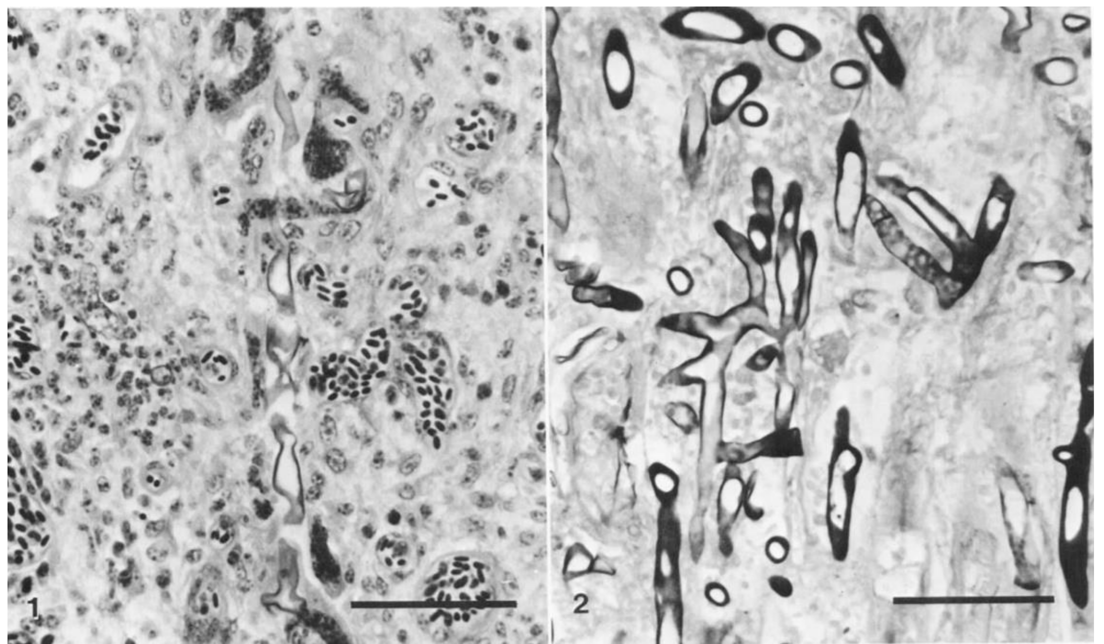
Dawson *et al.* (1976) described a case of mucormycosis in an African gray parrot (Psittacus erithacus) involving air sac and kidney. The causal fungus, ***Absidia corymbifera***, had produced sporangia in the air-sac lesion.



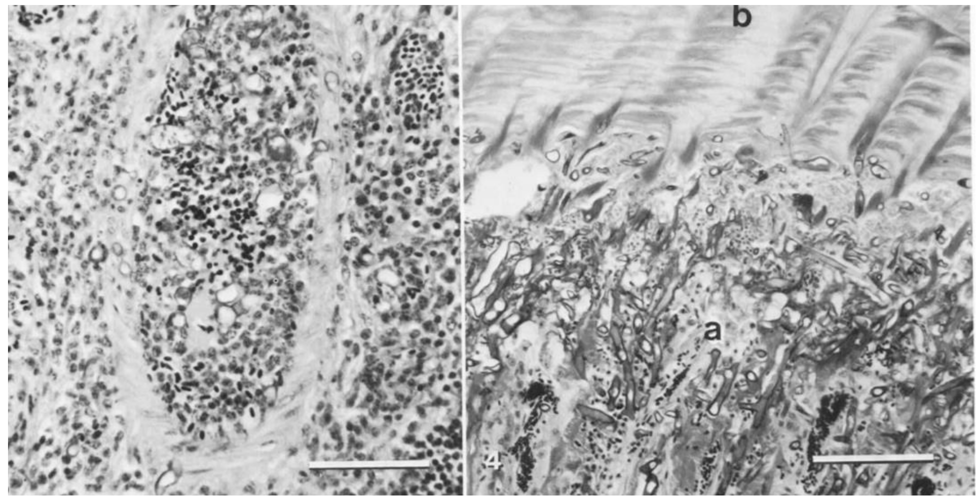
Dawson *et al.* (1976)

Panigrahy *et al* (1979) described deaths of a pigeon caused by air-sac infection with ***Absidia corymbifera***. The air sacs were thickened and contained a grayish gelatinous exudate.

Mitchell *et al.* (1986) reported an alimentary and disseminated mucormycosis due to ***Rhizopus microsporus*** in a group of young canaries (Serinus canarius). The disease was associated with the feeding of damp germinated seed from which Rhizopus microsporus was isolated. The birds were concurrently treated orally with tetracycline for suspect chlamydiosis. Lesions in infected birds consisted of granulomatous and necrotizing ventriculitis, enteritis, proctitis, cloacitis, peritonitis, and hepatitis. Focal and extensive caseous necrosis, infiltration by lymphocytes, plasmacytes and heterophils, and focal fibrinous exudation were significant features of the response. Hyphae, morphologically consistent with a fungus of the class Zygomycetes, were demonstrated in the lesions.



1. CloacaVrectal wall; fungal hyphae surrounded by multinucleate macrophages. PAS. Bar = 50 pm,. **2.** Fungal mycelium in the wall of the ventriculus. Methenamine silver. Bar = 50 pm. **Mitchell *et al.* (1986)**



.3. Thrombosis of small artery in cloaca1 wall, invasion of mycelium into vessel. PAS. Bar = 50 pm.**4.** Ventriculus; fungal mycelium in the glandular layer (a). Cornified layer (b). PAS. Bar-= 100 pm. **Mitchell *et al.* (1986)**

***Tsujioka et al.* (1988)** described an outbreak of pulmonary mucormycosis in broiler chickens with *Absidia Corymbifera.* The poultry farm where this disease outbreak occurred had been raising 3, 000 19 day old chicks and 7, 000 12 day old chicks when diagnosticians visited in March 1984. The outbreak was seen in the former group only. Its poultry house was using powdery diatomite as litter, the other sawdust. From 15 days of age onwards the chicks had shown depression, listlessness or collapse and some birds had died. In the course of 5 days 191 chicks (6.3%) died or were killed. A number of organisms of *Absidia corymbifera* were isolated from the lungs of all chicks examined. Microscopically, the lungs were congested and granulomatous lesions were scattered the secondary and tertiary bronchiolar walls. In the macrophages of the bronchioles deposits of diatomite crystals were recognized. A total of 33 chicks in 3 groups, 7, 14 and 28-day-old, were inoculated with a spore suspension into the bronchus. As a result, granulomatous lesions, which resembled those of the field case, were found at a high rate.

Connie *et al.* (1994) examined four-year-old chattering lory because of progressive neurologic defects culminating in seizures. Results of clinical pathologic testing, including plasma electrophoresis, suggested systemic inflammatory disease. On necropsy, massive numbers of fungal hyphae showing morphologic characteristics consistent with the order Mucorales were seen in the pulmonary parenchyma. Evidence of hyphal invasion of cerebral blood vessels and dissemination of the organism in the cerebrum was also seen. The fungal organisms were identified as Absidia sp. by direct fluorescent antibody testing.

Desmidt *et al.* (1998) described a case of mucormycosis combined with chlamydiosis in an African grey parrot (Psittacus erithacus erithacus). The clinical signs included diarrhoea, an unsteady gait and a twisted neck. Smears of the spleen, liver and contents of the cloaca stained strongly positive for Chlamydia species. Histologically, hyphae typical of Zygomycetes were observed invading through the walls of blood vessels of the spleen. Rhizomucor pusillus was isolated as a pure culture from the intestines, lungs and liver.

Quesada *et al.* (*2007)* isolated ***Mucor ramosissimus*** associated with feather loss in canaries (Serinus canarius). Three canaries showing feather loss on legs, dorsum, neck, and head, and hyperkeratosis on the feet were sacrificed because of their poor corporal condition and submitted to the Unit of Histology and Anatomic Pathology at the Veterinary School of Las Palmas de Gran Canaria. Histologically, skin revealed pronounced epidermal and follicular infundibular hyperplasia associated with orthokeratotic hyperkeratosis. Numerous fungal spores were observed on the stratum corneum of the epidermis and within feather follicles, associated with destruction of the feathers. This fungus was identified as *Mucor ramosissimus*. To the best of authors' knowledge, this is the first report of dermatitis and feather loss associated with *Mucor ramosissimus*, not only in canaries but also in birds.

References:

1. Bigland CH, Graesser FE, Pennifold KS: An osteolytic mucormycosis in a penguin. Avian Dis 5:367-370, 1961.

2. Caretta, G.; Piontelli, E., 1971: Pulmonary avian mucormycosis due to Mucor racemosus Fres. Atti Istituto Botanico della Universita e Laboratorio Crittogamico Pavia 7(6): 33-39
3. Connie J. Orcutt and Tracy E. Bartick, Mucormycotic meningoencephalitis and pneumonia in a Chattering Lory (Lorius garrulus). *Journal of the Association of Avian Veterinarians,* 8, 2 (1994), 85-89
4. Dawson, Ch. O. E. B. Wheeldon and Pauline E. McNeil, Air sac and renal mucormycosis in an African Gray Parrot (Psittacus erithacus).*Avian Diseases*20, 3 (Jul. - Sep., 1976), 593-600
5. Desmidt,M., P De Laender, D De Groote, M Rysselaere, P De Herdt, R Ducatelle, F Haesebrouck. Rhizomucor pusillus mucormycosis combined with chlamydiosis in an African grey parrot (Psittacus erithacus erithacus). Vet Rec 1998 Oct;143(16):447-8
6. Hanssen I. Pulmonary phycomycosis in captive rock ptarmigan (lagopus mutus) and willow ptarmigan (lagopus lagopus) chicks. Acta Vet Scand. 1975;16(1):134-6.
7. Migaki G, Langheinrich KA, Garner FM. Pulmonary mucormycosis (phycomycosis) in a chicken. Avian Dis. 1970 Feb;14(1):179-83.
8. MITCHELL,G. D. ESNOUF, AND R. PRITCHARD. Mucormycosis in Canaries (Serinus canarius) Fed Damp Germinated Seed Vet. Pathol. 23~625-627 (1986)
9. Panigrahy B, et al: Candidiasis in cockatiel nestlings and mucormycosis in a pigeon. Avian Dis 23:757-760, 1979.
10. Quesada, Ó , F. Rodríguez, P. Herráez, D. Seara, and A. Espinosa de los Monteros (*2007*) Mucor ramosissimus associated with feather loss in canaries (Serinus canarius). Avian Diseases: June 2007, Vol. 51, No. 2, pp. 643-645.
11. *TAKASHI TSUJIOKA*, *TOHRU MIZOGUCHI*, *YOSHIFUMI TSUCHIYA*, *KEIJI MOCHIZUKI*., YOSHIFUMI TSUCHIYA[1)], KEIJI MOCHIZUKI An Outbreak of Pulmonary Mucormycosis in Broiler Chickens with *Absidia Corymbifera*. Journal of the Japan Veterinary Medical Association, 41 (1988) 4, 255-258

3.3. Avian Dactylariosis (mycotic encephalitis):

Dactylariosis is a fungal disease characterized mainly by nervous signs in turkey poults and chicks causing severe losses. It is a relatively new fungal disease occasionally causing outbreaks.

Clinical Signs

- Clinical signs and gross lesions are not specific, making diagnosis difficult.
- Birds between initial 1-5 weeks of age are susceptible.
- Nervous signs: torticollis, incoordination, tremors, paralysis, and death.
- Eye lesion in few cases ocular lesion develops and produce blindness.
- Dyspnea: in rare cases pulmonary granulomas develop and cause dyspnea as in Aspergillosis, mortality 3-20 %.
- Mortality during disease outbreak ranges between 3-20%, mainly due to neurological disease

Transmission

- The source of infection are environments characterized by high temperature (> 43° C) and low PH (<5), such conditions exist in piles

of wet litter have undergone that natural heating process. Inhalation of spores from moldy litter or moldy egg incubators.

Lesions

- The lesions differ from the mycotic encephalitis of aspergillosis by having more malacia and haemorrhages and having a far larger number of giant cells.
- Severe large, hardened, circumscribed cerebellar and cerebral lesion (necrosis that is gray or yellow).
- Pulmonary granulornas are minimum, indistinguishable from those of Aspergillosis.
- Occular lesions appear similar to Aspergillosis grossly.

Diagnosis

- Signs, lesions, demonstration of pigmented hyphae with characteristic oval two - celled brownish conidia and large number of giant cells from brain lesions are diagnostic.
- Sabouraud Dextrose Agar (SDA) with suitable antibiotics and incubation at 45°C is suitable for fungal isolation from brain samples.
- Colonies produce brown color pigment diffusing into the surrounding medium and have characteristic diploid conidia

Differenttial diagnosis

- The disease should be differentiated from viral (ND, AE), bacterial meningitis, mycotic (Aspergillosis), nutritional (crazy chick disease).

Control

- There is no effective treatment for dactylariosis so prevention of exposure to mouldy litter especially that had undergone heating process and decontamination of incubators by fumigation is the only means of prevention.

Zoonotic aspect

- Infections jn immunocompromised humans was first reported in 1986. Since then, the fungus has been increasingly reported as an agent of human disease especially in recipients of solid organ transplants.
- Infection has a long onset and can involve a variety of body sites. Treatment of infection often involves a combination of antifungal drug therapy and surgical excision.

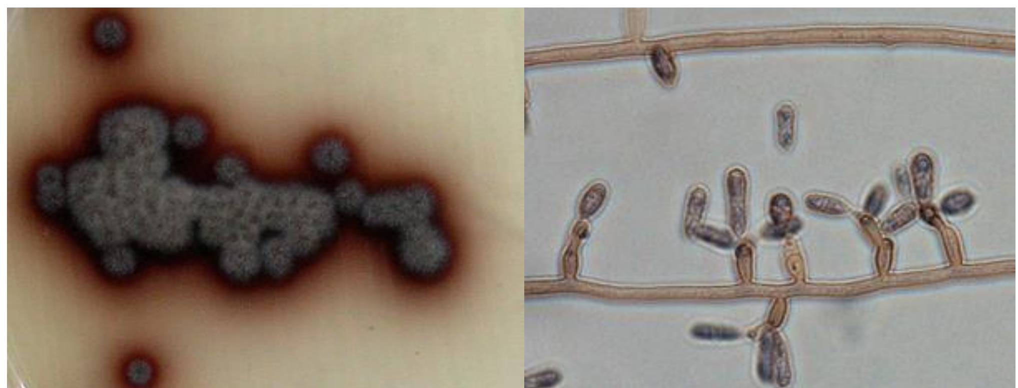
Aetiology:

Verruconis gallopava (W.B. Cooke) Samerpitak & de Hoog, Fungal Diversity 65: 117 (2014)

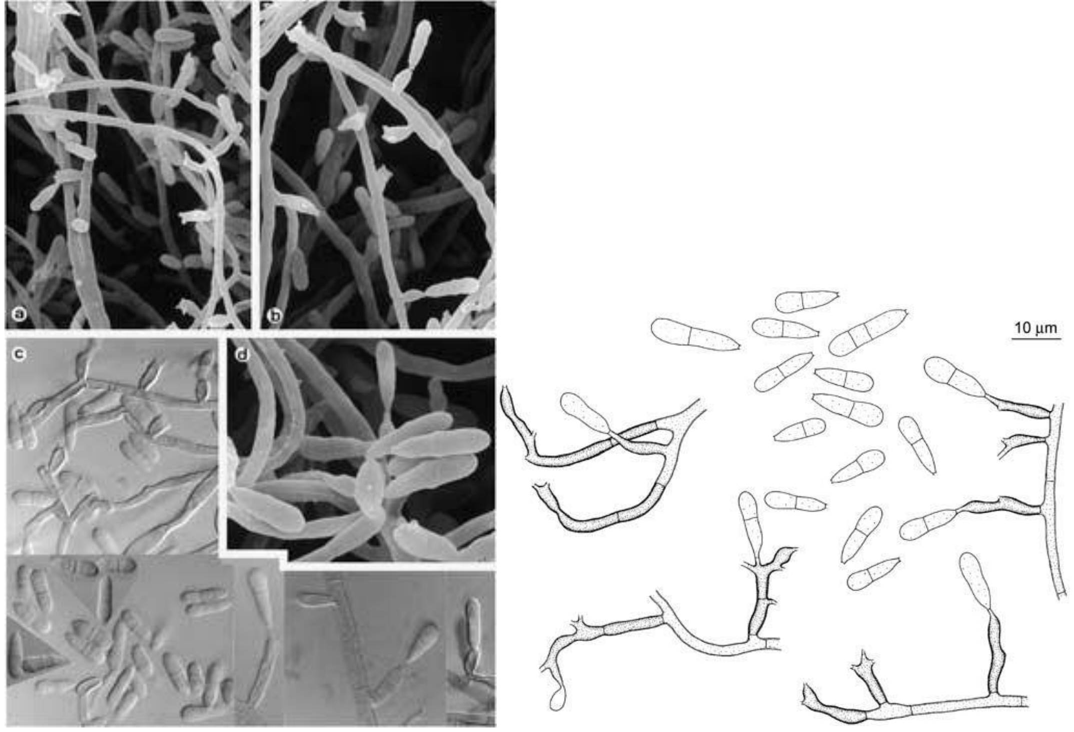
Synonyms:

≡Diplorhinotrichum gallopavum W.B. Cooke, Sabouraudia 3 (3): 241 (1964) ≡Dactylaria gallopava (W.B. Cooke) G.C. Bhatt & W.B. Kendr., Canadian Journal of Botany 46 (10): 1257 (1968) [MB#329619]
≡Ochroconis gallopava (W.B. Cooke) de Hoog, Fung. Path. Hum. Anim.: 181 (1983) [MB#115609]
≡Dactylaria constricta var. gallopava (W.B. Cooke) Salkin & D.M. Dixon, Mycotaxon 29: 379 (1987) [MB#131896]
≡Scolecobasidium gallopavum W.B. Cooke, Mycopathologia 12: 492 (2013) ≡Scolecobasidium gallopavum (W.B. Cooke)G.Y. Sun & Lu Hao, Mycopathologia 12: 492 (2013)

On OA, colony attaining 5.5 cm, smooth to felty, dry, flat, brown to reddish brown; a pink pigment is exuded into the agar. On MEA, colony attaining 3.2 cm, smooth to velvety, dark grey at the center and lighter near the edge; a pink pigment is exuded into the agar. Hyphae brown, with rather thick walls. Conidiophores flexible, mostly cylindrical to acicular, with 0(-1) thin septa, poorly differentiated, bearing a few conidia near the apex on fragile denticles. Conidia two-celled, verruculose to nearly smooth-walled, subhyaline to pale brown, clavate, constricted at the septum, 11-18 — 2.5-4.5 µm; apical cell wider than basal cell. Conidial secession rhexolytic, frills remaining on denticle and on conidial base. Cardinal temperatures: growth abilities ranging from 15-50 oC, with optimal growth at 35 oC; growth with 5 % MgCl2 and 5 % Na



Culture of *Verruconis gallopava*. www.mycology.adelaide.edu.au



Verruconis gallopava. Mycobank

Reports:

Georg *et al.* (1964) described a dematiaceous fungus, *Diplorhinotrichum gallopavum* sp. Nov as an agent of encephalitis in young turkey poults. In the outbreak described, at least 600 poults, of a flock of 4,000, were affected. The infection was proven by isolation of the fungus from the brains of 9 of the turkey poults, and by demonstration of dematiaceous mycelium in the brain tissue. The disease was probably acquired through contact with old sawdust used as litter for the birds. Attempts to reproduce the disease experimentally failed.

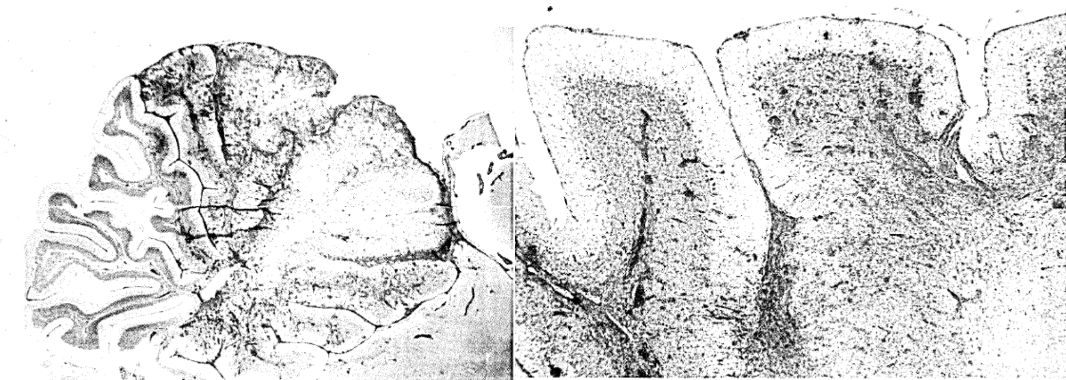
Blalock ***et al.*** **(1973)** reported a case of mycotic encephalitis as the cause of death in a flock of Nicholas turkey poults in South Carolina. Symptoms included leg paralysis and torticollis, with mortality reaching 20%. Grossly visible large granulomatous lesions occurred in the brains. The eyes of some birds contained opaque areas. The fungus Dactylaria (Diplorhinotrichum) gallopava was isolated from the brains and eyes. Similar symptoms, lesions, and mortality were produced in 1-day-old turkey poults by intratracheal inoculation of a spore suspension of D. gallopava. Histopathological studies of the granulomatous areas of the brain and lung revealed massive inflammatory cellular infiltration and coagulative necrosis with extensive giant cell formation. Hyphal elements were demonstrated in these areas. The fungus can be identified by the gross appearance of the colony and by demonstration of the characteristic two-celled spores. Careful cultural studies must be done to differentiate encephalitis caused by D. gallopava from that caused by Aspergillus fumigatus or Arizona hinshawii.

Merrill Ranck ***et al.*** **(1974)** reported a fatal encephalitis of chickens, caused by the thermophilic fungus Dactylaria gallopava, which affected over 200 birds in a flock of 65,000 broilers. The disease was reproduced experimentally by inoculating spore suspensions into 1-day-old chicks via the left posterior thoracic air sac, the left maxillary sinus, and also intracerebrally. Gross and microscopic lesions were found in the brains, air sacs, lungs, eyes, and livers. The brain lesions were like those in the natural outbreak, and D. gallopava was recovered from the inoculated chickens. The brain lesions were compared with those in birds with aspergillosis.

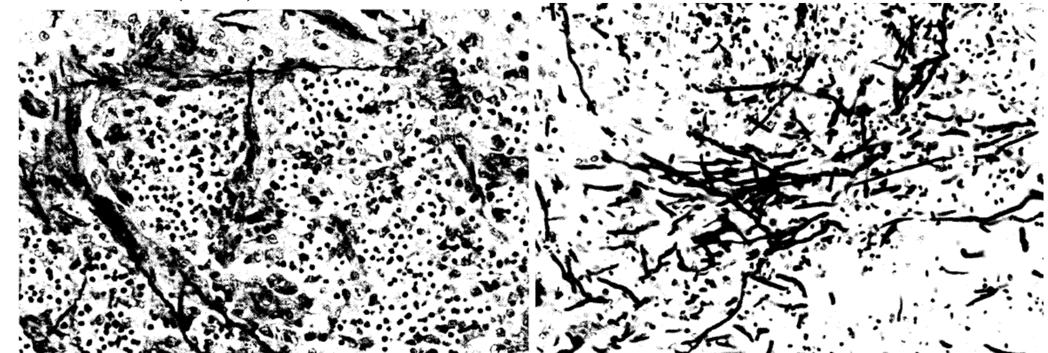
Waldrip ***et al.*** **(1974)** described the first outbreak of dactylariosis in chickens from Georgia. The mortality rate among 60,000 birds comprising six separate flocks of broilers was 3-5%. Diagnosis in each flock was confirmed by histopathology of brain tissue and isolation of ***Dactylaria gallopava*** from birds. When a dilution technique and an incubation temperature of 42 C were used, D. gallopava was isolated from 2 of 75 litter samples from five broiler houses, the first recorded isolation of D. gallopava from chicken litter. It is suggested that the wood chips and sawdust in litter may introduce the fungus into broiler houses.

Randall et al. (1981) mentioned that an outbreak of mycotic encephalitis caused by a fungus resembling ***Dactylaria gallopava*** was encountered in two flocks of broilers that were placed on bark litter. The chicks showed a variety of nervous signs. Gross abnormalities were confined to the brains. Cerebellar oedema and haemorrhage were prominent features in some chicks. Oedema was also present over the cerebral hemispheres and haemorrhage was noted in some of the brain stems. A few small, pearly nodules up to 1 mm in diameter were seen later in the outbreak in the lungs of some birds. No abnormalities were found in the air sacs. Fungal isolates were obtained from both the brains and the litter; others were isolated from the litter alone but were not identified. The predominant growth from the brain was of a brown velvety fungus. The growth of this organism only became apparent following incubation at 37°C for 48 hours and after standing at room temperature for a further 2 days on the laboratory bench. Owing to overgrowth with other fungi in several of the initial cultures, the presence of this organism was best appreciated from an

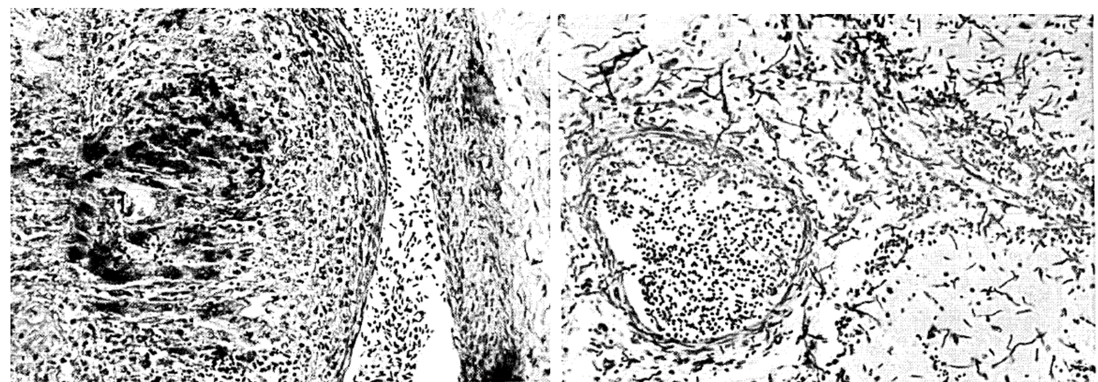
examination of the base of the culture plate where a reddish pigment could be seen diffusing into the medium. This feature was most noticeable around portions of embedded brain rather than on plates where the tissue had been smeared over the surface of the agar. A more exuberant growth was obtained after incubation of sub-cultures at 43°C. This fungus was not isolated from the lungs and small intestinal contents. A severe non-purulent meningoencephalitis was present in 10 out of 11 brains examined from broilers. Destruction of between one and two-thirds of the cerebellum and an accompanying reaction in brain stem was observed in most of these. Lesions were rapidly spreading and frequently accompanied by haemorrhage, some being centred on the basal medullary white matter with subsequent spread into the folia. Areas of cerebellum were necrotic and in several specimens little tissue reaction was encountered except near the margin of the lesion where an extensive, glial, giant cell and macrophage response was usually present. Numerous thin, septate, branching hyphae were visible throughout all the lesions on examination of PAS and Grocott-Gomori methanamine silver-stained sections . Fairly long fungal filaments could be followed in the tissue and their walls had a yellowish tint when viewed in unstained 12 /um-thick sections. Hyphae were usually ensheathed by newly formed giant cells in zones where there was more cellular reaction. Fungal migration through the walls of blood vessels and the presence of phagocytosed hyphae within giant cells were prominent features. Interfolial meningitis was common, particularly in the depths of the sulci. Dense perivascular cuffs were present in most brain stems.



Extensive destruction of anterior cerebellum as seen in a midsaggital section. PAS-haematoxylin x *10.*Thin *rim of viable cerebellum at the periphery of an extensive necrotic lesion (lower right). The inflammatory response is represented by the dark-staining areas and foci. PAS-haematoxylin* **x** *40.*
Randall et al. (1981)



Newly-formed giant cells ensheathing thin hyphae in the granular layer of cerebellum. Note pyknotic nuclei of intervening nerve cells. PAS-haematoxylin x *400. Extensive growth of fungal hyphae throughout a cerebellar lesion. Grocott-Gomori methenamine silver-haematoxylin* x *250.* **Randall et al. (1981)**



Active mural granuloma containing a few small hyphal fragments within a pulmonary blood vessel. PAS-haematoxylin x 400. *Mycotic invasion of leptomeninges and molecular layer of cerebellum from a meningeal vein. Experimental reproduction in 6-dayold chick. PAS-haematoxylin* **x** *250.* **Randall et al. (1981)**

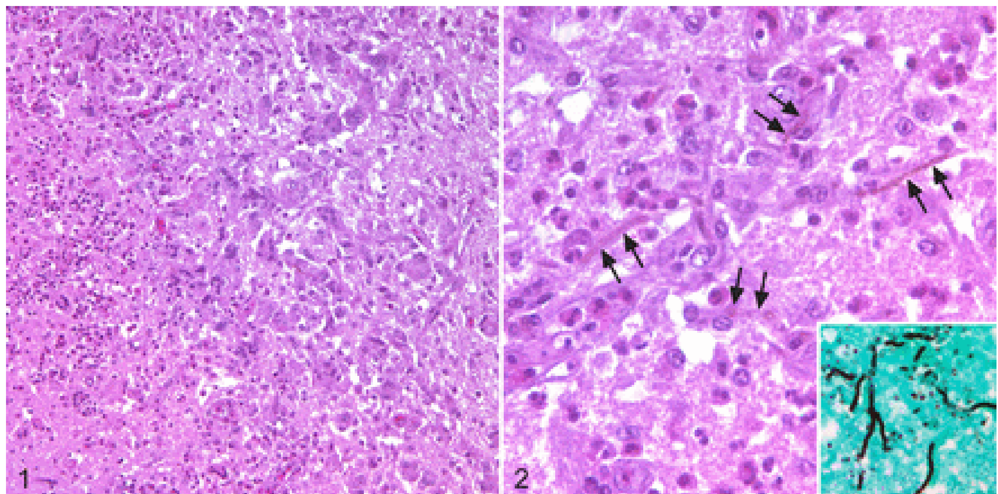
Shane *et al.* (1985) isolated ***Dactylaria gallopava*** from brain tissue of 1-to-3-week-old quail chicks. Successive batches demonstrated elevated (15-20%) mortality preceded by incoordination and lateral recumbency. Chicks exhibited cerebellar and cerebral encephalitis characterized by brown-red discoloration of affected brain tissue. Decontamination of setters and hatchers resulted in abrupt cessation of mortality in subsequent placements, implicating incubators as the source of infection.

Karesh *et al.* (1987) reported encephalitis caused by ***Dactylaria gallopava*** in two 17-to-18-day-old grey-winged trumpeters (Psophia crepitans). One of the chicks was housed in a tropical exhibit, and the other was in an adjacent room. Fir bark litter and aerosol infection were the suspected source and route of infection. The occurrence of this disease in a species other than the domestic chicken and turkey suggests the presence of a broader avian population at risk than previously indicated. Adult trumpeters and both young and old passerines housed in the same exhibit were not affected.

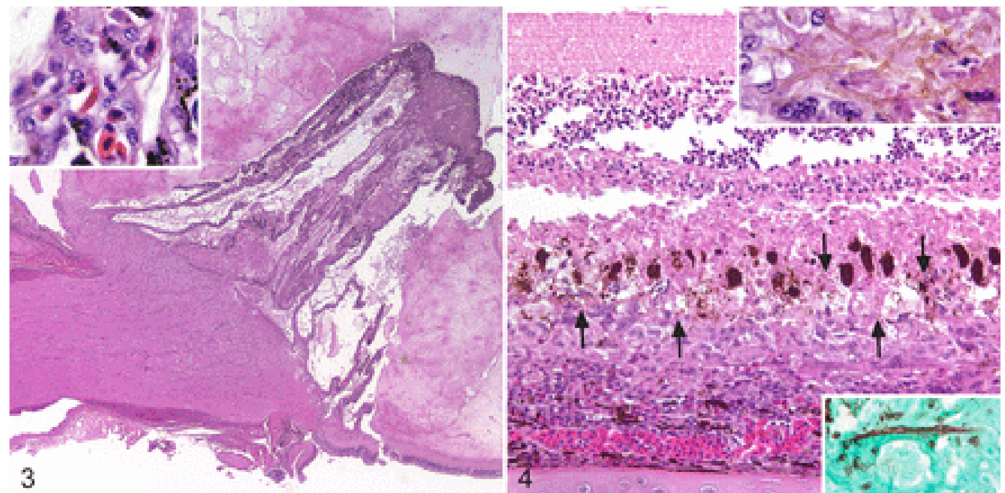
Salkin *et al.* (1990) mentioned that ***Dactylaria constricta var. gallopava*** (Cooke) Salkin et Dixon was found to cause fatal encephalitis in a 28-day-old, captivity-bred snowy owl chick (Nyctea scandiaca). The previously healthy bird suddenly developed ataxia, severe torticollis, and extensor rigidity of the legs. Since the animal did not improve with antibiotic or vitamin-mineral supplement therapy, the chick was euthanized 5 days after the onset of neurologic signs. At necropsy, all tissues except the brain were grossly normal. Cultures inoculated with blood from the brain and heart yielded a dematiaceous mould that subsequently proved to be D. constricta var. gallopava. This was the first report of natural central nervous system infection caused by D. constricta var. gallopava in a snowy owl.

Ossiboff *et al.* (2015) mentioned that 2 elegant crested tinamou chicks (Eudromia elegans), aged 27 and 50 days, respectively, died following acute onset of weakness and neurologic disease. Microscopically, the cerebral hemispheres of both chicks and the optic lobes of 1 chick contained multifocal granulomatous and heterophilic inflammation and necrosis with intralesional pigmented, thin-walled, fungal hyphae. In 1 chick, hyphae extended along the optic nerve into the globe and were associated with severe granulomatous and heterophilic inflammation of the choroid, retina, pecten, and vitreous. In both chicks, polymerase chain reaction amplification of the fungal 28S large subunit ribosomal RNA was positive with 99% sequence identity to

Ochroconis gallopava. While a well-characterized fungal infection of domestic poultry, ochroconiasis has rarely been reported in exotic avian species, and this is the first histologic characterization of ocular ochroconiasis in any avian species.



1. Severe granulomatous and heterophilic inflammation and necrosis disrupt the neuro-parenchyma. (HE). **2.** Thin-walled, 2.0- to 2.6-mm-wide, faint brown-yellow, septate hyphae (arrows) are present within areas of cerebral inflammation and necrosis. HE. Inset: Grocott's methenamine silver **Ossiboff *et al*. (2015)**



3. Heterophilic and granulomatous inflammation extends along the optic nerve and into the choroid, retina, pecten, and vitreous chamber. Inset: Heterophils and macrophages infiltrate the pecten (HE). **4.** Pigmented hyphae (arrows) with associated inflammatory cells expand the choroid and outer layers of the retina. HE. Inset, upper right: High magnification of pigmented hyphae within the retina. HE. Inset, lower right: Grocott's methenamine silver. **Ossiboff *et al*. (2015)**

References:

1. Blalock HG, Georg LK, Derieux WT. Encephalitis in turkey poults due to *Dactylaria* (*Diplorhinotrichum*) *gallopava:* a case report and its experimental reproduction. Avian Dis. 1973;17 (1):197–204.
2. Georg,**L. K.,** B.W. Bierer and W. Bridge Cooke. Encephalitis in Turkey poults due to a new fungus species. Sabouraudia: Journal of Medical and Veterinary Mycology 3, 3, 239-244, 1964
3. Karesh WB, Russell R, Gribble D. *Dactylaria gallopava* encephalitis in two grey-winged trumpeters (*Psophia crepitans*). Avian Dis. 1987;31 (3):685–688.

4. Merrill Ranck, F., Lucille K. Georg and Dennis H. Wallace Dactylariosis: A Newly Recognized Fungus Disease of Chickens.*Avian Diseases,*18, 1 (Jan. - Mar., 1974), 4-20
5. Randall , C.J., D.M. Owen & K.S. Kirkpatrick (1981) Encephalitis in broiler chickens caused by a hyphomycete resembling dactylaria gallopava , Avian Pathology, 10:1, 31-41,
6. Ossiboff RJ, Clancy MM, Terio KA, Conley KJ, McAloose D. Cerebral and Ocular Ochroconiasis in 2 Elegant Crested Tinamou (Eudromia elegans) Chicks. Vet Pathol. 2015 Jul;52(4):716-9.
7. Randall , C.J., D.M. Owen & K.S. Kirkpatrick (1981) . Encephalitis in broiler chickens caused by a hyphomycete resembling dactylaria gallopava , Avian Pathology, 10:1, 31-41,
8. Salkin IF, Dixon DM, Kemna ME, et al Fatal encephalitis caused by*Dactylaria constricta* var. *gallopava* in a snowy owl chick (*Nyctea scandiaca*). J Clin Microbiol. 1990;28 (12):2845–2847.
9. Shane SM, Markovits J, Snider TG 3rd, Harrington KS. Encephalitis attributed to dactylariosis in Japanese quail chicks (Coturnix coturnix japonica). Avian Dis. 1985 Jul-Sep;29(3):822-8.
10. Waldrip, D.W. A. A. Padhye, L. Ajello and M. Ajello. Isolation of Dactylaria gallopava from Broiler-House Litter. *Avian Diseases* Vol. 18, No. 3 (Jul. - Sep., 1974), pp. 445-451

4. Avian Mycotoxicosis

4.1. Aflatoxicosis (AF)

Introduction

Aflatoxicosis represents one of the serious diseases of poultry, livestock and other animals. The cause of this disease in poultry and other food-producing animals has been attributed to the ingestion of various feeds contaminated with ***A. flavus***. This toxigenic fungus is known to produce a group of extremely toxic metabolites, of which aflatoxin B I (AFB I) is most potent. Avian species especially chicks, goslings, ducklings and turkey poults are most susceptible to AFB 1 toxicity. The toxic effects of AFB 1 are mainly localized in liver as manifested by hepatic necrosis, bile duct proliferation, icterus and hemorrhage. Chronic toxicity in those birds is characterized by loss of weight, decline in feed efficiency, drop in egg production and increased susceptibility to infections. The incidence of hepatocellular tumors, particularly in ducklings, is considered to be one of the serious consequences of aflatoxicosis. Even though prevention and avoidance are the best way to control aflatoxicosis, natural contamination of crops with *A. flavus* is sometimes unavoidable. Such aflatoxin-contaminated feeds can be decontaminated using various methods which mainly focus on physical removal or chemical inactivation of the toxins in the feeds. Moreover, dietary additives such as activated charcoal, phenobarbital, cysteine, glutathione, betacarotene, fisetin and selenium have also been reported to be effective in the reduction of aflatoxicosis in poultry **(Dalvi, 1986)**

Historical

- The mysterious Turkey-X disease of 1960 which resulted in loss of several thousand turkey poults in the United Kingdom. The cause of enormous mortality in turkey poults was alarmed by several authors [**Blount,1960, Smith,1960**, **Stevens *et al.*, 1960** and **Swarbrick,1960**]
- Affected birds lost appetite, became lethargic, and died within 7 days after the onset of symptoms. Livers of diseased turkeys were severely damaged.
- A similar disease of ducklings and young pheasants was reported from England (**Asplin and Camaghan 1961).**
- A common factor in all disease outbreaks was the inclusion of Brazilian groundnut meal in the affected birds' diets (**Asplin and Carnaghan 1961).**
- Experimental feeding trials showed that chickens were much less susceptible to the disease than were turkey poults, ducklings or pheasant chicks **(Asplin and Carnaghan 1961).**
- The suspected toxic factor was found to be extractable by using chloroform [**Allcroft *et al.,* 1961**].

- **Sargeant et al. (1961)** demonstrated that an isolate of the common mould ***Aspergillus flavus*** Link ex Fries was in fact the responsible agent.
- The name "**aflatoxin**", using first letter from "**A**spergillus" and the first 3 letters from "**fla**vus" was proposed [**Patterson, 1962**].
- Aflatoxin was in the same year isolated in crystalline form in the Netherlands [**Van der Zijden *et al.,* 1962**],
- Detection of aflatoxins in extracts of contaminated peanut meal was facilitated by their intense blue or green fluorescence in ultraviolet light, and soon thereafter purified metabolites with identical physical and chemical properties were isolated from *A. flavus* cultures (**Nesbitt *et al.* 1962; Van der Zijden *et al.* 1962**).
- Aflatoxin was separated into two components, B (blue fluorescence) and G (green fluorescence) in the United Kingdom [**Nesbitt *et al.,* 1962**].
- Aflatoxin B was further separated into into B_1 and B_2 and its chemical structure was established
- A similar disease of **ducklings** was reported from Kenya. The ducklings' feed ration contained a groundnut meal produced in eastern Africa, indicating that the problem was not solely associated with Brazilian groundnut meal (**Allcroft and Camaghan 1962**).
- The disease in poultry was reported from
    - Spain (**Camaghan and Allcroft 1962**),
    - Austria (**Kohler and** Swaboda 1962),
    - Hungary (**Derzsy *et al.* 1962**).
    - Australia have described acute disease in poultry fed imported groundnut meal (**Gardiner and Oldroyd 1965, Hart 1965).**
    - India (**Gopal et al. 1969**).

Aflatoxins

Aflatoxins are a group of extremely toxic metabolites produced by the common moulds *Aspergillus flavus* and *Aspergillus parasiticus.* The aflatoxins consist of about 20 similar compounds belonging to a group called the difuranocoumarins.

1.1. Natural occurrence of aflatoxins

- The occurrence of aflatoxins in agricultural commodities depends on such factors as region, season and the conditions under which a particular crop is grown, harvested or stored.
- AF have generated the greatest public health concern because of the effects that AF-contaminated feeds may have on the growth and health of poultry, and also their possible transmission to humans via meat and egg contamination.
- AF are particularly present in corn, which comprises between 50% and 60% of most poultry diets
- **Crops** grown under warm and moist weather in tropical or subtropical countries are especialy more prone to aflatoxin-contamination than those in

temperate zones. Groundnuts and groundnut meal are by far the two agricultural commodities that seem to have the highest risk of aflatoxin contamination (**Wyllie and Morehouse, 1977; Patterson, 1983).**

- Corn, cottonseed, Brazil nuts, copra, various tree nuts, and pistachio nuts are the other commodities quite susceptible to the invasion of aflatoxin producing fungi. Although these commodities are important as substrates,
- Frequent contamination of corn and other commodities with high levels of aflatoxins has been a serious problem all over the world resulting in significant economic losses to farmers and a health hazard to farm animals and humans as well.
- The moisture content of the substrate and temperature are the main factors regulating the fungal growth and toxin formation.
    - A moisture content of 18% for starchy cereal grain s and 9-10% for oil-rich nuts and seeds has been established for maximum production of the toxin **(WHO, 1979).**
    - The minimum, optimum and maximum temperatures for aflatoxin production have been reported to be 12 ~ , 27 ~ , and 40-42~ respectively (**Christensen and Nelson, 1976).**

1.2. Types of aflatoxins

1. Aflatoxin B1 blue fluorescence in UV light
2. Aflatoxin B2 blue fluorescence in UV light
3. Aflatoxin B2a is the hydrated form at C2 – C3 double bond of B2
4. Aflatoxin G1 green fluorescence in UV light
5. Aflatoxin G2 green fluorescence in UV light
6. Aflatoxin G2a is the hydrated form at C2 – C3 double bond of G2
7. Aflatoxin M1 is a metabolite of B1 in milk
8. Aflatoxin M2 is a metabolite of B2 in milk
9. Aflatoxin M2a is a metabolite of B2 in milk
10. Aflatoxin GM1
11. Aflatoxin GM2
12. AflatoxinGM2a
13. Aflatoxin H1 is the reduction form of B1
14. Aflatoxin P1 is the phenolic derivative from o-demethylation of B1
15. Aflatoxin.Q1 is a monohydroxylated metabolite of B2
16. Aflatoxin R0 (aflatoxicicol) hydroxy group instead of a carbonyl group at ring E
17. Aflatoxin R B1 has hydroxy group instead of a carbonyl group at ring E
18. Aflatoxin R B2 has hydroxy group instead of a carbonyl group at ring E
19. Aflatoxin B3 is also called parasiticol
20. Aflatoxin BO (AFB1-*exo*-8,9-epoxide) results from epoxidation

1.3. Fungi producing aflatoxins

Recent data indicate that aflatoxins are produced by 13 species assigned to three sections of the genus *Aspergillus* **(Varga *et al.*, 2009, 2011):**

- **section *Flavi***
    1. *Aspergillus flavus,*
    2. *Aspergillus pseudotamarii,*
    3. *Aspergillus parasiticus,*
    4. *Aspergillus nomius,*
    5. *Aspergillus pseudonomius*
    6. *Aspergillus pseudocaelatus*
    7. *Aspergillus bombycis,*
    8. *A. parvisclerotigenus,*
    9. *Aspergillus minisclerotigenes,*
    10. *Aspergillus arachidicola*,
- **Section *Nidulantes***
    1. *Emericella astellata,*
    2. *E. venezuelensis,*
    3. *E. olivicola*
- **Section *Ochraceorosei***
    1. *Aspergillus ochraceoroseus,*
    2. *Aspergillus rambellii*

Several species claimed to produce aflatoxins have been synonymised with other aflatoxin producers, including

- *A. toxicarius* (=*A. parasiticus*),
- *A. flavus* var. *columnaris* (=*A. flavus*)
- A. *zhaoqingensis* (=*A. nomius*).

Compounds with related structures include sterigmatocystin, an intermediate of aflatoxin biosynthesis produced by several Aspergilli and species assigned to other genera, and dothistromin produced by a range of non-*Aspergillus* species

Description of some aflatoxin producing fungi

1. Aspergillus flavus Link, 1809 Synonyms: Monilia flava (Link) Pers., (1822)

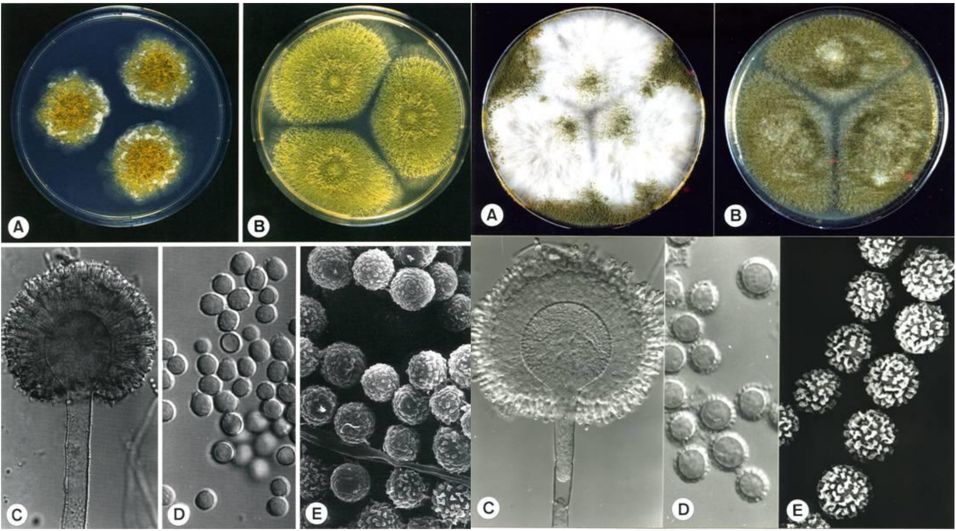
Synonyms:

- Monilia flava (Link) Pers., (1822)
- Sterigmatocystis lutea Tiegh., (1877)
- Aspergillus flavus var. proliferans Anguli, Rajam, Thirum., Rangiah & Ramamurthi, (1965)

Morphology

A. flavus is known as a velvety, yellow to green or brown mould with a goldish to red-brown reverse. On Czapek dox agar, colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, mostly 300-400 um in diameter, later splitting to form loose columns .The conidiophores are variable in length, rough, pitted and spiny. They may be either uniseriate or biseriate. They cover the entire vesicle, and phialides point out in all directions. Conidia are globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 mm in diameter. Based on the characteristics of the sclerotia produced, A. flavus isolates can be divided into two

phenotypic types. The S strain produces numerous small sclerotia (average diameter ,400 mm). The L strain produces fewer, larger sclerotia (Cotty, 1989). Within theS strain, some isolates, termed SB, produce only B aflatoxins, whilst others, named SBG, produce both B and G aflatoxins.



Aspergillus flavus S. S. Tzean and J. L. Chen *Aspergillus parasiticus*, S. S. Tzean and J. L. Chen

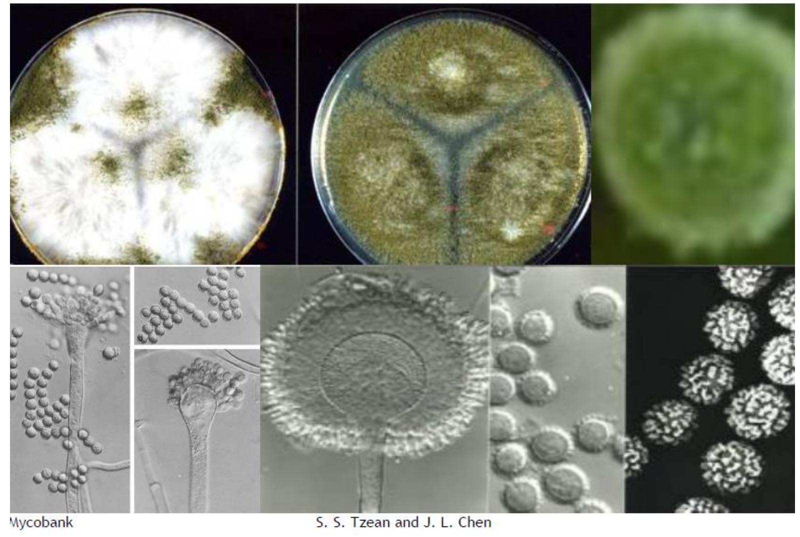
2. Aspergillus parasiticus Speare, 1912

Synonyms:

Aspergillus flavus subsp. parasiticus (Speare) Kurtzman, Smiley, Robnett & Wicklow,1986

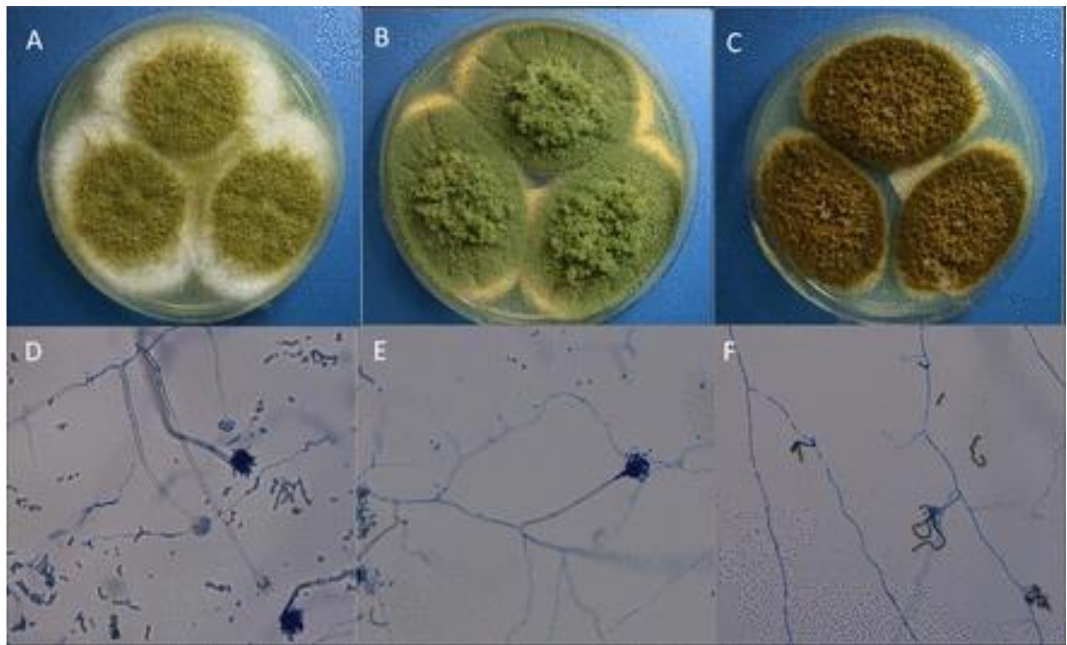
Aspergillus chungii Y.K. Shih, (1936)

Morphology Colony diameters on Czapek's Agar larger than 9 cm in 10 days at 25°C, distinctly floccose, sporulation abundant at margin; mycelium fimbriate, white; reverse uncolored; conidial heads mostly radiate or splitting into fine columns or rarely globose, small, primuline yellow, or wax yellow to yellowish citrine; stipes smooth to roughened, colorless, 86-2140 × 6.8-24.0 μm; vesicles globose to pyriform, 19.0-94.0 μm wide. Aspergilla mostly biseriate, occasionally uniseriate; metulae covering 1/2 to the whole surface of the vesicle, 9.5-21.4 × 4.8-12.7 μm; phialides 8.3-15.1 × 3.8-6.0 μm; conidia globose to subglobose, 5.5-8.3 × 4.4-7.1 μm, irregularly roughened to very roughened. Colony diameters on Malt Extract Agar 6.5-7.0 cm in 10 days at 25°C, floccose to plane; mycelium white; conidial heads distinctly radiate, occasionally loosely columnar, yellowish oil green, serpentine green to grass green, or cedar green; reverse colorless;



3. Aspergillus nomius Kurtzman, B.W. Horn & Hesselt., Antonie van Leeuwenhoek 53 (3): 151 (1987)

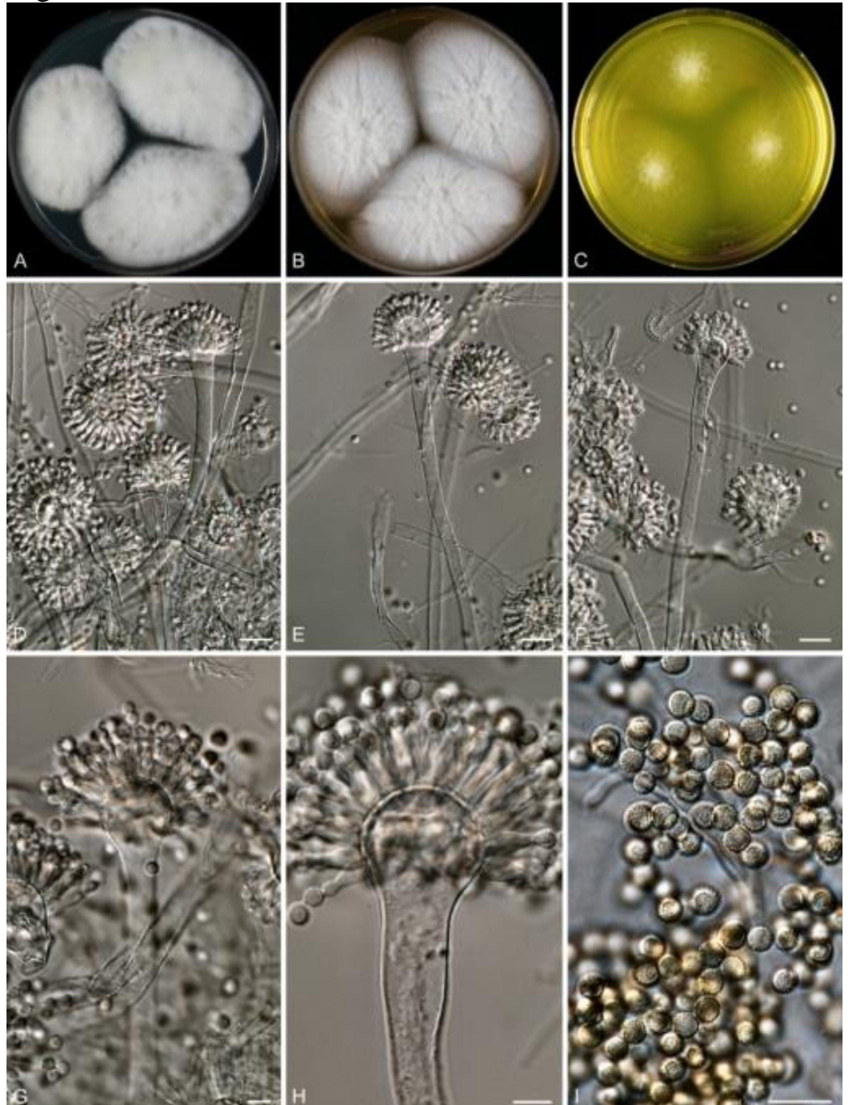
On SDA coloniese are velvety with floccose tufts and yellowish green conidia. The reverse of the colonies was dull yellow or orange-brown. The conidiophores were hyaline, with globose to subglobose vesicles and biseriate phialides. The conidia were globose to subglobose.



Aspergillus nomius, Tam *et al*., 2014

4. Aspergillus pseudonomius Varga, Samson & Frisvad, Studies inMycology 69: 67 (2011)

Colonies on YES, MEA, OA and CYA attain a diam of 6-6.5 cm in 7 d at 25 °C; growing rapidly on CYA at 37 °C, with a diam of 6-7 cm. On CREA a typical acid production. Colony surface floccose with dominant aerial mycelium with poor sporulation. Reverse not coloured. Sclerotia not observed. Conidial heads uniseriate. Stipes hyaline, smooth, variable in length, mostly (250-)400-600(21000) µm; diam just below vesicles 5-8 mm. Vesicles globose to subglobose, 15-30 µm in diam, fertile upper 75 % of their surface; Conidia globose to subglobose, echinulate, greenish, 4-5 µm. Isolates grow well at 25, 37 and 42C



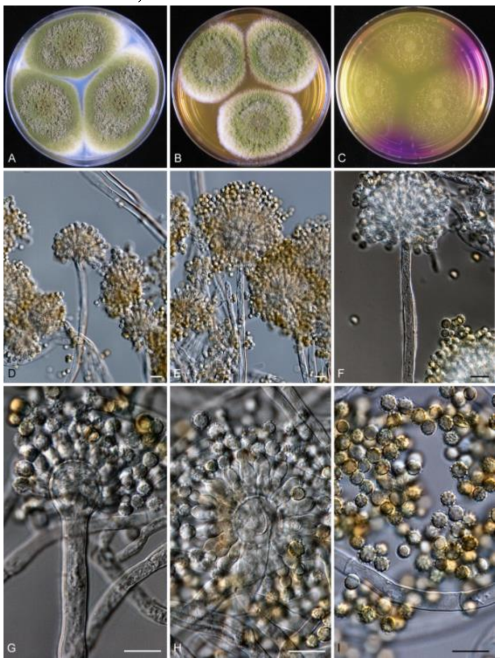
Aspergillus pseudonomius Varga, Samson & Frisvad, sp. nov. MycoBank MB560398

5. Aspergillus pseudocaelatus Varga, Samson & Frisvad, Studies in

Mycology 69: 63 (2011

Colonies on YES, MEA, OA and CYA attain a diam of 6-6.5 cm in 7 d at 25 °C; growing rapidly on CYA at 37 °C, with a diam of 6-7 cm. On CREA a typical acid production. Colony surface velvety with abundant conidial heads, olive to olive

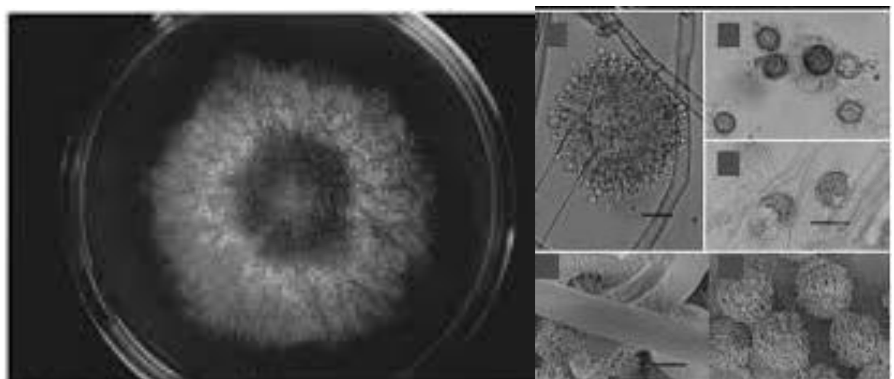
brown en masse. Reverse greenish yellow without diffusible pigments. Sclerotia not observed. Conidial heads uniseriate or biseriate. Stipes hyaline, smooth-walled, 5-8 µm wide variable in length, mostly (250-)400-600(21000) µm; Vesicles globose to subglobose, 17-22 mm in diam. Conidia globose to subglobose, echinulate, greenish, 4.5-5 µm. Isolates grow well at 25, 37 and 42 °C.



Aspergillus pseudocaelatus www.researchgate.net

6. Aspergillus pseudotamarii Yoko Ito, S.W. Peterson, Wicklow & T. Goto, Mycological Research 105 (2): 237 (2001)

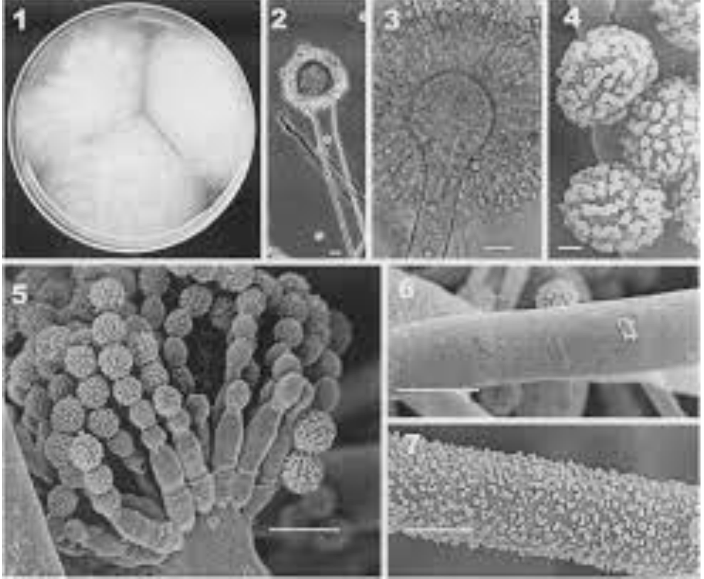
Colonies on Czapek-Dox Agar fr-7 em diam in 7 d at 25 °C; colonies at 37 0 in 7 days of 3.0-3.5 cm diam; at 42 0 spores do not genninate. Colony surface is mostly velvety consisting of abundant conidial heads. Conidial heads orange brown at 7 d. eventually shifting to light brown in mature cultures. Colony reverse pale yellow brown; diffusible pigment of the same colour seen in the agar medium. Sclerotia dark brown to black, globose to subglobose,. Conidial heads globose to radiate, often splitting into several columns, 500-770 Ilm diam. Stipe hyaline. finely roughed. Vesicles globose to subglobose., Conidia globose to subglobose, echinate; variable in diameter, ; loose outer wall surrounds a firm inner wall. Colonies on malt extract agar 6-7 em diam in 7 d, with colony surface mostly floccose and conidial heads olive green.



Aspergillus pseudotamarii, journals.cambridge.org

7. Aspergillus bombycis S.W. Peterson, Yoko Ito, B.W. Horn & T. Goto, Mycologia 93 (4): 691 (2001)

Aspergillus bombycis is a new species resembles A. flavus, but produces B and G aflatoxins. It is distinguished from A. flavus and A. nomius by differences in growth rates at 37 and 42 C, from A. nomius by roughness of the stipe, and from both of these species by differences in the nucleotide sequences in the beta-tubulin, calmodulin, norsolorinic acid reductase, ITS, and lsu-rDNA genes



Aspergillus bombycis, Peterson et al., 2001

.

1.4. The four major naturally known aflatoxins produced by the *Aspergillus* species of mould include

- AFB1, AFB2, AFG1 and AFG2 where the “B” and “G” refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates
- subscript numbers 1 and 2 indicate major and minor compounds, respectively.
- B designation of aflatoxins B1 and B2 result from the exhibition of blue fluorescence under UV-light,
- G designation refers to the yellow-green fluorescence of the relevant structures under UV-light
- The metabolic products of aflatoxins, M1 and M2 were first isolated from milk of lactating animals fed on mouldy grains contaminated with aflatoxin hence, the M designation.
- These toxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds.
- Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1 and G1, respectively. Whereas,
    - aflatoxin M1 is 4-hydroxy aflatoxin B1 and
    - aflatoxin M2 is 4-dihydroxy aflatoxin B2.

- Of the four major aflatoxins (B1, B2, G1 and G2),
    - G2 occurs in high quantities though less toxic while
    - AFB1 is the most toxic of all the aflatoxin. The World Health

- Organization (WHO) classifies AFB1 as a class 1 carcinogen
- The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of AFB1> AFG1> AFB2>AFG2
- The extent of toxicity depends on the organ affected especially the liver.
- The lethal toxicity of aflatoxin B1 varies in different animals from extremely susceptible (Sheep, Rat, Dog) to resistant species (Monkey, Chicken, Mouse).

  - Of all the above-named aflatoxins, aflatoxin B I (AFB1) is the most acutely toxic to various species.
  - Toxigenic *A. flavus* isolates generally produce only aflatoxins B1 and B2, whereas *A. parasiticus* isolates generally produce aflatoxins B1, B2, G1 and G2 (**Davis and Diener, 1983**).
  - Although aflatoxins B1, B2 and G1 are common in the same food sample,
    - AFB1 predominates (60-80% of the total aflatoxin content).
    - Generally AFB2, AFG1 and AFG2 do not occur in the absence of AFB1.
    - In most cases AFG1 is found in higher concentrations than AFB2 and AFG2 (**Weidenborner, 2001**).

Metabolism and mechanisms of action of aflatoxin B1

- The absorption from the gastrointestinal tract should be complete since very small doses, even in the presence of food, can cause toxicity. After the absorption, highest concentration of the toxin is found in the liver **(Mintzlaff *et al.*, 1974).**
- Once in liver, aflatoxin B1 is metabolized by microsomal enzymes to different metabolites through hydroxylation, hydration, demethylation and epoxidation
  - Hydroxylation of AFB1 at C4 or C22 produces, AFM1 and AFQ1, respectively.
  - Hydration of the C2 – C3 double bond results in the formation of AFB2a which is rapidly formed in certain avian species AFP1 results from o-demethylation
  - AFB1 – epoxide is formed by epoxidation at the 2,3 double bond.
  - Aflatoxicol is the only metabolite of AFB1 produced by a soluble cytoplasmic reductase enzyme system.
- The liver is the target organ for toxic effects of aflatoxin B1. As a result, metabolism of proteins, carbohydrates and lipids in liver is seriously impaired by AFB1. The toxin inhibits RNA polymerase and subsequent protein synthesis at a faster rate in ducks than in rats probably because of faster liver metabolism of AFB1 in ducks than in rats (**Smith, 1965**).
- In day-old chicks, AFB1 reduces the activity of liver UDP glucose-glycogen transglucosylase resulting in depletion of hepatic glycogen stores **(Shankaran *et al.*, 1970).**
- On the other hand, there is lipid accumulation in the liver of chickens and ducklings exposed to aflatoxin **(Carnaghan *et al.*, 1966; Shank and Wogan, 1966).**

- With regard to its toxic effects on liver microsomal enzymes, AFB1 is known to decrease microsomal glucose-6-phosphatase activity **(Shankaran *et al.*, 1970)**
- Stimulation of microsomal enzyme activity by inducers seems to be unaffected by AFB1 (**Kato *et al.*, 1970).**
- Since aflatoxin inhibits protein synthesis, it is conceivable why aflatoxin reduces resistance of poultry to infection with *Pasteurella multocida, salmonella sp.,* Marek disease virus, Coccidia and *Candida albicans* **(Smith *et al.*, 1969; Hamilton and Harris,1971).**
- Another effect of aflatoxin is that it causes anticoagulation of blood. This is probably because AFB1 inhibits synthesis of factors II and VII involved in prothrombin synthesis and clotting mechanism (**Bababunmi and Bassir, 1969**).

Susceptability of poultry to aflatoxins

Chick embryo, goslings, ducklings and turkey poults have been reported to be most susceptible as opposed to female rats being most resistant (**Newberne, 1974; WHO, 1979; Cavalheiro, 1981; Malkinson et al., 1982).**

- **Susceptibility of poultry to aflatoxins** varies among species, breeds and genetic lines
    - ducklings and turkey poultry are the most sensitive species to aflatoxins.
    - goslings, quails and pheasants show intermediate sensitivity
    - chickens appear to be the most resistant (**Leeson et al., 1995**).
    - chicks can tolerate 3 ppm in the diet without showing any observable adverse effects (**Diaz & Sugahara, 1995).**
    - chickens are not only highly resistant to the adverse effects of AFB1 but some studies have reported a modest enhancement in the body weight of chickens exposed to dietary aflatoxins, a finding that has been characterized as an hormetic-type dose-response relationship (**Diaz *et al.,* 2008).**

    - The susceptibility ranges from ducklings > turkey poults > goslings > pheasant chicks > chickens (**Muller et al., 1970**).
    - Ducklings are 5 to 15 times more sensitive to the effects of aflatoxins than are laying hens,
    - when laying hen strains are compared,
        - certain strains of hens may be as much as 3 times more sensitive than other strains (**Jones et al., 1994**).

- At low levels of feed contamination with AF (<500 ppb), exposed chickens show anorexia, decreased growth rate, poor food utilization, decreased weight gain, decreased egg production, increased susceptibility to environmental and microbial stressors, and increased

- AF toxic effects are mainly localized in the liver and are characterized by hepatobiliary damage and increased hepatic enzyme activity

Aflatoxicosis in poultry

Effect of aflatoxicosis on reproduction and hatchability

- Aflatoxins causes delayed maturation of both males and females (**Doerr, 1979; Doerr and Ottinger, 1980).**
- Aflatoxicosis in white leghorn males resulted in decreased feed consumption, body weight, testes weight, semen volume and decreased plasma testosterone values (**Sharlin *et al*., 1980**).
- Aflatoxicosis in broiler breeder males reduction in body weight and mild anemia with no alterations in semen characteristics were observed (**Wyatt, 1991; Briggs *et al.,* 1974).**
- Aflatoxicosis in mature laying hens caused enlarged and fatty liver and marked decrease in egg production (**Hamilton and Garlich, 1972**).
- Aflatoxicosis in mature broiler breeder hens caused severe decline in hatchability was recorded after consumption of aflatoxin (**Howarth and Wyatt, 1976).**
- Hatchability declines before egg production and is the most sensitive parameter of aflatoxicosis in broiler breeder hens (**Howarth and Wyatt, 1976**).
- The immediate and severe decline in hatchability was found to arise from an increase in early embryonic mortality rather than infertility of the hens. The cause of the increasedembryonic mortality is the transfer of toxic metabolites from the diet of the hen to the egg **(Wyatt, 1991).**
- The delayed response in egg production is thought to occur due to reducing synthesis and transport of yolk precursors in the liver **(Huff *et al.,* 1975).**

Effect of aflatoxicosis on hematological and biochemical alterations

- Aflatoxin causes hematopoietic suppression and anemia observed as decreases in total erythrocytes, packed-cell volume and hemoglobin (**Reddy *et al.,*1984; Huff *et al.,* 1986; Mohiuddin *et al.,* 1986).**
- Total leucocytes are increased and differential leucocytic counts vary among studies with concurrent lymphopenia **(Tung *et al.,* 1975a; Lanza *et al.,* 1980**),
- Aflatoxin produces hemolytic anemia by decreasing the circulating mature erythrocytes. Lysis of erythrocytes will result in above the normal levels of cellular debris in circulation and consequently the spleen appear congested because of an unusually high concentration of inorganic iron and debris from the circulation **(Tung *et al*., 1975a, Wyatt,1991).**
- Several biochemical parameters are affected by aflatoxin exposure. Aflatoxin decreases total serum proteins, alpha, beta and gamma globulins, with IgG being more sensitive than IgM **(Tung *et al*., 1975a**).
- Total serum proteins contents are depressed due to reduced values of alpha and beta globulins and albumen, while gamma globulins are affected more variably **(Pier, 1973).**

- Serum lipoproteins, cholesterol, triglycerides, uric acid and calcium are also decreased **(Garlich *et al*., 1973; Doerr *et al*., 1983; Reddy *et al*.,1984; Huff *et al*., 1986).**
- Aflatoxin alters both the extrinsic and common clotting pathways in chickens.
- Aflatoxins causes biochemical changes in thromboplastin clotting factors V, VII and X and reduces plasma prothrombin and fibrinogen and consequently increases whole blood clotting and prothrombin times (Doerr *et al.*, 1974, 1976).
- Dietary aflatoxin produced a malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia and decreased concentrations of bile salts and pancreatic lipase, trypsin, amylase and Rnase **(Osbrone *et al.,*1982**).
- In another experiment, the specific activities of pancreatic chymotrypsin, amylase and lipase, but not trypsin were increased significantly by aflatoxin **(Richardson and Hamilton, 1987**).

Effect of aflatoxicosis on production parameters

- In poultry, aflatoxin impairs all important production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, male and female reproductive performance.
- Some influences are direct effects of intoxication, while others are indirect, such as from reduced feed intake (**Calnek et al., 1997**).

- **The direct and indirect effects of aflatoxicosis include**
    - increased mortality from heat stress in broiler breeders **(Dafalla et al, 1987a**),
    - decreased egg production in leghorns (**Bryden et al., 1980)**
    - anemia, hemorrhages and liver condemnations (**Lamont, 1979)**
    - paralysis and lameness (**Okoye et al., 1988**),
    - impaired performance in broilers (**Jones et al., 1982**),
    - increased mortality rate in ducks (**Bryden et al., 1980**),
    - impaired ambulation and paralysis in quail (**Wilson et al., 1975**),
    - impaired immunization in turkeys **(Hegazy et al., 1991**)
    - increased susceptibility to infectious diseases (**Bryden et al., 1980 and Calnek et al., 1997).**
- **Immunosuppressive effects of aflatoxins**
    - Aflatoxin induces immunosuppression and increases susceptibility of toxicated birds to bacterial, viral and parasitic infections.
    - Immunosuppression caused by AFB1 has been demonstrated in chickens and turkeys as well as laboratory animals (**Sharma, 1993**).
    - Aflatoxin decreases the concentrations of immunoglobulins IgM, IgG and IgA in birds (**Giambrone et al., 1978).**
    - The presence of low levels of AFB1 in the feed appears to decrease vaccinal immunity and may therefore lead to the occurrence of disease even in properly vaccinated flocks (**Leeson et al., 1995**).
    - Reduced antibody production was recorded following injection of sheep red blood cells in chickens experiencing aflatoxicosis. (**Thaxton et al. (1974)**

- Chickens fed AFB1 and vaccinated against Marek's disease showed a significantly higher frequency of gross and microscopical lesions of Marek's disease than did chickens fed aflatoxin-free diet .(**Batra et al., 1991)**
- Cell-mediated immune response and effector cell function are also affected during aflatoxicosis (**Leeson et al.,1995).**
- Aflatoxin decreased complement activity in chickens (**Campbell et al., 1983 and Stewart et al., 1985)**
- Aflatoxin decreased complement activity in turkeys (**Corrier, 1991**).
- Chang and Hamilton (1979a) demonstrated reduced chemotactic ability of leucocytes, impaired phagocytosis of heterophils and impaired cellular and serum factors required for optimal phayocytosis in aflatoxicated chickens.
- Chickens receiving aflatoxin-contaminated diets showed higher susceptibility to
    - Marek's disease (**Edds and Bortell, 1983),**
    - infectious bursal disease virus (**Giambrone et al., 1978a & b**),
    - congenitally acquired salmonellosis (**Wyatt and Hamilton, 1975**)
    - duodenal and cecal coccidiosis (**Edds et al., 1973**)

Aflatoxicosis in chickens

- **Susceptibility** of chickens to toxic effects of AFB1 varies with several factors such as breed,strain, age, nutritional status, amount of toxin intake and also the capacity of liver microsomal enzymes to detoxify AFB1 (**Edds, 1973; Veltmann, 1984).**
- **Acute toxicity** of aflatoxins in chickens may be characterized by hemorrhage in many tissues and liver necrosis with icterus.Although number of field cases of aflatoxicosis in chickens has been diagnosed in various countries, the most severe spontaneous outbreak occurred in North Carolina, in which 50% of a flock of laying hens died within 48 hr of being fed highly toxic maize containing 100 ppm aflatoxin (**Hamilton, 1971**).
- **The necropsy** revealed that liver damage was the most important lesion showing paleness, occasional white pinhead-sized foci and petechial hemorrhages while gallbladder and bile ducts were distended.
- **Levels of aflatoxin** B1 in mouldy feed normally vary from 0 to 10 ppm.
    - **At low levels of feed contamination**, exposed chickens show, in general, weakness, failure to gain weight with concomitant decline in feed efficiency and egg production. Hepatic damage is manifested by enlarged and putty-colored liver, petechial hemorrhages, marked vacuolation of hepatic cells and bile duct proliferation. (**Smith and Hamilton, 1970; Doerr *et al.,*1983).**
    - **Feed levels of AFB1 as low as 250-500 ppb** given to New Hampshire chickens have been reported to result in liver damage, decreased hemoglobin, and hypoproteinemia (**Brown and Abrams, 1965).**
    - **Aflatoxin levels ranging from 1-1.5 ppm**: in experimental trials have caused growth retardation in chickens. Mortality was low but markedhepatic damage was manifested by enlarged and hemorrhagic liver (**Carnaghan *et al.,* 1966).**
    - **Relatively, high dietary levels** of aflatoxin B1 (**0-10 ppm**) given to Rock type broiler chickens have been reported to cause substantial

decrease in weight gain, feed efficiency and hepatic microsomal drug metabolizing enzymes with concomitant increase in serum glutamic oxalacetic transaminase activity reflecting liver damage (Dalvi and McGowan, 1984; Dalvi and Ademoyero, 1984).

Aflatoxicosis in ducks

- **Lethal aflatoxicosis** in ducklings occurred as inappetance, reduced growth, abnormal vocalizations, feather picking, purple discoloration of legs and feet and lameness. Ataxia,convulsions and opisthotonus preceded death **(Asplin and Carnaghan, 1961).**
- **At necropsy**, livers and kidneys were enlarged and pale. With chronicity, ascitis and hydropericardium developed accompanied by shrunken firm nodular liver, distention of the gall bladder and hemorrhages, distended abdomen due to liver tumors and secondary ascitis (**Asplin and Carnagham, 1961; Calnek *et al.,* 1997, Hetzel *et al.,* 1984).**
- **Microscopic lesions in the liver** were fatty change in hepatocytes, proliferation of bile ductules and extensive fibrosis accompanied by vascular and degenerative lesions in pancreas and kidney, bile duct hyperplasia (**Asplin and Carnagham, 1961 and Calnek *et al.,* 1997**).
- Bile duct carcinoma are also reported (Hetzel *et al.,* 1984) in aflatoxicated Campbell ducks.

Aflatoxicosis in turkeys

- **The initial clinical signs** reported during the outbreak of Turkey “x" disease were anorexia and weight loss followed by depression, ataxia and recumbency. Affected birds died within a week or two and at the time of death frequency had opisthotonus characterized by arched neck, head down back and legs extended backwards (**Hamilton *et al.,* 1972).**
- Along with decreased feed conversion and weight gain, reduced spontaneous activity, unsteady gait, recumbency, anemia and death (**Siller and Ostler, 1961; Wannop, 1961;Giambrone *et al.,* 1985 ; Richard *et al.,* 1987**).
- **At necropsy**, the body condition was generally good but there was generalized congestionand edema. The liver and kidney were congested, enlarged and firm, the gall bladder was full, and\ the duodenum was distended with catarrhal content (Siller and Ostler, 1961;Wannop, 1961; Calnek *et al.,* 1997).

Aflatoxicosis in Japanese quail

- decreased feed conversion, egg production, egg weight, hatchability and exterior and interior egg quality were detected (**Sawhney *et al.,* 1973a & b).**
- **Dhanasekaran *et al.,* (2009)** reported that histopathological analysis of aflatoxin ingested hens reveals that lesions were observed in tissues of liver, kidney, intestine.
- **Jayabharathi and Mohamudhaparveen (2010)** tested the aflatoxicosis in hens. Haematological analysis showed the decreased haemoglobin than the control group

Summary of effects of aflatoxins on the health of poultry

Hepatotoxic effects	• Jaundice (yellow skin)
Teratogenic effects	• Birth defects of the offspring
Carcinogenic effects	• Higher incidence of cancer in exposed animals
Pathological changes	• Weight variation of the internal organs: • Enlargement of the liver, • spleen and kidneys (fatty liver syndrome) • Bursa of Fabricius and thymus reduction. • Change in the texture and coloration of the organs (liver, gizzard)
Decreased performance	• Decreased feed intake (anorexia) • Decreased daily weight gain • Decreased slaughtering weight • Decreased egg production • Inhomogeneous flocks
Hematopoietic effects	• Hemorrhages • Anemia
Immunosuppression	• Decreased resistance to environmental and microbial stressors • Increased susceptibility to diseases
Neurotoxic effects	• Nervous syndrome (abnormal behaviour)
Dermal effects	• Impaired feathering.
Residues	• Residues present in the liver, meat and eggs
Dermal effects	• Paleness of the mucous membranes and legs (pale bird syndrome)
Decreased Performance	• Decreased hatchability of eggs

http://www.thepoultrysite.com/focus/biomin/2255/biomin-mycofix-the-effects-of-125-ppm-t2-toxin-on-performance-lesions-and-general-health-of-male-broiler

Reports

Smith (1960) wrote a letter to the editor of the Vet. Rec. on his observations on Turkey X disease , a "disease" in turkey poults associated with commercial ration. Mortality of turkey poults often ceased after a change of feed.

Stevens *et al.* (1960) presented a preliminary observations on Turkey X disease. The authors encountered 45 outbreaks of "disease" in turkey poults associated with high mortality. Birds died in good condition after a short illness and mortality rates ranged from **10** to **70 %.** Affected poults were usually about 4 weeks old, but birds 12 to **15** weeks old were sometimes involved. The consistent post-mortem findings were engorgement and congestion of the kidneys. Other lesions often present included enteritis, distention of the gizzard **by** coarse material, haemorrhages or necrotic foci in the liver and, less commonly, haemorrhages on the pancreas, white fleck. on

the air sacs and generalized oedema. More than one commercial ration was involved, but mortality often ceased after a change of feed.

Swarbrick (1960) presented his observations on Turkey X disease. The most striking post-mortem lesions were generalized oedema with large quantities of fluid in the peritoneal cavity, and in most of the birds, around the coronary of band of the heart. Extensive swelling of the kidneys, the surfaces of which were covered with petechial haemorrhages was very evident. Enteritis of various parts of the alimentary canal was also a prominent feature.

Blount (1960a) described an outbreak of a disease of turkey poults in England. He emphasized that the disease outbreaks were associated with certain feeds.

Blount (1960b) mentioned in his letter to the editor of the Vet. Rec. some comments on the implication of rations in turkey X disease. Outbreaks of turkey X disease were not associated with rations containing milo.

Asplin and Carnaghan (1961) proved experimentally the toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Ducklings were highly susceptible to the toxic principle in these meal, and it was suggested that they are eminently suitable for screening suspected samples of groundnut meal and for other experimental work connected with this type of toxicity. A toxic agent was found in certain Brazilian and East African groundnut meals, and evidence was presented which suggested that the toxic principle in these meals is identical. The gross and microscopic lesions in ducklings and chickens fed on toxic groundnut meals were described and the similarities and differences between the lesions in these birds and turkeys and in large animals were discussed.

Blount (1961b) emphasized in his letter to the editor of the Vet. Rec. the importance of labelling of poultry foods in relation to turkey X disease.

Blount (1961b) reported upon turkey X disease occurring in the United Kingdom with information on its aetiology.

Carnaghan (1961) described various outbreaks of **Turkey X** disease in ducklings and pheasant chicks in the U.K. during 1961. Indian groundnut meal in the birds' rations was associated with many of these outbreaks. Results of feeding experiments suggested that certain consignments of Indian groundnut meal contained a toxic principle similar to that found in Brazilian and East African samples in 1960. The toxicity of the Indian groundnut meal samples tested was considerably less than any of the toxic Brazilian or East African groundnut meals examined.

Carnaghan and Sargeant (1961) gave day-old ducklings in groups of six two turkey diets which had been associated with outbreaks of Turkey "X" disease. The diets had about 6 % Indian groundnut meal. Other groups were given similar amounts of Indian groundnut meal known to be non-toxic. Those given the toxic meal did not grow well and five in each group died within 5 weeks. Gross and microscopical lesions, were similar to those produced by toxic Brazilian and East Gross African groundnut meals, lesions, were similar to those and East Gross African and microscopical groundnut meals, were found. Extracts of the Indian meals in amounts

equivalent to 100, 200 and 750 g in 5, 5 and 11 days did not kill day-old ducklings, but liver lesions were found post- mortem.

Gibson (1961) emphasized that outbreaks of Turkey "X"disease have not always been associated with the inclusion of groundnut meal in the diet. It is not considered that listing of ingredients of proprietary foods would serve any practical purpose.

Gibson and Harris (1961) considered that there is sufficient evidence that recent heavy losses in flocks of turkeys in the United Kingdom were caused **by** poisoning by Brazilian groundnut meal

Lancaster *et al.* (1961) wrote the following letter to the editor of Nature: a new disease, called 'turkey *X* disease' has been described since the widespread outbreaks of deaths in turkey poults in 1960[1]. Post-mortem examination of dead poults from field outbreaks revealed acute hepatic necrosis, associated with generalized bile duct proliferation. Siller and Ostler[2] directed attention to the similarities of the lesions to those of *Senecio*-alkaloid poisoning in the fowl described by Campbell[3].

1. Blount, W. P. , *Turkeys*, **9**, 52 (1961).
2. Siller, W. G. , and Ostler, D. C. , *Vet. Rec.*, **73**, 134 (1961).
3. Campbell, J. G. , *Proc. Roy. Soc. Edin.*, **66**, 111 (1955–57).
4. Allcroft, R. , Carnaghan, R. B. A. , Sargeant, K. , and O'Kelly, J. , *Vet. Rec.*, **73**, 428 (1961).
5. Carnaghan, R. B. A. , and Sargeant, K. , *Vet. Rec.*, **73**, 726 (1961). Sargeant, K. , Allcroft, R. , and Carnaghan, R. B. A. , *ibid.*, **73**, 865 (1961).

Sargeant *et al. et al.* (1961a) wrote the following letter to the editor of Nature: Large numbers of turkey poults[1] and ducklings[2] died on British farms in 1960 as a result of consuming groundnut (*Arachis hypogaea*) meal imported from Brazil. Afterwards, outbreaks of disease associated with the feeding of Brazilian groundnut meal were reported in cattle[3], pigs[4], and sheep (Buxton, J. C., personal communication). More recently it has been shown that some samples of groundnut products from a number of other producing countries are toxic to animals[5].

1. Blount, W. P. , *Turkeys*, **9**, 52 (1961).
2. Asplin, F. D. , and Carnaghan, R. B. A. , *Vet. Rec.*, **73**, 1215 (1961).
3. Loosmore, R. M. , and Markson, L. M. , *Vet. Rec.*, **73**, 813 (1961).
4. Loosmore, R. M. , and Harding, J. D. J. , *Vet. Rec.* (in the press).
5. Carnaghan, R. B. A. , and Sargeant, K. , *Vet. Rec.*, **73**, 726 (1961). Sargeant, K. , Allcroft, Ruth , and Carnaghan, R. B. A. , *ibid.*, **73**, 865 (1961).
6. Allcroft, Ruth , Carnaghan, R. B. A. , Sargeant, K. , and O'Kelly, J. , *Vet. Rec.*, **73**, 428 (1961). Sargeant, K. , O'Kelly, J. , Carnaghan, R. B. A. , and Allcroft, Ruth , *ibid.*, **73**, 1219 (1961).
7. Milner, M. , and Geddes, W. F. , *Storage of Cereal Grains and their Products*, edit. by Anderson, J. A., and Alcock, A. W., 165 (Amer. Assoc. Cereal Chem., St. Paul, Minnesota, 1954).

Sargeant *et al.* (1961b) described a method for the fractionation of Brazilian groundnut meal. All the toxic material, 0.4 %by weight of the original, was found in the fraction insoluble in methanol and water, extracted with chloroform and separated with petrol and water. A suspension in water was made so that I mL was equivalent in toxicity to 40 g meal. Ducklings were killed in under 24 h by I mL of that suspension and it was lethal at lower concentrations. The equivalent of 0.5 g meal caused liver damage. The corresponding fraction from Indian groundnut meal was not toxic.

Turkey poults were less susceptible than were ducklings and the mortality and lesions were identical to those in field outbreaks of Turkey "X" disease. It was confirmed that the toxic substance is neither a pyrrolizidine alkaloid nor the N-oxide of such an alkaloid. It may be derived from a micro-organism. A similar toxic substance has since been found in samples of groundnuts from India, Uganda and Tanganyika, French West Africa, Nigeria, the Gambia and Ghana.

Sargeant *et al.* (1961c) reported that the toxic extract of a Brazilian groundnut meal was further purified and a fluorescent method of identification after chromatographic separation was devised. The toxic substance was isolated from a fungus, Aspergillus flavus. When the fungus was grown on sterilized groundnuts and fed to ducklings, it resulted in typical liver lesions in ducklings.

Wannop *et al.* (1961) reported cases **cf** Turkey "X" disease seen in chickens, ducks and turkeys, which were given compound feeds containing no groundnut meal.

Archibald *et al.* (1962) demonstrated that examination of chickens after death showed liver damage typical of groundnut poisoning. Birds less than 5 weeks old were more affected than older ones, and their mortality was higber. I twas confirmed that diets of al affected chickens contained 5 % of Brazilian groundnut meal.

Carnaghan and Alcroft (1962) wrote a letter to the editor of the Vet. Rec., which highlighted the possible hazards of incorporation of toxic groundnut meal in animal feeds.

de Iongh *et al.* (1962) investigated the factor in groundnut meal responsible for "turkey X disease. Toxin-containing extracts of either toxic groundnut meals or Aspergillus flavus cultures were resolved by thin-layer chromatography into several zones which were fluorescent when viewed in ultraviolet light. The separated fluorescent materials were administered to ducklings. The fraction B, was toxic to ducklings. The combined fractions (B, and B2) showed greater toxicity than B, alone, indicating some toxicity also due to B2. The B, fraction from mold cultures and B, from extracts of toxic groundnut meals had the same R, values, and identical ultraviolet-absorption spectra. It is concluded that the extracts from cultures of A. flavus contained at least two substances toxic to ducklings.

Derzsy *et al.* (1961) mentioned that two diseases, virus hepatitis and toxic liver damage caused by feeding groundnut meal caused considerable losses of ducks. The preventive inoculation of ducklings on infected premises with hyperimmune serum completely prevented losses caused by virus hepatitis. Food mashes containing groundnut meal imported from Brazil, Africa and India caused heavy losses among young ducks but intoxications of the same origin occurred also among young chickens and turkeys. In young ducklings such a meal of high toxicity caused an acute liver degeneration, but usually the condition was of a subacute or chronic character. Pathologically an inclination to regeneration was characteristic for the condition, but in more adult ducks cirrhotic livers were often encountered. The only possibility of the control of this condition is to stop the feeding of the toxic groundnut meal immediately after loss of appetite was observed. Loss of appetite may usually be observed in such cases several days before the first deaths occurred.

Allcroft and Carnaghan (1963a) reviewed the biological effects of toxic groundnut meal (meal containing aflatoxin) in various birds and animals. Ducklings are the most susceptible to the toxin. They are suitable for bioassay of aflatoxin. Turkey poults are less suscaptible, while chickens are comparatively resistant.

Allcroft and Carnaghan (1963b) fed rations containing toxic groundnut meal excreted in the milk a toxic factor having a biological effect in ducklings similar to that caused by aflatoxin. -Precipitation of protein fractions of the milk showed that the toxin was present only in the rennet-precipitated casein fraction which also included the fat; none was found in the protein-free filtrate. Its presence was not detected in samples of bulked milk supplies from collection centres in Britain; nor was it found in liver from a cow or a pig, or clotted blood and serum from a cow, or pullet eggs, from animals fed rations containing toxic groundnut meal.

Allroft and Loosmore (1963) described the clinical and pathological effects of a disease in poultry, pigs, cattle and laboratory animals associated with the feeding of toxic batches of groundnut meal. The toxic factor is a hepatotoxin and is produced by infection of groundnuts by a toxigenic strain of Aspergillus flavus which has been found in some batches of groundnuts from all major groundnut-producing countries.

Gardiner *et al.* (1964) diagnosed aflatoxicosis in broiler flocks fed on rations containing 14 **%**groundnut meal in Western Australia. Post-mortem examination revealed very pale, sometimes almost white livers and kidneys which were firmer than normal. Aflatoxin B1 content of three groundnut meal samples was assayed at 2800, 2200 and 2500 lag kg**-'.** The pathology in ducklings, chickens and laying hens fed the original ration, or synthetic rations with added groundnut meal, is described. In chickens after 21 days feeding on the toxic ration, pale areas were noticed in skeletal muscle, which were seen to be large areas of necrosis with a diffuse increase in sarcolemmal nuclei. Toxigenic strains of *Aspergillus flavus* were isolated from the aflatoxin-contaminated samples of groundnut meal.

Hart (1965) reported that in an outbreak of poisoning in turkeys in Australia the feed had 5 % of groundnut meal. The groundnut meal had more than 2000 .tg kg[1] aflatoxin B, estimated by thin-layer chromatography, and water extracts were poisonous to ducklings. Other samples of groundnut meal imported into Australia had 1600 to 2000 gg kg' aflatoxin and a local product had 2700 to 3300 gg kg' aflatoxin.

Gopal *et al.* (1969) reported occurrence of aflatoxicosis in poultry in Mysore State, India, The disease was first recognised at the Government Poultry Breeding Unit, Hebbal, Bangalore in 1966 wherein 2219 chicks died in one week. Subsequently, several sporadic incidences were found in various poultry farms in the State. The disease was predominent in younger stocks, possibly due to the increased percentage of protein in the form of toxic groundnut cake.

Mabee and Chipley (1973) investigated the **metabolism of AFB_1** during continuous exposure. The effects of administering low levels of aflatoxin B(1)-(14)C by crop intubation daily for 14 days to **broiler chickens** were determined. Studies on the

distribution of (14)C in the blood, selected organs, tissues, and excreta were conducted. No toxic effects were observed in broiler chickens during the 14 days of the experiment. The broiler chickens excreted 90.64% of the (14)C administered. Of the (14)C retained, 11.04, 9.83, 4.30, 12.52, 31.66, and 30.63% were detected in the blood, liver, heart, gizzard, breast, and leg, respectively. Chemical assay of those samples demonstrating radioactivity revealed that 81.2% of the radioactivity in these substrates was not extractable by classical extraction procedures while approximately 10% was extractable. Treatment of aqueous extracts for conjugated steroids by treatments with beta-glucuronidase revealed that 31.5% of the (14)C detected in the aqueous extract was a liberated glucuronide conjugate of aflatoxin M(1)-(14)C.

Chu and Ueno (1977) obtained antibody against aflatoxin B1 after one multiple-site injection of bovine serum albumin-aflatoxin B1 conjugate into rabbits. The antibody has greatest binding efficiency for aflatoxin B1, less efficiency for B2, G1, and Q1, and least for aflatoxicol, G2, and M1. Sterigmatocystin, coumarin, and 4-hydroxycoumarin did not give a cross-reaction with the antibody. The sensitivity of the binding assay for detection of aflatoxin B1 is in the range of 0.2 to 2.0 ng per 0.5-ml sample. Detailed methods for the preparation of the conjugate, production of immune serum, and methods for antibody tiOr determination are described.

Ruff (1978) reported that **broiler chicks** (Hubbard x Hubbard) receiving 2.5 microgram of aflatoxin/g of diet and inoculated with **sporulated oocysts of** *Eimeria acervulina* gained significantly less weight than chicks receiving either aflatoxin or coccidia alone. Aflatoxin alone affected body weight more in females than males. Blood parameters, however, showed no sex-related differences to E. acervulina or aflatoxin. E. acervulina had no effect on packed cell volume, red cell number, or hemoglobin levels. Gross lesions in the intestine caused by the coccidia appeared the same with or without dietary aflatoxin. Either aflatoxin or E. acervulina alone reduced the plasma pigment. When both were present, depigmentation was greater with some strains of coccidia than with either alone. Aflatoxin alone reduced the plasma levels of cholesterol and protein. The effect of E. acervulina on cholesterol or protein, with or without aflatoxin, depended on the coccidial strain used.

Warren and Hamilton (1980) fed graded concentrations of dietary **ochratoxin** (0, 0.5, 1.0, 2.0, 4.0, and 8.0 microgram/g) and **aflatoxin** (0, 0.625, 1.25, 2.5, 5.0, and 10.0 microgram/g) to broiler chicks from hatching to 3 weeks of age. The breaking strength of the **large intestines** was decreased significantly ($P < 0.05$) by ochratoxin (2, 4, and 8 microgram/g), but not by aflatoxin. This fragility was accompanied by an increase in the weight of the large intestine relative to body weight of birds fed ochratoxin (4.0 and 8.0 microgram/g), whereas aflatoxin had no significant ($P < 0.05$) effect on this parameter. Lipid content of the large intestine was decreased significantly ($P < 0.05$) by aflatoxin (10.0 microgram/g) and increased by ochratoxin (8.0 microgram/g). Microscopic examination of cross sections of large intestines stained for collagen gave the impression of a great decrease in collagen content of birds fed ochratoxin, but not aflatoxin. The radial length of the collagenous longitudinal folds of the large intestine was decreased significantly ($P < 0.05$) by ochratoxin (2.0, 4.0, and 8.0 microgram/g). These observations, plus a field case characterized by intestinal ruptures causing carcass condemnations on the processing

line and by the occurrence of aflatoxin and ochratoxin in the chicken feed, suggest a novel way in which mycotoxins cause economic loss to agriculture.

Gaur *et al.* (1981) converted Aflatoxin B1 to aflatoxin B2a and then conjugated it to bovine serum albumin and horseradish peroxidase by a reductive alkylation method. Antiserum was developed in New Zealand white rabbits by multiple-site injection with the aflatoxin B2-bovine serum albumin conjugate. Antibody titers were indicated that the antiserum was most reactive with aflatoxin B1, and slightly cross-reactive with aflatoxins B2a, B2, and M1. Competitive ELISAs showed the antiserum to be equally specific for aflatoxins B2a and B, and less reactive with aflatoxins B2 and M1. The relative sensitivities of RIA and ELISA for aflatoxin B, quantitation were 100 and 10 pg per assay, respectively.

Osborne and Hamilton (1981) reported that dietary aflatoxin at concentrations of 1.25 microgram/g or above caused in **broiler chickens** a significant (P less than .05) decrease in the specific activities of pancreatic amylase, trypsin, lipase, RNase, and DNase. These enzymes are the primary **enzymes of digestion** for starches, protein, lipid, and nucleic acids. At concentrations of 2.5 microgram/g or above there was a compensatory pancreatomegaly that resulted in essentially normal total activity for trypsin, RNase, and DNase. Thus, aflatoxicosis was associated with reduced activity levels of enzymes that digest starch and lipid. This digestive deficiency could account for a malabsorption syndrome observed in field outbreaks of aflatoxicosis.

Dalvi *et al.* (1984) reported that aflatoxin B1 (AFB1) caused dose-dependent reductions in weight gain and feed consumption when day-old Hubbard X Hubbard broiler type chicks were maintained on a diet contaminated with either 0, 2.5, 5, or 10 ppm purified AFB1 for 8 weeks. Although changes in these parameters were detected at the 2.5 and 5 ppm, the most profound changes were evident at 10 ppm contamination. The concentration of cytochrome P-450 in hepatic microsomes, measured at the end of 8 weeks, also showed dose-dependent decreases. Cytochrome P-450 content in chickens receiving 2.5, 5, and 10 ppm AFB1 was 16, 28, and 65%, respectively, less than the control. Microsomal benzphetamine N-demethylase activity was not inhibited by 2.5 or 5 ppm, but ingestion of 10 ppm AFB1 reduced its activity by more than 40%. Serum glutamic oxalacetic transaminase (SGOT) levels of chickens receiving 10 ppm AFB1 increased by more than 100%, indicating substantial liver damage. However, birds simultaneously receiving 10 ppm AFB1 and activated charcoal (.1% in the feed) or either reduced glutathione (.05%) or phenobarbital (.05%, given intermittently) in their drinking water showed a trend of improvement in feed consumption (less than 10% reversal) and weight gain (less than 28% reversal) over the birds receiving 10 ppm AFB1 alone. The results also indicate that the simultaneous presence of these agents with AFB1 considerably prevented the inhibitory effect of AFB1 on the microsomal cytochrome P-450 and benzphetamine N-demethylase activity. Furthermore, these agents were able to provide moderate protection against AFB1-induced liver injury manifested by elevation of SGOT activity.

Giambrone *et al.* (1985a) conducted two separate experiments on Hubbard **broilers,** they noted a non-significant increasing trend in **ND** titers with increase in the AFB_1 content of ration from zero to 0.5 mg/kg in one of these experiments. Also, a higher ($P < 0.05$) response from fowl cholera vaccine was noted in the birds fed 0.5 mg AFB_1/kg diet. In the other experiment, higher ($P < 0.05$) ND, and fowl cholera titers were noted in birds fed 0.1 mg, and 0.2 mg AFB_1/kg diet, respectively. The

increase in titers against ND and fowl cholera in birds fed on AFB_1 contaminated ration was not seen in the birds fed on rations containing mixtures of AFB_1 and AFB_2.

Giambrone *et al.* (1985b) studied the effect of crude aflatoxin (AF) on the **growth, performance, and immune response** of **turkeys and broilers**. Crude AF, produced from a natural outbreak of Aspergillus flavus on corn, was ground and mixed in rations to contain either 0, 100, 200, 400, or 800 ppb of aflatoxin B1 (AFB1). Turkeys (Experiment 1) and broilers (Experiment 2) were used in identical experimental designs. In each, 200, 14-day-old birds were divided equally by sex into five groups of 40 and were fed one of five AF diets for 35 days. In Experiment 1, crude AF greater than or equal to 400 ppb was highly toxic to turkeys. These levels produced signs and lesions of aflatoxicosis as well as a significant decrease in weight gain and feed conversion during 5 weeks. In addition, microscopic lesions, indicative of aflatoxicosis, were evident as low as 100 ppb, and significant decreases in cell-mediated immunity were noted in the 200 ppb group birds. Experiment 2 indicated that chickens were less susceptible to crude AF than turkeys. Neither morbidity nor mortality occurred in broilers. Gross lesions consistent with AF toxicity were evident in birds given 800 ppb and microscopic lesions were observed in birds given 100 ppb. Feed conversion was significantly increased in the 800 ppb broilers only. Cell-mediated immunity, measured by a delayed hypersensitive skin test, was significantly decreased in broilers receiving AF at 200 ppb or greater. Neither humoral immunity nor the development of the acquired immunity to Newcastle disease or fowl cholera vaccination were decreased in turkeys or broilers given AF.

Wolzak *et al.* (1986) conducted a feeding trial to determine the **levels of aflatoxins deposited in the tissues** of **hens** fed a diet contaminated with 3310 micrograms AFB1/kg and 1680 micrograms AFB2/kg for 4 wk. At the end of aflatoxin feeding, the livers were pale, enlarged and haemorrhagic and the ovaries were significantly smaller than those from control hens and contained only small ova. Only a small fraction of the aflatoxins consumed was deposited in the tissues, either as the original compounds or as their metabolites, which were widely distributed in all tissues. The highest levels of aflatoxins were detected in the gizzard, kidneys and liver, with average total concentrations of less than 3 micrograms/kg. The lowest residue levels were detected in the breast, blood serum and leg, with breast muscle having a total concentration of less than 0.1 microgram/kg. Two days after removal of the contaminated feed, aflatoxin residues in all tissues had decreased markedly, with no aflatoxins being detected in the heart or spleen. No aflatoxin residues were detected in the breast, leg, gizzard and ovaries of hens killed 8 days after withdrawal, or in the kidneys and blood at 16 days. However, one of seven hens had measurable amounts of AFB2 in the liver 32 days after withdrawal. Although few residues were detected in most tissues after 8 days on the aflatoxin-free diet, variation existed between tissues and between individual hens in the amount of time required to achieve tissue clearance.

Chotinski *et al.* (1987) studied the effect of aflatoxin B1 on the content of SH-groups and the **activity of leucinamino peptidase, Mg (Na+, K+), ATP, and glucoamylase** in the mucous membrane of **the small intestine** of birds. The experiment was carried out with three groups of male **broilers**. The controls were given starter and finisher mixtures with 21.6 and 19.5 per cent protein, with no aflatoxin B1. The birds of the test groups (II and III) were offered one and the same mixture containing 0.250 and 0.600 ppm of aflatoxin B1. By the end of the finisher period on the 49th day mucosa

homogenate was used to determine the content of SH-groups and the activity of leucinamino peptidase, Mg (Na+, K+), ATP, and glucoamylase. It was found that rates of 0.600 ppm in the feed suppressed the activity of Mg (Na+, K+) ATP in the mucosa of the small intestine. Lower amounts (0.250 ppm) produced no effect on the activity of this enzyme. Leucinamino peptidase, glucoamylase, and the SH-groups did not change essentially their activity at the two rates of the toxin.

Richardson K.E., Hamilton (1987) mentioned that it was hypothesized that aflatoxin causes malabsorption and its toxicity is enhanced by a low protein diet, **digestive enzymes** formed in the pancreas apparently are influenced by aflatoxin. This hypothesis was investigated in a 2 X 2 factorial experiment. Six groups of 10 egg-type chickens per treatment were analyzed for the absence and presence of aflatoxin (0 and 4 micrograms/g diet) and for normal (12.75%) and low (10.00%) protein in soy-dextrose diets. The **specific activities of pancreatic chymotrypsin, amylase, and lipase,** but not trypsin, were increased significantly (P less than .01) by aflatoxin. Lowering dietary protein had no effect by itself except to increase amylase activity. Low protein and aflatoxin interacted to lessen but not prevent the effect of aflatoxin on chymotrypsin and amylase. Calculation of total pancreatic activities revealed that aflatoxin increased trypsin, chymotrypsin, amylase, and lipase to 107, 169, 113, and 119%, respectively, of control values on the low protein diet, whereas values were 99, 175, 115, and 115%, respectively, on the normal protein diet. Neither aflatoxin nor low protein altered significantly (P less than .05) the lipid content of fecal material. Thus, aflatoxicosis in egg-type chickens is characterized by a surplus of some digestive enzymes and by normal fecal lipids in contrast to the specific deficiency of amylase and lipase and steatorrhea reported earlier in meat-type chickens. Whereas malabsorption caused by aflatoxin in broilers can be accounted for in part by impaired digestion, this mechanism apparently does not occur in egg-type chickens.

Micco *et al.* (1988) performed a study to determine **aflatoxin residues in tissues and organs** of male **broilers and hens** that had been fed a diet contaminated with 50 micrograms/kg aflatoxin B1 (AFB1). Residue levels of AFB1, aflatoxicol (Ro), aflatoxin M1 (AFM1) and aflatoxin B2a (AFB2a) were determined by an HPLC method and, with the exception of AFB2a, were detected in the liver, kidney and thigh of both male broilers and hens. The highest levels found were for Ro in liver (1.10 and 0.60 micrograms/kg for male broilers and hens, respectively). On the other hand no detectable amounts of aflatoxins were found in any tissue after withdrawal periods of 14 and 33 days for male broilers and laying hens respectively.

Rao *et al.* (1990) mentioned that the clinical signs and gross lesions caused by **Eimeria uzura** (10(5) oocysts) in **Japanese quail** (Coturnix coturnix japonica) exhibited little or no influence in the face of intercurrent dietary aflatoxicosis (1 p.p.m. of aflatoxin B1 from Day 0 to 55). Similarly, no significant differences in the mucosal morphology of the intestine were evident histologically between the two groups of Japanese quail. The nervous signs of ataxia, leg weakness, incoordination of movement, torticollis and terminal opisthotonos were toxin-induced manifestations. In the aflatoxic quail, hypoplastic changes and selective depletion of lymphocytes were more prominent in the bursa of fabricius. Increased relative mean weights of liver, kidney, spleen, crop, proventriculus and gizzard were observed in birds due to aflatoxin sensitivity. The combination of E. uzura infection and aflatoxicosis in Japanese quail may cause significant weight loss, and increased oocyst production and reproductive potential.

SCHEIDELER (1993) conducted in vivo and in vitro trials to test the efficacy of four **aluminosilicates (AS) (Ethacal® feed component, Novasil, Perlite, and Zeobrite)** to sorb aflatoxin B_1 (AFB_1) and alleviate aflatoxicosis in **broiler chicks**. Percentage sorption capacity of AS to radiolabeled AFB_1 dissolved in methanol varied from 2 to 60%, whereas percentage sorption in intestinal contents varied from 0 to 40.0% according to type of AS tested. Intestinal contents alone sorbed 42% radiolabeled AFB_1. Novasil and Zeobrite exhibited the highest rates of sorption (55 and 60%, respectively) in methanol. An *in vivo* study compared the four types of AS in combination with 0 or 2.5 ppm AFB_1 fed to day-old chicks (two pens of six chicks per treatment) to 3 wk of age. Diet effects on body weight, liver lipid, bone ash, and serum Ca, P, Na, K, and Cl were measured. The AFB_1significantly decreased 2- and 3-wk body weight, and a significant interaction effect of AS and AFB_1 on bird weight occurred at 2 and 3 wk of age. Three of the four AS tested alleviated the growth depression caused by AFB_1. Liver lipids percentage was increased in the AFB_1-treated chicks, but this effect was suppressed by three of the AS. Bone ash was not affected by AFB_1 and was increased by Novasil and decreased by Ethacal®. Ethacal®, Novasil, Perlite, and Zeobrite all tended to decrease serum Cl, regardless of AFB_1 treatment

Fernandez *et al.* (1994) fed two groups of 32 **hens and broiler chickens** with 2.5 and 5 mg of aflatoxin (AF) kg^{-1} feed for a period of 32 days. During this contamination 16 birds were sacrificed and aflatoxin and its metabolites were detected using thin-layer chrotnatography and fluorescence densisometry. The **tissues** analysed (liver, muscle, kidney, gizzard and eggs) gave a wide range of concentrations, the lowest was found in ben muscle (0.05 μg kg^{-1} of AFB_1) and the highest in gizzards from the 5 mg kg^{-1} group of the hens (9.01 μg kg^{-1} of AFB_1). Metabolites of AFB_1, AFM, and AFB_{2a} appeared in the liver but not in other tissues. In broiler's tissues, the following metabolities were isolated: AFM_1 and AFB_{2a}, in liver, aflatoxicol in muscle and AFM_1 and AFB_{2a} in kidneys, all having concentrations lower than AFB_1. Aflatoxicol was isolated from one egg sample (0.32 μg kg^{-1}). For both types of birds, aflatoxin clearance time was only 24 h for muscle and kidneys. In livers from the 5 mg kg^{-1} group, AFM_1 and AFB_{2a} were still found 4 days after removal of the contaminated feed. In **eggs and gizzards, aflatoxin residue** was still detected on the 8th day of the clearance period although in low quantities. In the broiler's gizzards, clearance time was only 24 h. These results suggest that aflatoxin transfer to edible tissues is very small and the danger of contaminations to humans is also very small, except in the case of gizzards.

Rao *et al.* (1995) conducted a study to assess the influence of dietary aflatoxin on **Eimeria uzura** in **Japanese quail** (Coturnix coturnix japonica). Quail receiving 1 part per million (ppm) of dietary aflatoxin B1 and inoculated with 10(5) sporulated oocysts of E. uzura gained significantly less weight than chicks receiving either aflatoxin or coccidia alone. Increased morbidity, mortality and decreased efficiency of feed utilization were also evident. The combination of E. uzura infection and aflatoxicosis resulted in reduction in packed cell volume (PCV) and haemoglobin (Hb). The combination of E. uzura infection and aflatoxicosis in Japanese quail may influence the course of coccidial infection due to the additive effects of aflatoxin.

Edrington *et al.* (1997) conducted a study to evaluate the effectiveness of a **superactivated charcoal (SAC)** in alleviating mycotoxicosis. Two experiments were

conducted in which 432 male broiler chicks (216 per experiment) were fed diets containing 4 mg aflatoxin (AF) or 6 mg T-2 toxin/kg of diet, with and without 0.5% SAC, from 1 to 21 d of age. Feeding AF and T-2 toxin significantly decreased BW gain over the 21-d experimental period. Inclusion of SAC in the diet containing AF resulted in BW gains that were intermediate between gains of chicks fed AF and those of controls. No benefits were seen in BW gain when SAC + T-2 toxin was fed. Feeding AF increased relative weights of liver, spleen, and kidney; however, only liver weight in Experiment 1 was similar to controls when SAC was included. Of the blood parameters altered by AF (decreased cholesterol, inorganic phosphorus, total protein, and urea nitrogen, and increased mean corpuscular volume and hematocrit in Experiment 1; decreased albumin and total protein, and increased creatine kinase in Experiment 2) only urea nitrogen, hematocrit, and inorganic phosphorus (Experiment 1) and hematocrit (Experiment 2) were comparable to controls when SAC was included in the diet. Feeding T-2 toxin decreased serum cholesterol, total protein, urea nitrogen, and mean corpuscular volume; however, only cholesterol and mean corpuscular volume were improved with the addition of SAC (Experiment 1). Oral lesions were observed in birds fed T-2 toxin with no difference in severity when SAC was added in Experiment 1, however in Experiment 2, birds fed SAC + T-2 had a significantly lower lesion scores than those fed T-2 alone. Mortality was noted in both experiments but was not influenced by SAC treatment. These findings suggest that the addition of dietary SAC is marginally effective in alleviating some of the toxic affects associated with AF, but was of little benefit when T-2 toxin was fed to growing broiler chicks.

Okotie-Eboh *et al.* (1997) conducted 2 x 3 factorial experiments, where 240 **broiler** chicks were fed diets containing 0, 0.01, and 0.02% **beta-carotene or canthaxanthin** with or without 5 ppm aflatoxin to determine the effects of these two carotenoids on the health and well-being of broilers subjected to aflatoxin poisoning. Neither beta-carotene nor canthaxanthin was effective at overcoming the growth-depressing effects of aflatoxin. Relative liver weights were significantly higher in broilers receiving dietary aflatoxin in the presence of beta-carotene but not canthaxanthin. beta-Carotene and canthaxanthin had no effect on antibody production against infectious bursal disease (IBD). Interestingly, secondary antibody production against IBD was enhanced by the presence of aflatoxin in the diet. Canthaxanthin significantly increased the concentrations of cholesterol, total protein, uric acid, and triglyceride, all of which were significantly depressed by aflatoxin. beta-Carotene did not effect any of the measured blood analytes. There was a **significant interaction between canthaxanthin and aflatoxin** with respect to creatine kinase activity. Creatine kinase activity decreased as dietary canthaxanthin increased in the presence of aflatoxin. The data suggest that beta-carotene is not effective at ameliorating aflatoxicosis in broiler chickens but that canthaxanthin may be somewhat effective with respect to certain clinical blood chemistry indicators.

Bailey *et al.* (1998) conducted experiments to determine the efficacy of three inorganic sorbents, S1, S2, and S3, to reduce the toxicity of aflatoxins (AF) and T-2 toxin in male broiler chickens from day of hatch to 21 d of age. The compounds had been reported to bind to AF and T-2 toxin in vitro. S1 and S2 were the same basic compound that had been stored for different lengths of time following activation. In Experiments 1, 2, and 3, the appropriate diets were produced to contain no mycotoxins, the specific adsorbent at 0.5% of diet, AF alone at 5 mg/kg of diet, T-2 alone at 8 mg/kg of diet, AF at 5 mg/kg of diet plus the specific sorbent at 0.5% of

diet, or T-2 at 8 mg/kg of diet plus the specific sorbent at 0.5% of diet. The specific sorbents used were: 1) Experiment 1, S1; 2) Experiment 2, S1 and S2; and 3) Experiment 3, S3. In Experiments 1 and 3, S1 and S3, respectively, showed no protection against AF or T-2 toxin as measured by BW gain, when compared to AF alone group. In Experiment 2, S1 showed no protection; however S2 reduced the effects of AF on BW gain by 25% as compared to AF alone diet. The data demonstrate that under the conditions of our experiment: 1) one of the sorbents provided some protection against aflatoxicosis; 2) there was variability in protection against aflatoxicosis between two different samples of the same sorbent that had been stored for different lengths of time following activation; 3) protection by the sorbents against the effects of T-2 toxin was not observed.

Kiran *et al.* (1998) conducted a study in order to evaluate the efficiacy of a **polyvinylpolypyrrolidone for protection against aflatoxicosis in broiler chick**s. For this purpose 132 day-old broiler chicks (Hybro) were used. They were divided into four groups, each of 33 chicks. Group 1: control; Group 2: 3 g of polyvinylpolypyrrolidone (PVPP) per kg of diet; Group 3: 2.5 mg of aflotoxin (AF) per kg of diet; Group 4: 2.5 mg of AF per kg of diet plus 3 g of PVPP per kg of diet. The chicks were maintained on these treatments for 21 days, and then 15 broilers from each treatment group were killed for pathological examination. Hepatic lesions in broilers of AF treatment group were characterized as diffuse and severe hydropic degeneration, bile duct hyperplasia and periportal fibrosis. In the AF plus PVPP group, the liver of eight broilers showed slight or moderate hydropic degeneration. Grossly, the bursa of Fabricius was atrophied and sections revealed necrosis and depletion of lymphocytes from follicles in 12 broilers of the AF group and two of the AF plus PVPP group. In the spleen of six chicks from the AF treatment group Iymphoid areas were depleted. Thymuses from nine chicks fed the AF-alone diet showed atrophy and depletion of lymphocytes from the cortical and medullary areas. Similar changes were observed in the thymuses of four chicks from the AF plus PVPP group. The severity of atrophy in the lymphoid organs was more pronounced in the AF group than in the AF plus PVPP treatment group. In this study it was found that both the number of affected broilers and the severity of lesions were significantly decreased in the AF plus PVPP treatment group compared with AF-alone treatment. These findings suggested that PVPP can diminish the toxicity of aflatoxin in broiler chicks.

McKenzie *et al.* (1998) evaluated the capability of electrochemically produced **ozone** to **degrade AFB1 in naturally contaminated whole kernel corn** and confirm detoxification in turkey poults. Corn was procured from the southern coastal areas of Texas and HPLC revealed 1,220 +/- 73.3 ppb AFB1. Control and contaminated corn were treated for 92 h with O3 at 200 mg/min in 30 kg batches; greater than 95% reduction of AFB1 in contaminated corn was achieved. One-day-old female turkey poults were fed 1) control corn, 2) control corn + O3, 3) AFB1 corn, or 4) AFB1 corn + O3 mixed in rations (46% by wt.) and consumed ad libitum for 3 wk. When compared with controls, turkeys fed AFB1 corn had reduced body weight gain and relative liver weight, whereas turkeys fed control corn + O3 or AFB1 corn + O3 did not differ from controls. Furthermore, alterations in the majority of relative organ weight, liver discoloration, serum enzyme activity, hematological parameters, and blood chemistry caused by AFB1 were eliminated (no difference from controls) by treatment with O3. These data demonstrate that treatment of contaminated corn with electrochemically produced O3 provided protection against AFB1 in young turkey

poults. It is important to note that treatment of control corn with O3 did not alter the performance of the turkey poults.

Qureshi *et al.* (1998) fed **broiler breeder hens** diets amended with 0 and 10 mg/kg (Trial 1) or 0, 0.2, 1, or 5 mg/kg (Trial 2) of aflatoxin (AF). Fertile eggs collected during 14 d of AF feeding were examined for AF residues. Various **immunological** endpoints were examined in chicks hatched from these eggs. **Eggs** collected at 7 d of AF feeding (Trial 1) had 0.15 to 0.48 ng/g of AFB1 and 0.22 to 0.51 ng/g of aflatoxicol, whereas eggs collected at 14 d of AF feeding had 0.05 to 0.60 ng of AFB1/g and 0.19 to 1.20 ng of aflatoxicol/g. In both trials, AF dietary exposure resulted in embryonic mortality and reduction in hatchability compared to controls. The AF progeny chicks in Trial 2 had total anti-SRBC antibodies similar to the controls during the primary antibody response. However, at 5 and 7 d after secondary SRBC injection, the antibody levels in the 1 and 5 mg/kg AF groups were lower than those of controls. Depression in anti-Brucella abortus antibodies occurred only in chicks from the 5 mg/kg AF group. Furthermore, phagocytosis of SRBC and reactive oxygen intermediate production by macrophages from AF progeny chicks were reduced as compared with the control chicks. The findings of this study imply that the progeny chicks from hens consuming a AF-amended diet may be increasingly susceptible to disease owing to suppression of humoral and cellular immunity.

Amaya-Farfan (1999) indicated that aflatoxin B1 (AFB1) exerts a chronic carcinogenic and an acute toxic effect on animals. Whereas the mechanism for carcinogenicity is known, no mechanism has been proposed for the toxic action. Among the most prominent signs of aflatoxicosis in several species, including birds and mammals, are hypolipidaemia, hypocholesterolaemia, and hypocarotenaemia, associated with severe hepatic steatosis and weight loss. We suggest that these signs of acute imbalance of lipid metabolism can be the result of the chemical modification (blocking) of key lysyl residues on the LDL protein B-100 by the activated AFB1 molecule. Modified LDLs are not recognised by their specific receptors and thus are rejected by peripheral cells. Upon return to the liver, the modified particles bind to the sinusoidal lining cells. Lipid starvation of peripheral tissues takes place while fat accumulates in the liver. This abnormal state is maintained and reinforced by further modification of nascent apoproteins, which in turn become unable to receive a lipid load for as long as aflatoxin continues to be available in the liver.

Amer *et al.* (1998) studied the **kinetic behaviour of ceftiofur sodium** in aflatoxin treated chickens for 30 days and in non-treated chickens, following oral, intramuscular and intravenous administrations of 10 mg kg(-1) bodyweight of ceftiofur. Aflatoxicosis resulted in a more significant decrease in ceftiofur serum concentration in the treated than in non-treated chickens following oral and intravenous administrations. The kinetic behaviour showed that following intravenous injection the elimination half life time t0.5 (el) was significantly shorter in the treated chickens (1.75+/-0.03 hours) than in non-treated chickens (4.23+/-0.05 hours). Following oral administration, the kinetic behaviour revealed a longer absorption half-life [t0.5 (ab), 62.74+/-1.59 minutes] in the treated chickens than in non-treated (50.46+/-5.07 minutes), with lower Cmax 23.25+/-0.42 microg ml(-1) at long tmax (3.05+/-0.07 hour) in treated chickens than in non-treated (Cmax 27.83+/-1.28 at tmax 2.39+/-0.07 hours).

Ledoux *et al.* (1999) conducted in vitro and in vivo studies to evaluate the efficacy of a **hydrated sodium calcium aluminosilicate** (Improved **Milbond-TX, IMTX**) to alleviate the toxic effects of aflatoxin (AF) B1 in chicks. In vitro results indicated that IMTX was able to bind 100% of AFB1 at pH 3 to 9. In the in vivo study, five pen replicates of six chicks were assigned to each of four dietary treatments, which included: 1) basal diet containing neither IMTX nor AFB1 (control); 2) basal diet supplemented with 1% IMTX; 3) basal diet supplemented with 4 mg AFB1/kg diet; and 4) basal diet supplemented with 1% IMTX and 4 mg AFB1/kg diet. The addition of IMTX to chick diets at a level of 1% did not negatively affect chick performance, organ weights, or serum chemistry, or cause pathological changes. Improved Milbond-TX completely prevented the reduced performance, changes in organ weights, serum chemistry changes, and gross pathology observed in chicks fed AFB1. The IMTX dramatically reduced the incidence and severity of the hepatic histopathology changes associated with aflatoxicosis and completely prevented the renal lesions of aflatoxicosis. These results indicated that IMTX was effective in preventing the toxic effects of AF that may be present in poultry rations at levels up to 4 mg/kg feed.

Parlat *et al.* (1999) evaluated **clinoptilolite (CLI, a natural zeolite**), incorporated into the diet at 50 g/kg, for its ability to reduce the deleterious effects of 2.0 mg total aflatoxin (AF;83.06% AFB1, 12.98% AFB2, 2.84% AFG1 and 1.12% AFG2)/kg diet on growing Japanese quail chicks from 10 to 45 d of age. A total of 40 Japanese quail chicks were divided into 4 treatment groups (control, AF, CLI, AF plus CLI) each consisting of 10 chicks. **The performance** of the birds was evaluated. The AF treatment significantly decreased food consumption and body weight gain from the 3rd week onwards. The adverse effect of AF on food conversion ratio was also significant from week 4 of the experiment. The addition of CLI to an AF-containing diet significantly reduced the deleterious effects of AF on food consumption, body weight gain and food conversion ratio. Food consumption was reduced by 14% in quail chicks consuming the AF diet without CLI, but by only 6% for quail chicks consuming the AF plus CLI diet. Similarly, overall body weight gain was reduced by 27% in birds consuming the AF diet without CLI, but by only 8% for birds consuming the AF plus CLI diet. The addition of CLI to the AF-free diet significantly decreased food consumption and body weight gain during week 4, but these parameters were similar to the controls in week 5. No mortality was observed in any of the groups. These results suggest that CLI effectively diminished the detrimental effects of AF on the variables investigated in this study.

Çelik *et al.* (2000) studied the protective action of an enzyme-linked polyvinylpolypyrrolidone (**PVPP, Mycofix Plus**) against **the immunosuppressive effect of afatoxins (AF)** by determination of peripheral blood T-lymphocyte proportions and splenic plasma cell counts. Histological changes in lymphoid organs were also investigated by light microscopy. One-d-old broiler chicks (Hybro) received 2.5 mg/kg diet AF (83.06% AFB1, 12.98% AFB2, 2.84% AFG1, 1.12% AFG2) with or without PVPP (3g/kg diet) until 21 d of age. When compared with controls, AF treatment significantly decreased peripheral T-lymphocyte counts. AF caused a slight decrease in splenic plasma cell counts. The addition of PVPP to an AF-containing diet significantly increased T-lymphocyte counts. Splenic plasma cell counts were numerically intermediate between control and AF groups. 3. The dietary addition of

PVPP to AF-free diet did not significantly alter either T-lymphocyte or splenic plasma cell counts.

Ibrahim *et al.* (2000) investigated the ameliorative effect of graded levels of dietary **sodium bentonite** (0.2, 0.4 and 0.6 per cent wt/wt of feed) on in vitro-impaired phagocytosis and suppressed immune response to Newcastle disease vaccine during aflatoxicosis (AF) in broiler chicks. Both percentage and mean of phagocytic activities were decreased significantly ($P < 0.05$) in chicks fed 2.5 mg aflatoxin per kg feed. The addition of sodium bentonite was significantly effective in ameliorating the negative effect of AF on the percentage and mean of phagocytosis. The presence of AF alone in the diet depressed the **immune response** of chicks as measured by haemagglutination inhibition (HI) test. Sodium bentonite was also effective in ameliorating the suppressive effect of AF on the HI -titre in chicks vaccinated against Newcastle disease. The best results obtained when sodium bentonite was added at the rate of 0.4 per cent wt/wt of feed to the AF-containing diets.

Miazzo *et al.* (2000) evaluated **synthetic zeolites (NaX, NaY, NaA, and CaA)** in vitro for their ability to sorb aflatoxin (AF) B1 from an aqueous solution. Zeolite NaA (ZN) was selected to be tested in vivo because of its high affinity and its stable association with AFB1. This sorbent was incorporated into diets (1%) containing 2.5 mg/kg AFB1. Male broiler chicks from 21 to 42 d of age received ad libitum access to their respective diets and water. When compared with controls, BW gains were lower ($P < 0.05$) for broilers that were fed AF in their diets. No differences were found between the BW gains of chicks fed diets without AF and those of chicks fed AF + ZN, indicating almost total protection against the effects caused by AF. Liver weights were considerably higher in chicks fed a diet containing AF, compared with those of controls, nevertheless, no significant differences were found in feed:gain ratio among the groups. The findings of this research suggest that ZN can counteract some of the toxic effects of AF in growing broiler chicks

Oğuz *et al.* (2000) evaluated **clinoptilolite (CLI, a natural zeolite)** incorporated into the diet at 1.5 and 2.5 per cent for their ability to reduce the deleterious effects of 2.5 mg total aflatoxin (AF) kg(-1)diet on broiler chickens from 1 to 21 days of age. In total 360 broiler chicks were divided into six equal treatment groups (control, AF, CLI (1.5 per cent), AF plus CLI (1.5 per cent), CLI (2.5 per cent) and AF plus CLI (2.5 per cent)). When compared with the controls, AF treatment significantly decreased serum total protein, albumin, inorganic phosphorus, uric acid, total cholesterol and the values of haematocrit, red blood cell counts, mean corpuscular volume, haemoglobin, thrombocyte counts, percentage of monocyte counts; increased values of white blood cell and heterophil counts. The addition of CLI (1.5 per cent) and CLI (2.5 per cent) to the AF -containing diet reduced the adverse effects of AF and should be helpful in a solution to the aflatoxicosis problem in poultry.

Oğuz and Kurtoğlu (2000) examined the amelioration of aflatoxicosis in **broiler** chickens by feeding 2 concentrations of **natural zeolite (clinoptilolite).** Clinoptilolite (CLI), incorporated into the diet at 15 and 25 g/kg, was evaluated for its ability to reduce the deleterious effects of 2.5 mg total aflatoxin (AF; 76.40% AFB1, 16.12% AFB2, 6.01% AFG1 and 1.47% AFG in diet on growing broiler chicks from 1 to 21 d of age. A total of 360 broiler chicks were divided into 6 treatment groups (6 replicates of 10 broilers each): control, AF, CLI (15 g/kg), AF plus CLI (15 g/kg), CLI (25 g/kg), and AF plus CLI (25 g/kg). 2. Compared to controls, the treatment had significantly decreased body weight gain from week 1 onwards. The adverse effect of

AF on food consumption (8.0%) and food conversion ratio (8.3%) was also shown over the entire 21-d feeding period. 3. The addition of CLI (15 g/kg) to an AF-containing diet significantly reduced the deleterious effects of AF on food consumption and body weight gain. Food conversion ratio was also slightly improved by adding CLI (15 g/kg) to AF-containing diets. Food consumption, body weight gain and food conversion ratio values were rendered numerically intermediate between AF and control groups by the addition of CLI (25 g/kg) to the AF-containing diet. 4. The addition of CLI (both 15 and 25 g/kg) to the AF-free diet did not produce any significant changes compared with the controls, except for decreased total food consumption in the CLI (25 g/kg)-alone group. 5. These results suggest that CLI (15 g/kg) addition effectively diminished the detrimental effects of AF on the values investigated. Also, the lower dietary concentration of CLI (15 g/kg) was more effective than the greater concentration against the adverse effects of AF on the variables investigated in this study.

Quezada *et al.* (2000) studied the influence of age on the toxic effects of AFB(1) on **plasma, renal and hepatic enzymes**, under two protocols, in adult and in developing Arbor-Acres chickens. Protocol A: 100 male 4-week-old chickens (640 g), received AFB(1), 0.5, 1.0, or 2.0 microg/g of feed (daily p.o.), a fourth group received an aflatoxin-free diet. Five birds/group were slaughtered at 7, 14, 21 and 28 days of treatment. Body, hepatic and renal weights, succinate-dehydrogenase (SDH) and glutamate-dehydrogenase (GluDH) in plasma and liver were measured. Hepatic SDH and GluDH decreased ($P<0.05$). Protocol B: two groups of 24 male 1-week-old chickens (106 g) received either aflatoxin-free feed (n=24) or AFB(1) feed (2.0 microg/g). At days 7, 14, 21 and 28, the same parameters of Protocol A were measured. AFB(1) markedly reduced body weight gain (20-30%), plasma proteins, albumin, renal and hepatic protein content ($P<0.05$) and increased absolute and relative weights of the kidney ($P<0.05$). SDH and GluDH were reduced ($P<0.05$), while total renal gamma-glutamyltranspeptidase (GGT) increased ($P<0.05$). Results suggest that serum proteins, SDH and GluDH are sensitive early indicators of this toxicity that was more severe in developing chickens. Decrease in serum albumin might be used as an early and suitable indicator of the deleterious effect of this mycotoxin in developing chickens.

Raju and Devegowda (2000) conducted a study to evaluate the individual and combined effects of **aflatoxin B1 (AF), ochratoxin A (OA) and T-2 toxin (T-2)** on **performance, organ morphology serum biochemistry and haematology of broiler** chickens and the efficacy of esterified-glucomannan (E-GM), a cell wall derivative of Saccharomyces cerevisiae1026 in their counteraction. 2. Two dietary inclusion rates of AF (0 and 0.3 mg/kg), OA (0 and 2 mg/kg), T-2 (0 and 3 mg/kg) and E-GM (0 and 1 g/kg) were tested in a 2 x 2 x 2 x 2 factorial manner on a total of 960 broiler chickens from 1 to 35 d of age in an open sided deep litter pen house. 3. Body weight and food intake were depressed by all the mycotoxins, OA being the most toxic during early life. 4. Weights of kidney and adrenals were increased by AF and OA. Liver weight was increased by AF (17.8%), while OA increased gizzard weight (14.6%) and reduced bone ash content (8.1%). T-2 toxin showed no effect on these variables. 5. Serum cholesterol content was decreased and activity of serum gamma glutamyl transferase (GGT) was increased by AF and OA while serum protein content was decreased by AF. These effects were more pronounced at 21 d than at 35 d of age. Inconsistent responses were seen in the other variables: blood urea nitrogen (BUN) content, activities of serum alanine amino transferase and aspertate amino

transferase. Blood haemoglobin content was depressed by AF and T-2, whereas blood coagulation time was prolonged by OA. 6. Significant interactions were observed between any 2 toxins for their additive effects on body weight, food intake, bone ash content and serum GGT activity at 21 d. Conversely, antagonistic interactions were observed among any 2 of the toxins for their effects on variables such as serum protein and serum cholesterol content. Simultaneous feeding of all 3 mycotoxins did not show increased toxicity above that seen with any 2. 7. Esterified-glucomannan increased body weight (2.26%) and food intake (1.6%), decreased weights of liver (32.5%) and adrenals (18.9%) and activity of serum GGT (8.7%), and increased serum protein (14.7%), cholesterol (21.9%), BUN (20.8%) and blood haemoglobin (3.1%) content, indicating its possible beneficial effect on mycotoxicosis in broiler chickens.

Cheng *et al.* (2001) conducted a study to investigate if **carotenoids** could alleviate the adverse effects caused by aflatoxin with respect to **growth performance and immune response.** In two experiments, a total of 320 mule ducklings were assigned to 5 treatments, i.e. control, aflatoxin B(1) (AFB(1)) 200 ppb, AFB(1) +beta-carotene (BC) 200 ppm, AFB(1)+BC 400 ppm, and AFB(1)+astaxanthin (AS) 200 ppm. In experiment 1, the addition of beta-carotene or astaxanthin in the diet containing AFB(1) 200 ppb resulted in a significant decrease in average daily gain as compared with the control. AFB(1) 200 ppb alone and the addition of BC or AS on top of AFB(1) resulted in a significantly lower daily feed intake than for the control group. There were no significant differences in relative organ weights among treatment groups. Both treatments of BC 400 ppm and AS 200 ppm had significantly more macrophages harvested per duck than the control and AFB(1) 200 ppb treatments. However, there were no significant differences among treatments in percentages of phagocytotic macrophages and number of Candida albican phagocytized by phagocytotic macrophages. In experiment 2, blood biochemical parameters and antibody titers were evaluated. There were no significant differences among treatments in total bilirubin content and alkaline phosphatase activity in the serum or in antibody titers against fowl cholera. However, AFB(1) treatment had the highest activities of AST and ALT in the serum. The addition of BC 400 ppm on top of AFB(1) significantly reduced ALT activity as compared with the AFB(1) 200 ppb treatment. These results suggest that carotenoids could provide a slightly toxic alleviating effect on growth performance, enhance the chemotaxis ability of macrophages, and reduce ALT activity elevated by AFB(1).

Ortatatli and Oğuz (2001) examined the amelioration of aflatoxicosis in broiler chickens was examined by feeding two concentrations of **natural zeolite (clinoptilolite). Clinoptilolite (ClI),** incorporated into the diet at 1.5 and 2.5 per cent, was evaluated for the ability to reduce the deleterious effects of 2.5 mg total aflatoxin (AF) kg(-1)diet on growing broiler chicks from 1 to 21 days of age. A total of 360 broiler chicks were divided into six treatment groups [Control, AF, CLI (1.5 per cent), AF plus CLI (1.5 per cent), CLI (2.5 per cent), and AF plus CLI (2.5 per cent)] each consisting of 60 chicks. Compared to controls, the AF consuming chicks showed increases in the relative weights of liver and kidney; and gross-histopathologic hepatic lesions such as paleness, friability, diffuse hydropic degeneration and/or fatty change, bile-duct hyperplasia and periportal fibrosis. Glumerular hypertrophy, increases in the number of mesengial cells and hydropic degeneration of tubuler epithelium in kidneys of chicks fed diet AF alone were also observed. Atrophy and lymphoid depletion were seen in the thymuses and bursa of Fabricius from the chicks fed AF alone. The

additions of CLI (1.5 and 2.5 per cent) to the AF -containing diet moderately (significantly in some cases) decreased the number of affected broilers and/or the severity of lesions. The addition of CLI to the AF-free diet did not produce any significant changes compared with the controls. These results suggest that CLI was effective for the protection of AF-toxication in broilers and it could contribute to a solution of the AF problem in poultry production.

Parlat ***et al.*** **(2001)** examined the **amelioration of aflatoxicosis** in **Japanese quails** by the dietary addition of **live yeast (Saccharomyces cerevisiae; SCE**). Yeast incorporated into the diet at 1 g kg(-1) was evaluated for its ability to reduce the deleterious effects of 2.5 mg total aflatoxin (AF; 82.30 per cent AFB1, 2.06 per cent AFB2, 7.68 per cent AFG1 and 7.96 per cent AFG2) kg(-1)diet on growing Japanese quail chicks from 10 days to 45 days of age. Forty 10-day-old Japanese quail chicks were assigned to 2x2 factorial arrangement of treatments (control, AF, SCE, AF plus SCE) each consisting of 10 quails. The performances of birds were evaluated. The AF treatment significantly and dramatically decreased food consumption and body-weight gain from the first week onwards. The significant adverse effect of AF on the food conversion ratio was also determined from week 1 to the end of the experiments. The addition of SCE to the AF -containing diet significantly reduced these deleterious effects of AF on food consumption, body-weight gain and food conversion ratio. Compared to controls, the cumulative body weight gain was reduced by 37 per cent among the quails consuming AF without SCE, but increased 15 per cent for the birds fed AF plus SCE. Interestingly, the single inclusion of SCE to the AF-free diet provided significant improvements in all the investigated **growth performances** of birds (approximately 40 per cent) compared to controls.

Rosa ***et al.*** **(2001)** carried out in vitro studies, which indicated that a **sodium bentonite (SB)** from southern Argentina had a high ability to sorb aflatoxin B1 (AFB1) from aqueous solution. We evaluated this compound for its ability to reduce the effects of total aflatoxins (AF; 5 mg AFB1/kg) in the diet of growing broiler chickens from 30 to 52 d of age. The diets were amended with 0.3% Argentinean SB to determine the effect of this compound during aflatoxicosis. When compared with the controls, BW gains were significantly ($P < 0.05$) lower for broilers fed diets containing AF alone (1,865 vs. 1,552 g). No differences were found between the BW gains of broiler chickens fed diets without AF (1,785 g) and those of chickens fed AF + SB (1,809 g). These results suggest that effects of AF treatment were ameliorated when SB was used in the broiler chick diets. The AF significantly ($P < 0.05$) decreased feed efficiency. Liver, kidney, and pancreas relative weights increased in chickens fed the diet containing AF alone. Alterations in the levels of serum total protein, albumin (ALB), and globulins (GLOB) were observed for AF diets, and moderate protection was provided by the sorbent. The ALB:GLOB ratio decreased in both groups of birds fed with the AF-contaminated diet, and we observed a moderate increase in this ratio by 0.3% addition of SB. The histopathological findings in liver sections of broiler fed diets with AF + SB indicated a non-protective effect of this adsorbent, because a moderate hepatic steatosis was observed.

Valdivia ***et al*****. (2001)** performed a study to evaluate the capability of **dietary supplementation with** ***N*****-acetylcysteine (NAC**) to ameliorate the effects of subacute intoxication with AFB1 in broiler chickens. One hundred twenty male Hubbard 1-d-old chickens were allocated into one of four dietary treatments: 1) control group without treatment, 2) purified AFB1 added to diet (3 mg/kg of feed) for 21 d, 3) NAC (800 mg/kg BW, daily), or 4) AFB1 plus NAC at the same doses as Groups 2 and 3.

Broilers treated with AFB1 plus NAC were shown to be partially protected against deleterious effects on BW (57.8%), daily weight gain (49.1%), feed conversion index (21.4%), plasma and hepatic total protein concentration (45.2, 66.7%), plasma alanine aminotransferase (67.4%), hepatic glutathione-S-transferase (18.8%), and reduced glutathione liver concentration (75.0%). In addition, they showed less intense liver fading, friable texture, and microvesicular steatosis. In the kidney, thickening of glomerular basement membrane was also less severe in NAC+AFB1-treated chickens than in AFB1-treated chickens. Our results suggest that NAC provided protection against negative effects on performance, liver and renal damage, and biochemical alterations induced by AFB1 in broiler chickens. Effects of NAC alone on chick performance were also evaluated. Addition of NAC to diet (800 mg/kg BW) did not negatively affect feed consumption, conversion index, or serum chemistry and did not induce structural changes in the liver or kidney.

Klein *et al.* (2002) performed a study to test whether **dietary butylated hydroxytoluene (BHT)** protects against aflatoxicosis in turkeys. They supplemented the feed of 10-day-old male white **turkeys** with low (1000 ppm) and high (4000 ppm) BHT for 20 days. AFB(1) (1 ppm) was then added to the diets and continued for another 10 days. Birds in the AFB(1)-only group had a lower weight gain, a condition that had returned to near control in groups fed diets containing AFB(1) + BHT. Significant elevations in serum aspartate transaminase, alanine aminotransferase, and lactate dehydrogenase, which were evident in the AFB(1) group, were reversed in the AFB(1) + BHT groups. Histopathology revealed hepatic submassive necrotic lesions and biliary hyperplasia, the severity of which was lessened in the AFB(1) + BHT-treated birds. Hepatocellular hydropic degeneration was observed in the BHT-only group, but not in the AFB(1) + BHT groups. This condition associated with BHT treatment was found in a separate study to be reversible and without any long-term adverse effects. These results indicate that BHT counteracts many of the deleterious effects caused by AFB(1) and that this antioxidant may prove to be a viable feed additive for the reduction of aflatoxicosis in turkeys.

Ortatatli *et al.* (2002) conducted a study to determine the **pathological changes** in testes and epididymides and plasma testosterone levels of adult roosters during experimentally induced aflatoxicosis. In the study, 24 months of age, 32 Babcock breeder males were used, and they were divided into four groups each containing 8 animals. The groups were designed as follows; group 1: Control, no aflatoxin (AF), group 2: 5 ppm (parts per million) total aflatoxin (AF; B1, B2, G1, G2), group 3: 10 ppm AF and group 4: 20 ppm AF in the diet, and the birds were fed for 8 weeks. Grossly, it was seen that the testes of all AF-treatment groups birds were significantly ($P < 0.001$) atrophied when compared with those of control birds. Histopathologically, there was no spermatogenesis in the testes of 4, 5 and 6 cocks fed on a diet containing AF 5, 10 and 20 ppm, respectively. Furthermore, abnormal spermatozoa were observed in some of AF-treatment groups (in 2 cases in each of 5 and 10 ppm AF-treated groups, and in one case in 20 ppm AF-treated group). There were also mononuclear cell infiltration and/or focal lymphoid cell accumulation in the intertubular areas of the testes and epididymides in all AF-treatment groups. In conclusion, it has been shown that AF might totally or partially (dose related) suppress spermatogenesis, cause abnormality in spermatozoa and atrophy in testes. Furthermore, there was degeneration and desquamation in the epithelium and decrease in the size and thickness of the germinative layer of the seminiferous tubules, and lowered plasma testosterone levels in adult roosters.

Verma ***et al.*** **(2002)** conducted studies to evaluate protein and **energy utilisation in broilers** fed diets containing various levels of aflatoxin (AF; 0, 0.5, 1 and 2 mg kg^{-1}) and ochratoxin A (OA; 0, 1, 2 and 4 mg kg^{-1}) either singly or in different combinations. Total protein efficiency (TPE) was reduced by 50.97, 76.52 and 132.75% at 2 mg kg^{-1} AF and 2 and 4 mg kg^{-1} OA respectively. Co-toxicity at two levels, 1 mg kg^{-1} AF + 2 mg kg^{-1} OA and 2 mg kg^{-1} AF + 4 mg kg^{-1} OA, resulted in significant reductions of 78.58 and 127.43% respectively in TPE. AF at all three levels and OA at 2 and 4 mg kg^{-1} caused significant decreases in net protein utilisation (NPU). Co-toxicity at all three levels led to significantly lower NPU. The reduction in NPU ranged from 18.68% at 0.5 mg kg^{-1} AF to 75.12% at 2 mg kg^{-1} AF + 4 mg kg^{-1} OA. Significant reductions in metabolisable energy (ME) content were recorded at 1 and 2 mg kg^{-1} AF and all three levels of OA. ME content was reduced drastically when both toxins were fed simultaneously. It is suggested that both AF and OA adversely affect energy and protein utilisation in **broilers,** and this effect is exacerbated when both toxins are fed simultaneously.

Dersjant ***et al.*** **(2003)** evaluated the quantitative impact of dietary aflatoxin concentrations on **performance** of broilers, with special emphasis on low concentrations of the toxin. It was estimated that with each mg/kg increase of aflatoxin in the diet, the growth rate would be depressed by 16 % for pigs and 5 % for broilers.

Gathumbi ***et al.*** **(2003)** described a novel and highly sensitive immunochemical method for the rapid detection of aflatoxin B1 (AFB1) in chicken liver tissues. Liver tissues were homogenized with cold methanol-acetone (50:50), followed by AFB1 extraction with methanol-acetone-PBS (25:25:50). The tissue extracts were, with or without further purification by immunoaffinity chromatography (IAC), applied to a highly sensitive direct ELISA for determination of AFB1. The detection limits for this assay were 15 +/- 0.77 pg/mL when standards and samples were dissolved in methanol-PBS (10:90) and 17 +/- 2.0 pg/mL when methanol-acetone-PBS (5:5:90) solution was used. The average recoveries of AFB1 were 54.3 to 65.5% in artificially contaminated tissue samples at 1 to 5 ng/g. In samples spiked with AFB1 at 1 ng/g, the method had diagnostic sensitivity and specificity of 100% for samples processed with IAC and 91.7 and 100%, respectively, for samples without IAC purification. The test was successfully applied to the detection of AFB1 in liver tissues from chickens that were experimentally dosed with AFB1. It is hoped that this test will be applicable in rapid detection of aflatoxins in poultry meats and in diagnosis of aflatoxicosis in chicken.

Klein et al. (2003) performed a study to determine whether **BHT** has a similar effect in **turkeys**. Ten-day-old male turkeys were maintained on diets amended with 1000 or 4000 ppm of BHT for 10 days, then sampled. Hepatic microsomal CYP 1A activity as well as conversion of AFB(1) to the putative toxic metabolite, the exo-AFB(1)-8,9-epoxide (AFBO), were significantly lower compared with control. Conversely, dietary BHT significantly increased activities of several isoforms of hepatic cytosolic GST, as well quinone oxidoreductase (QOR). Western immunoblotting confirmed that dietary BHT increased expression of homologues to rodent GST isoforms Yc1, Yc2 and Ya. There was, however, no observable BHT-related increase in GST-mediated specific conjugation with microsomally-generated AFBO. In total, our data indicates that

dietary BHT modulates a variety of AFB(1)-relevant phase I and phase II enzymes, while having no measurable effect towards specific AFB(1) detoxification by GST.

OGUZ *et al.* (2003) addetotal aflatoxin (AF) and a natural zeolite (clinoptilolite ; CLI) to the broiler feed and development of humoral immunity against Infectious Bronchitis (IB) and Newcastle Disease (ND) was evaluated. A total of 576 1-d-old Ross broiler chicks (96 per each) were housed in six treatment groups [Control, CLI (15 g/kg diet), 50 ppb AF, 50 ppb AF plus CLI, 100 ppb AF, 100 ppb AF plus CLI] and fed for 42 days. Compared to controls, the antibody titres of IB were determined significantly lower ($p < 0.05$) in 50 and 100 ppb AF fed chicks from 20 to 42 days of age. The ND titres were also significantly lower ($p < 0.05$) in 100 ppb AF fed chicks, while no significant differences were seen in 50 ppb AF group compared to controls ($p > 0.05$). The addition of CLI to the AF-containing diets (50 and 100 ppb) significantly ameliorated ($p < 0.05$) the adverse effect of AF on humoral immunity. The single addition of CLI to the AF-free diet had no adverse effects in chicks, except the IB titres on 42nd day.

Shivachandra *et al.* (2003) divided 240 unvaccinated day-old broiler chicks, which had been found to be negative for **antibodies against FAV-4**, into four groups of 60 chicks each. Group A was fed aflatoxin at 1 ppm from 7 days to 7 weeks of age. Group V was infected intra-abdominally at 14 days of age with 0.2 ml of FAV-4, having a titre of 10(5.5) TCID50 per 0.2 ml. The combined group AV was given the aflatoxin and infected with FAV-4. The fourth group C served as the control. More pronounced clinical signs, a higher mortality rate (56.7%), and reductions in body weight gain and in the organ to body weight ratios of the bursa and spleen were recorded in group AV. A significant ($p < 0.01$) reduction in the HI antibody titre following vaccination against Newcastle disease, and of skin thickness in the delayed hypersensitivity test following sensitization with DNCB, indicated an additive immunosuppressive effect from aflatoxin and FAV-4 on the humoral and cell-mediated **immune responses** in group AV compared to groups A and V. Microscopically, marked depletion and degeneration of lymphocytes in the thymus, bursa, spleen and caecal tonsils were observed in group AV up to 5 weeks PI.

Wilkinson *et al.* (2003) used a mucosal vaccine in an effort to elicit serum IgG and intestinal secretory IgA against the mycotoxin aflatoxin B1 (AFB) in chickens. AFB was coupled to carrier proteins (BSA and porcine thyroglobulin) for use as a vaccine and ELISA coating antigen, respectively. Seven-day-old broiler chicks were divided into groups of 10 and immunized with one of four vaccine preparations: 1) AFB-BSA conjugate alone, 2) AFB-BSA linked to the B subunit of the recombinant heat-labile enterotoxin of *Escherichia coli* (rLT-B), 3) AFB-BSA admixed with rLT-B, or 4) AFB-BSA mixed with cholera toxin (CT). Each vaccine preparation was administered perorally, intrarectally, or intraperitoneally, with a booster immunization given 2 wk later. Sera and feces were collected weekly and assayed using isotype specific ELISA. All three routes of immunization elicited significant serum IgG responses; however, the intraperitoneal route was strongest for all vaccine preparations tested. The serum IgG immune response to the AFB-BSA conjugate was enhanced by co-administration of rLT-B but not by covalent coupling to rLT-B or coadministration with CT. Secretory IgA anti-CT and antirLT- B antibodies were detected in fecal supernatants, but no anti-AFB responses could be detected. As all 12 treatment groups produced significant levels of serum IgG anti-AFB, any of these approaches, including oral

administration without adjuvant, may afford the chicken some level of protection through simple immuno-interception of free AFB.

Jakhar and Sadana (2004) reported that feeding of aflatoxin B1 1 ppm to 2-week old **Japanese quail** for a period of 8 weeks produced **gross and microscopic changes** in the liver, skeletal muscles, heart and bursa of Fabricius. These included fatty changes, bile duct hyperplasia and lymphoid aggregation in liver; haemorrhages in thigh, breast muscles and myocardium; mild depletion of lymphocytes, cystic degeneration and fibrous tissue proliferation in bursa of Fabricius. More or less similar lesions were seen in quail chicks fed on aflatoxin with sodium selenite 5 ppm but these were of lesser intensity and appeared at later stages of the experiment thereby indicating that supplementation of selenium had some protective action against the toxic effect of aflatoxin B1 in Japanese quail.

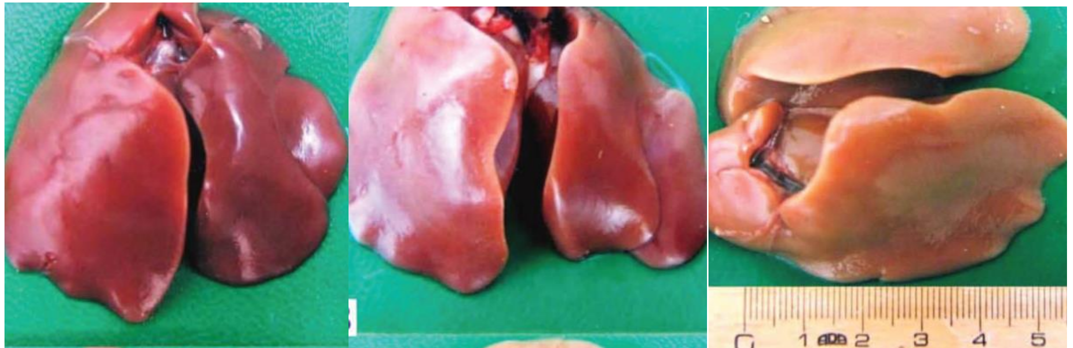
Kana *et al.* (2004) studied the effects of **dietary aflatoxin** (AF, 0.5, 1.0 and 2.0 mg/kg), ochratoxin (OA, 1.0, 2.0 and 4.0 mg/kg) or combinations of these **on body weight gain, feed efficiency, organ weights and immune response in broilers**. Significant growth depression, reduced food consumption and poor food conversion efficiency were recorded in broilers fed a diet containing the greater concentrations of AF (1 and 2 mg/kg) and OA (2 and 4 mg/kg). 3. The combination of 2 mg/kg AF and 4 mg/kg OA exerted the maximum adverse effect on growth, feed intake and feed efficiency, indicating a synergistic effect on performance. 4. AF at 2 mg/kg in the diet caused a significant increase in the relative weight of liver, whereas the relative weight of kidney was significantly increased at 4 mg/kg of OA. A significant decrease in the relative weight of the bursa of Fabricius was noted at the highest concentration of AF (2 mg/kg) and combinations of 1 and 2 mg/kg AF and 2 and 4 mg/kg OA. 5. Cell mediated immunity (CMI), in terms of mean skin thickness (MST) sensitive to dinitrochlorobenzene (DNCB), was significantly reduced in chicks given the combination of 2 mg/kg AF and 4 mg/kg OA. Haemagglutination (HA) titre against sheep red blood cells (SRBCs) peaked at 42 d of age. At 42 and 47 d of age, a significant decrease in HA titres was recorded in chicks given 4 mg/kg OA or a combination of AF (1 or 2 mg/kg) and OA (2 or 4 mg/kg). 6. AF at a dietary concentration of 1 mg/kg or more and OA at 2 mg/kg or more, either alone or in combination, caused severe reductions in growth and **immune response**.

Pimpukdee *et al.* (2004) evaluated **NSP** for its ability to bind aflatoxin B1 (AfB1) in vitro and to prevent the onset of aflatoxicosis and vitamin A depletion in broiler chicks in vivo. Isothermal analyses were conducted with NSP and AfB1 to quantitate and characterize critical sorption parameters at equilibrium, i.e., ligand saturation capacities, affinity constants, and thermodynamics of the sorption process. In vitro results indicated that AfB1 was tightly sorbed onto the surface of NSP, which provided a high capacity and high affinity for the ligand. Thermodynamics favored sorption of AfB1 to NSP. The process was exothermic and spontaneous with a mean heat of sorption equal to approximately -50 kJ/mol, suggesting chemisorption (or tight binding). In addition to the in vitro studies, the effectiveness of NSP as an aflatoxin enterosorbent to attenuate the onset of aflatoxicosis in broiler chicks was determined at 3 different inclusion levels in the diet (0.5, 0.25, and 0.125%). NSP alone was not toxic to chicks at a level as high as 0.5% in the total diets (based on body and organ weights, feed intake, and hepatic vitamin A levels). NSP in the diet significantly protected chicks from the effects of high level exposure to aflatoxins (i.e., 5 mg/kg) and preserved hepatic vitamin A levels, even at lower dietary intake of clay.

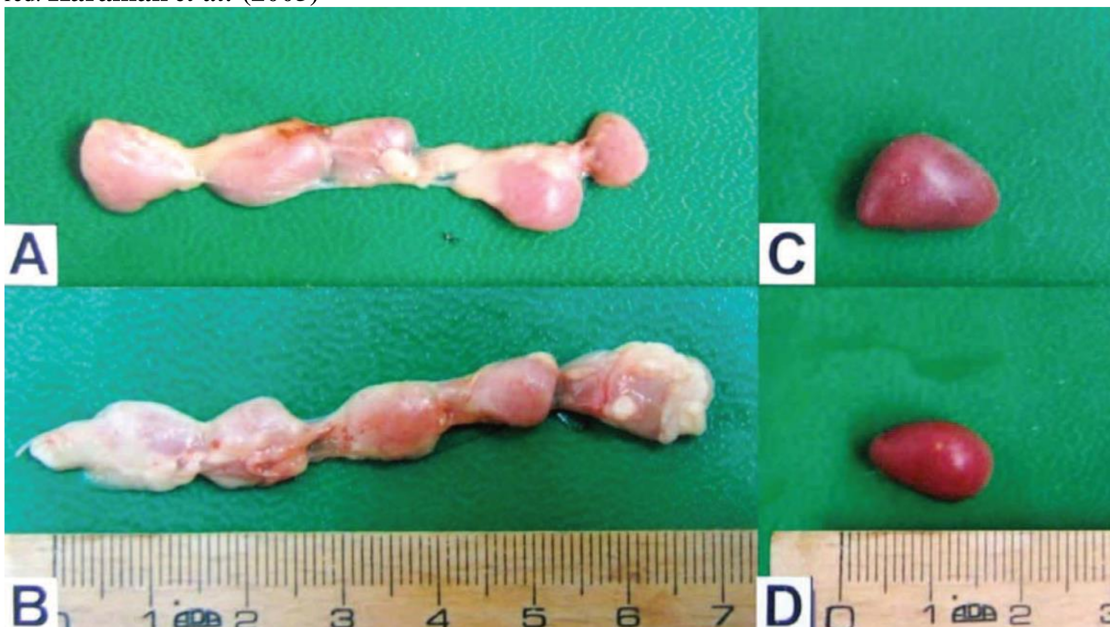
Tedesco *et al.* (2004) focused their studies on the effects of a **silymarin-phospholipid complex** in reducing the toxic effects of aflatoxin B1 (AFB1) in broiler chickens. Twenty-one 14-d-old male commercial broilers were randomly allotted to 3 groups and treated as follows: basal diet alone [Group C (Control)]; AFB1 at 0.8 mg/kg of feed [Group B1]; AFB1 at 0.8 mg/kg of feed plus silymarin phytosome, a silymarin complexed form with phospholipids from soy, at 600 mg/kg of BW [Group B1+Sil]. Considering the whole growth cycle, BW gain and feed intake were lower in AFB1-treated birds with respect to controls ($P < 0.05$). In the B1+Sil group, BW gain and feed intake were higher with respect to birds receiving AFB1 alone ($P < 0.05$), and not different from the control birds. Serum biochemistry showed no difference among groups, except for a decrease of alanine amino transferase (ALT) in chicks treated only with AFB1. Alanine amino transferase activity in AFB1 plus silymarin phytosome treated birds was not different from the controls. No treatment differences were noted on liver weight. In conclusion, our results suggest that silymarin phytosome can provide protection against the negative effects of AFB1 on performance of broiler chicks.

Citil *et al.* (2005) designed a study to evaluate the effect of **L-carnitine supplementation** on the plasma malondialdehyde (MDA) and whole blood reduced glutathione (GSH) concentrations in experimentally-induced chronic aflatoxicosis in quails. For this purpose, a total of 80 **quails** up to 8 weeks old were divided into four equal groups. Group I served as control, Group II was given L-carnitine at the dose of 200 mg/litre in the drinking water for 60 days, Group III was given 60 microg total aflatoxin/kg diet for 60 days, and Group IV was given both 60 microg total aflatoxin/kg diet and 200 mg L-carnitine/litre in the drinking water for 60 days. Aflatoxin treatment caused a significant increase in plasma MDA and a significant decrease in blood GSH concentrations. On the other hand, there was a significant decrease in plasma MDA and a significant increase in whole blood GSH in the L-carnitine-supplemented group. The present study demonstrated that L-carnitine brought about the inhibition of lipid peroxidation by enhancing antioxidant capacity in quails with chronic aflatoxicosis.

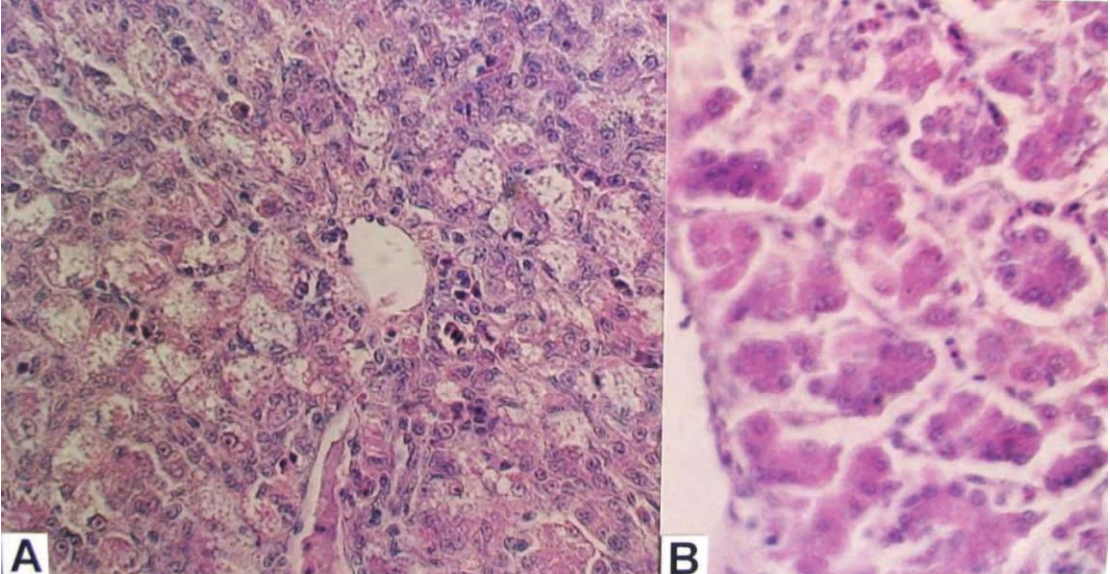
Karaman *et al.* (2005) evaluated the efficacy of **yeast glucomannan (Mycosorb**), incorporated into the diet at 0.5 and 1 g/kg, in reducing the detrimental effects of 2 mg aflatoxin/kg diet on growing **broiler chicks** from 1 to 21 d of age. A total of 240 male broiler chicks (Ross-308) was divided into 6 treatment groups [Control, Aflatoxin (AF), Yeast glucomannan (YG; 0.5 g/kg), AF plus YG (0.5 g/kg), YG (1 g/kg), and AF plus YG (1 g/kg)]. Ten chicks from each of the 6 groups were slaughtered and pathological examinations were performed on the liver, bursa of Fabricius, thymus, spleen and kidney. The aflatoxin treatment caused moderate to severe hydropic/fatty degeneration in the hepatocytes of the liver and the tubular epithelium of the kidneys, and follicular depletion in the bursa of Fabricius, thymus and spleen. Yeast glucomannan added to the aflatoxin-containing diet at 0.5 and 1 g/kg diminished the severity of pathological changes, slightly and moderately, respectively. The number of affected organs was also reduced in the group given 1 g/kg yeast glucomannan, compared to the aflatoxin group. These results show that yeast glucomannan effectively diminished the adverse effects of aflatoxin on the pathological changes and that the higher concentration of yeast glucomannan (1 g/kg) was more effective than the lower concentration (0.5 g/kg) and itself had no adverse effect.



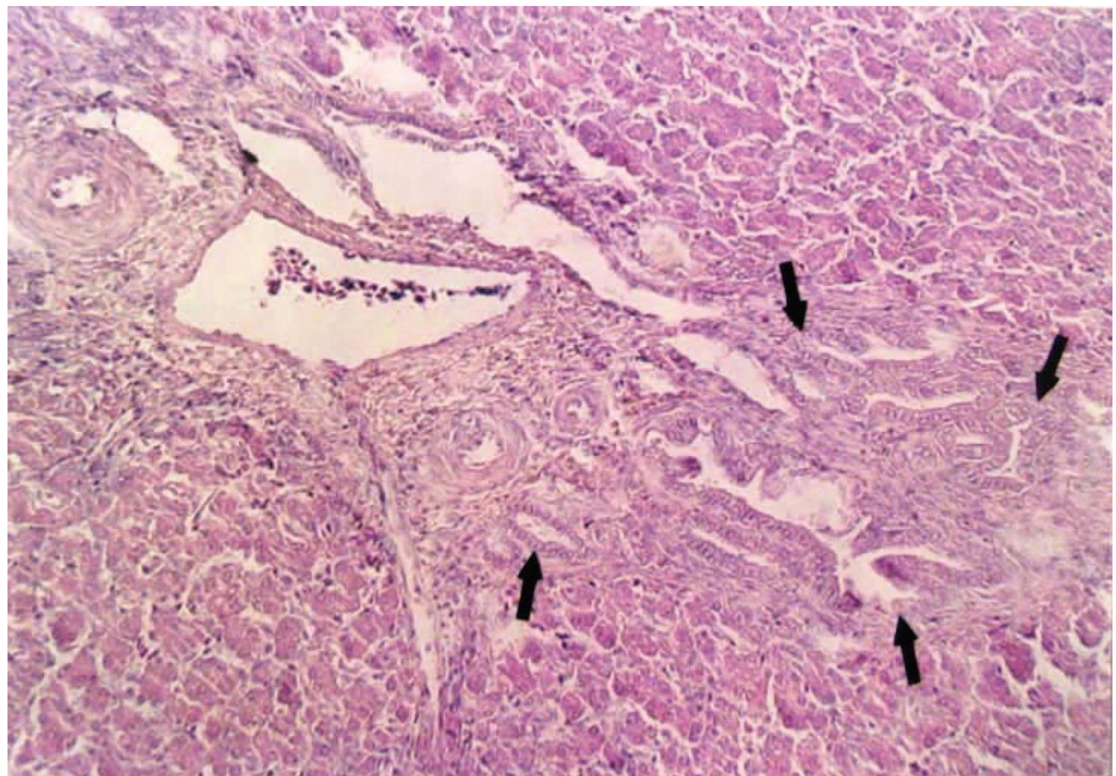
Comparative appearance of livers. (A) Control group. Normal liver. (B) AF plus YG (1 g/kg) group. The liver is slightly affected when compared to C. (C) AF-treated group. Severely affected liver is enlarged and pale yellow red. **Karaman *et al.* (2005)**



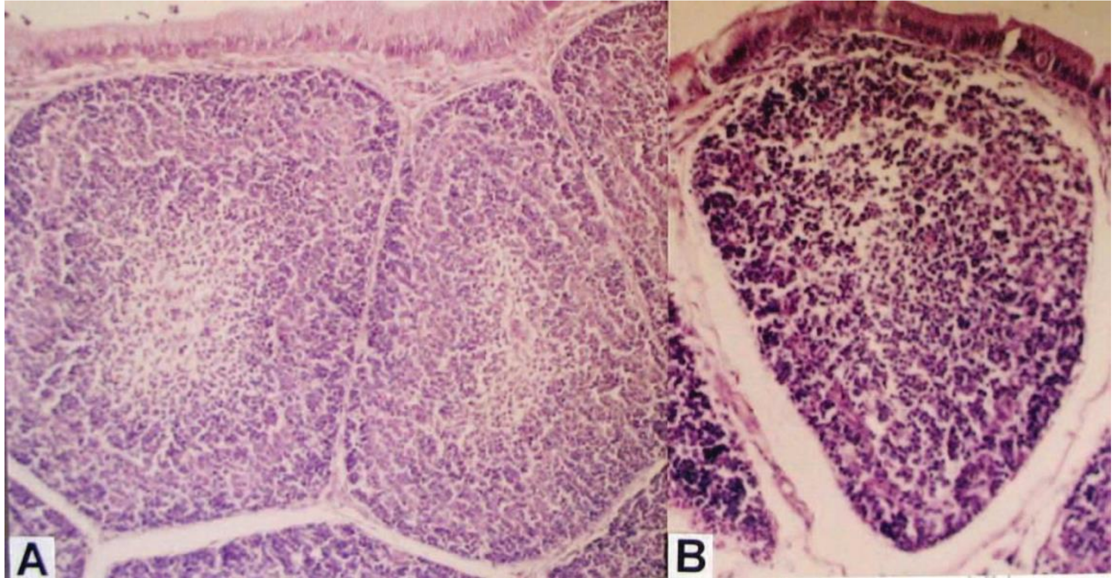
Thymus and spleen. (A) Atrophied thymus in AF-treated group. (B) Control group. (C) Enlarged spleen in AF-treated group. (D) Control group. **Karaman *et al.* (2005)**



Comparative micrographs of livers. (A) AF-treated group. Severe hydropic degeneration in centrilobuler hepatocytes. (B) AF plus YG (1 g/kg) group. There is no hydropic degeneration, but some hepatocytes are arranged in acinar pattern. H & E_400. **Karaman *et al.* (2005)**



Liver of AF-treated group. Bile-duct proliferation (arrows) in portal triad. H & E_160. **Karaman *et al.* (2005)**



Bursae of Fabricius from AF-treated group (A) and AF plus 1 g/kg YG group (B). Note: Severe lymphoid depletion is visible in the centre of follicles in the AF-treated group. H & E_160 **Karaman *et al.* (2005)**.

Miazzo *et al.* (2005) evaluated **sodium bentonite (SB)** for its ability to reduce the deleterious effects of **fumonisin B1 (FB1) and aflatoxin B1 (AFB1**) in **broiler** diets. It was incorporated into the diets (0.3%) containing 2.5 mg/kg AFB1, 200 mg/kg FB1, or a combination of 2.5 mg/kg AFB1 and 200 mg/kg FB1. Aflatoxin B1 significantly diminished body weight gain, whereas FB1 or the combination of FB1 and SB had no effect. Addition of SB in the diets significantly diminished the inhibitory effects of dietary AFB1. Feeding AFB1 alone caused significant increases in the relative weights of most observed organs. Feeding FB1 alone did not alter relative weights of any organs. In the combined diet (AFB1 plus FB1) relative

weights of the liver, kidney, gizzard, and spleen were increased. Addition of SB to the diet containing AFB1 diminished the relative weights of liver, kidney, and spleen. Addition of SB to diets containing AFB1 and FB1 only decreased liver weights. In relation to the control, lower serum levels of total protein, albumin, and globulins were observed for all AFB, containing diets without SB addition, whereas all other treatments were not altered. Livers of birds fed diets containing AFB1 and a combination of AFB1 and FB1 were enlarged, yellowish, friable, and had rounded borders. The histopathology of them, stained with hematoxylin and eosin, showed multifocal and varied cytoplasmatic vacuolization with perilobular location. Incorporation of SB reduced the incidence and severity of the hepatic histopathology changes associated with aflatoxicosis.

Otim *et al*. (2005) carried out a study to investigate the **immunosuppressive effects** of infectious bursal disease virus (IBDV) and aflatoxin in indigenous **chickens** of Uganda. Newcastle disease (ND) seronegative chicks were randomly allocated to two treatment groups. Group A chicks were injected intramuscularly at the age of 3 weeks every 2 days up to four times with 0.250 mg aflatoxin B1 per bird, group B was infected occulo-nasally with IBDV 3 days prior to vaccination, while group C was left as a control group. All the chicks from the three groups were then vaccinated with Hitchner B1 vaccine at 21 days of age followed by a secondary vaccination with La Sota vaccine 3 weeks later. Humoral and cell-mediated immune responses were assessed by measuring antibody levels and delayed hypersensitivity reaction post vaccination. Growth performance in the three groups was assessed by weekly body weights while evidence of excretion of vaccinal ND virus was detected by reverse transcription-polymerase chain reaction.A significant ($P < 0.05$) reduction in the haemagglutination inhibition of ND antibody titre following initial priming with Hitchner B1 and subsequent booster with La Sota vaccines and a delayed hypersensitivity test following sensitization with dinitrochlorobenzene showed aflatoxin to be a more potent immunosuppressant than IBDV. Aflatoxin exerted its maximum effects during primary antibody response in the second and third weeks post vaccination. Aflatoxin and IBDV did not affect growth rates ($P > 0.05$) but prolonged La Sota vaccine virus excretion in faeces. Under our experimental conditions, aflatoxin and IBDV do not significantly affect the **immune response** of rural chickens to **ND vaccination**.

Sehu *et al.* (2005) conducted a study to evaluate the toxic effects of aflatoxin (AF) on **growth performance** of **quail,** and to determine the preventive efficacy of **MYCOTOX (oxicinol, tymol, micronised yeast).**. One hundred and eighty 1-d-old quail (Coturnix coturnix japonica) of both sexes were weighed and randomly divided into 4 experimental groups each with 5 replicates of 9 birds. There were 4 dietary treatments: (1) control with 0 mg AF/kg diet and 0% MYCOTOX; (2) 0 mg AF/kg diet and 0.5% MYCOTOX; (3) 2.5 mg AF/kg diet and 0% MYCOTOX; (4) 2.5 mg AF/kg diet plus 0.5% MYCOTOX. The chicks were maintained on these treatments to 3 weeks of age. Quail consumed the diets and water ad libitum. 4. Body weight (BW) gains in groups receiving AF alone were the lowest at all periods. Feed intake was lowest in the group consuming the AF diet. The addition of MYCOTOX to the AF diet did not prevent or reduce the toxic effects of AF on feed intake at any time period. Feeding diets containing MYCOTOX alone did not change feed intake significantly. With the exception of the 1 to 7 d period, feed conversion of chicks fed the AF diet was similar to those of the other experimental groups. 5. Bursa of Fabricius weight decreased, whereas the relative weights of liver, kidney and spleen

increased in quail consuming diets containing AF and AF plus MYCOTOX. Liver colour was normal in the control and MYCOTOX alone group, but was lighter in groups fed AF. 6. The results indicated that MYCOTOX was not effective in preventing the deleterious effects of AF.

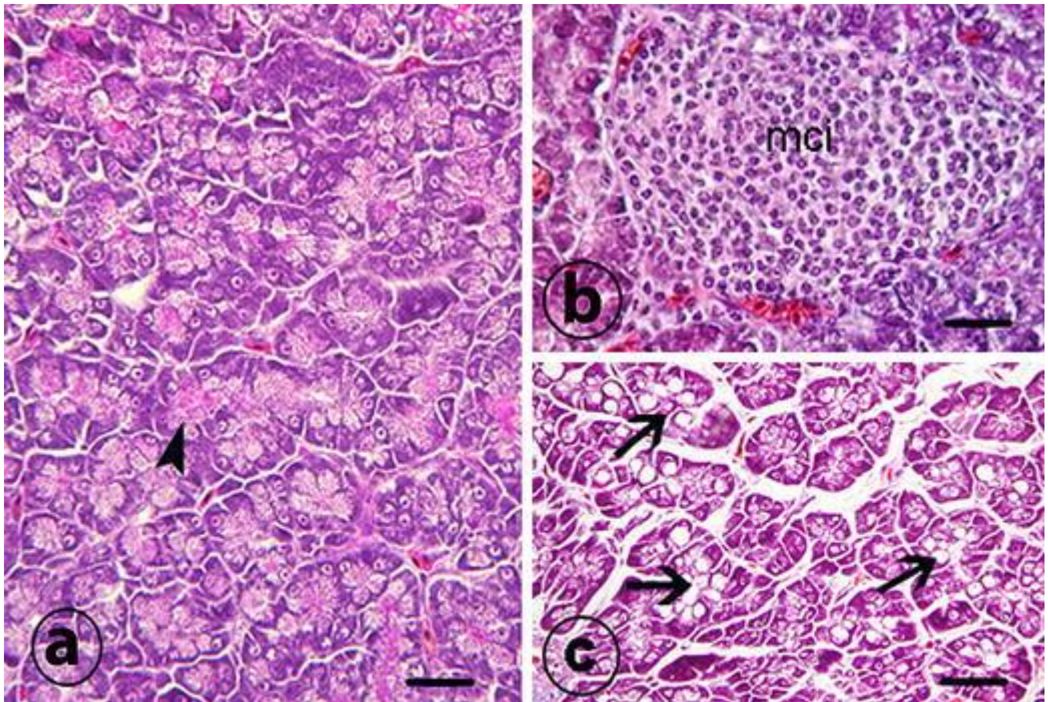
Raju *et al.* (2005) evaluated the addition of **sunflower oil (SFO**) at 30 or 60 g/kg or three vegetable oils, namely SFO, soybean (SBO) or groundnut (GNO), at 30 g/kg to isocaloric and isonitrogenous **broiler chicken** diets for possible counteractive effects against aflatoxin (AF) (0.3 microg B1/g diet) from 0 to 42 d of age. 2. Body weight, food intake and serum concentration of protein were lower in the AF group than in the control, whereas in the SFO and SBO supplemented groups they were comparable with those of the control. Sunflower oil at both concentrations exerted similar effects on growth. Groundnut oil did not improve growth or food intake in AF-fed birds. 3. The serum concentration of cholesterol and triglycerides decreased with AF feeding and was increased by supplementation of any of the three oils both in the control and in AF-fed groups. 4. Liver and giblet weight and liver fat content were increased by AF; these effects were countered by dietary oil inclusion, except for liver weight at 60 g/kg SFO. Weights of pancreas and gall bladder were increased by AF. Oil supplementation reduced the weight of pancreas in chickens given AF. 5. Humoral immune response was depressed by AF and dietary oil supplementation (particularly SFO or SBO) countered this effect. Other variables, namely, serum gamma glutamyl transferase activity, bone mineralisation, weights of lymphoid organs, kidney and adrenals, ready-to-cook yields and fat content in muscle and skin showed little or no effect of dietary oil supplementation. 6. It is concluded that dietary inclusion of SFO or SBO at 30 g/kg may alleviate the adverse effects of 0.3 microg/g of AF B1 in commercial broiler chickens. Groundnut oil, although showing beneficial effects on some biochemical variables, failed to improve growth performance.

Bintvihok and Kositcharoenkul (2005) performed a trial to study toxic effects of aflatoxins and reducing toxic effects of **calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers.** Two hundred and seventy 1-day-old hybrid Arbor Acor **broiler chickens** were fed conventional feed for 3 days. Broilers were then randomly divided into nine groups of 30 birds each. The nine dietary treatments consisted of (1) conventional feed as a negative control diet, (2) 0.25% calcium propionate, (3) 0.5% calcium propionate, (4) 50 ppb aflatoxin B1, (5) 50 ppb aflatoxin B1 plus 0.25% calcium propionate, (6) 50 ppb aflatoxin B1 plus 0.5% calcium propionate, (7) 100 ppb aflatoxin B1, (8) 100 ppb aflatoxin B1 plus 0.25% calcium propionate, (9) 100 ppb aflatoxin B1 plus 0.5% calcium propionate. Test diets were offered for 6 weeks continuously and the birds were sacrificed. Decreased body weight gain, feed consumption and feed conversion ratio were observed in aflatoxin treated groups whereas aflatoxin B1-calcium propionate supplemented diet groups increased, in comparison to the control group. Significant difference was observed after 4 weeks of feeding. Serum samples were tested for gamma glutamyl transferase (gamma-GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Gamma-GGT, AST and ALT were significantly increased in aflatoxin treated groups, in comparison among the dietary treated groups. Muscle and liver tissues were analyzed for aflatoxin residues. The residual levels of aflatoxin B1 and aflatoxin M1 were significantly higher in liver than in muscle. The levels in the liver and the muscle were highest in the aflatoxin B1-supplemented groups and lower in the aflatoxin B1-calcium propionate supplemented groups. Results of this study indicate that addition of calcium propionate to diets containing

aflatoxin B1 appears to be effective in reducing toxicity. Aflatoxin contamination in broiler feed may cause economic losses by lowering body weight gain. Therefore, lower levels of aflatoxin B1 in the chicken feeds should be required if all acceptable risk is to be avoided. Additionally, the risk of aflatoxins in broiler as a food appears to remain very low, although the levels of aflatoxins in human foods should be kept as low as possible to reduce the incidence of hepatic cancer.

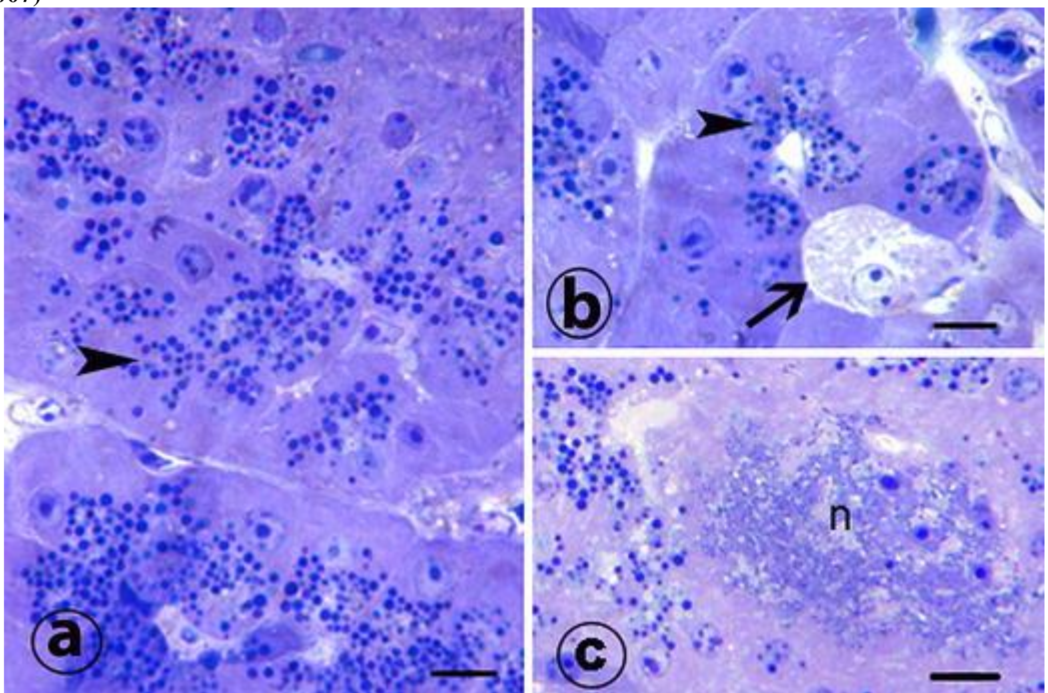
Lawson ***et al.*** **(2006)** documented for the first time the presence of **hepatic aflatoxin residues** in British **wild birds**: two passerine species, the house sparrow (Passer domesticus) and greenfinch (Carduelis chloris). Further research is required to investigate the source of the dietary aflatoxins and their pathological significance, if any, for wild birds in Britain

Simsek *et al.* (2007) carried out an investigation to assess the effects of aflatoxin (AF) on the **exocrine pancreas** in **quails** by means of light and **electron microscopy**. A total of 30 quails were divided into three groups, each composed of ten animals. Total AF was incorporated into the diet of these groups, at doses of 0, 2.5, and 5.0 mg of AF/kg feed, ppm, respectively. The quails were raised in cages with electrical heating and 24-h lighting for a period of 3 weeks. Ad libitum access was provided to feed and drinking water. Pancreas samples were taken for light and electron microscopic examination from animals that were killed by means of cervical dislocation at the end of the study. Light microscopic examination demonstrated mild mononuclear cell infiltration of exocrine tissue and vacuolisation of acinar cells in the group fed on AF at 2.5 ppm. On the other hand, electron microscopic examination demonstrated degranulation of the rough endoplasmic reticulum (rER) of acinar cells, decrease in the number of zymogen granules and free ribosomes and polisomes, and dilatation of capillaries in the group fed on AF at a dose of 2.5 ppm. Numerous degenerative acinar cells were determined in the group fed a diet containing 5.0 ppm AF, in addition to the findings common with the other group exposed to the toxin.

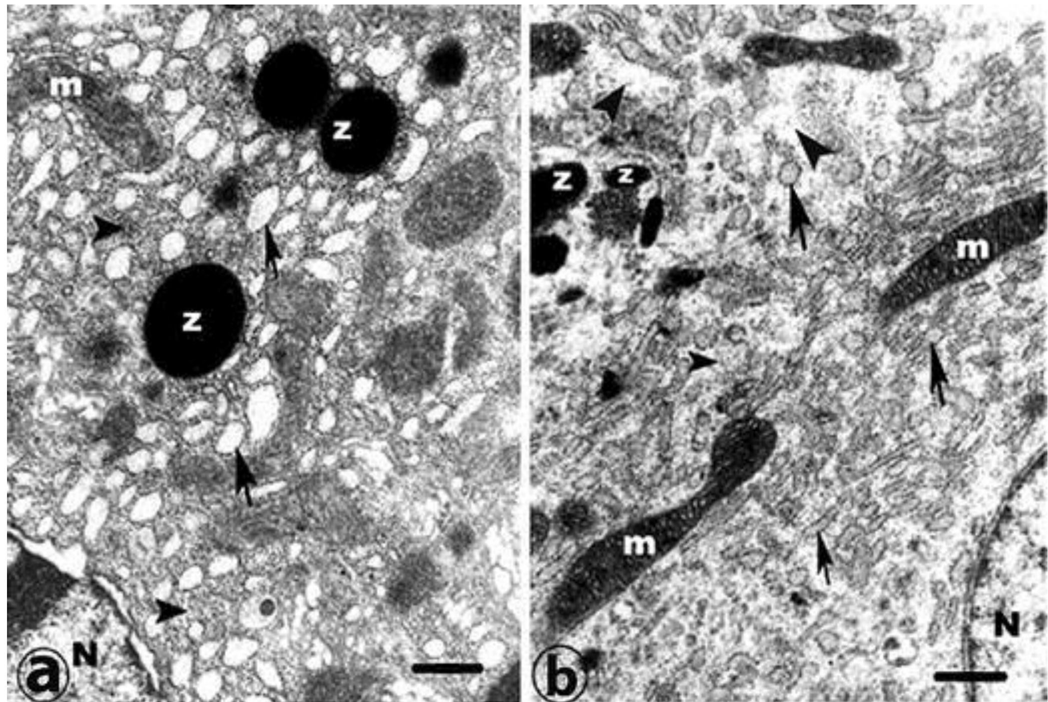


Light micrograph of sections of exocrine pancreas. **a** Control group. **b**–**c** Treated with 2.5 ppm AF group. Zymogen granules (*arrows head*), vacuolisation in the asinar cells (*arrows*), mononuclear cell

infiltration in the exocrine pancreas (*mci*). Triple (magnification × 350). *Bar* 28 μm **Simsek *et al.* (2007)**

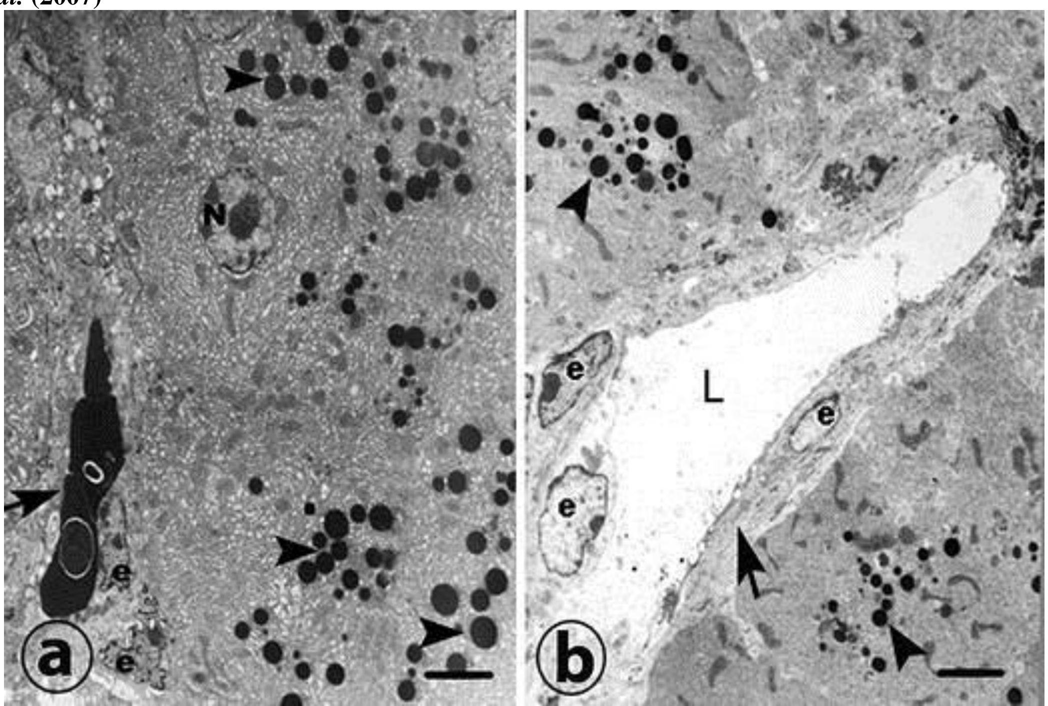


Semi-thin sections of exocrine pancreas. **a** Control group, **b**–**c** treated with 5 ppm AF group. Zymogen granules (*arrows head*), the lost of cellular integrity in the acinar cell (*arrow*), necrotic exocrine area (*n*). Toluidine blue (magnification × 1,250). *Bar* 8 μm Electron microscopic examination demonstrated the cytoplasm of pancreatic acinar cells of the control group to be rich in rER (Fig. 3a), free ribosomes, and polisomes (Fig. 3a). In pancreatic acinar cells of the group fed on AF at a dose of 2.5 ppm, the diameter of the cisterns pertaining to the rER, which were fewer when compared to the control group, was determined to be smaller (Fig. 3b). The number of free ribosomes and polisomes (Fig. 3b, arrow heads) were decreased in the experimental groups. **Simsek *et al.* (2007)**

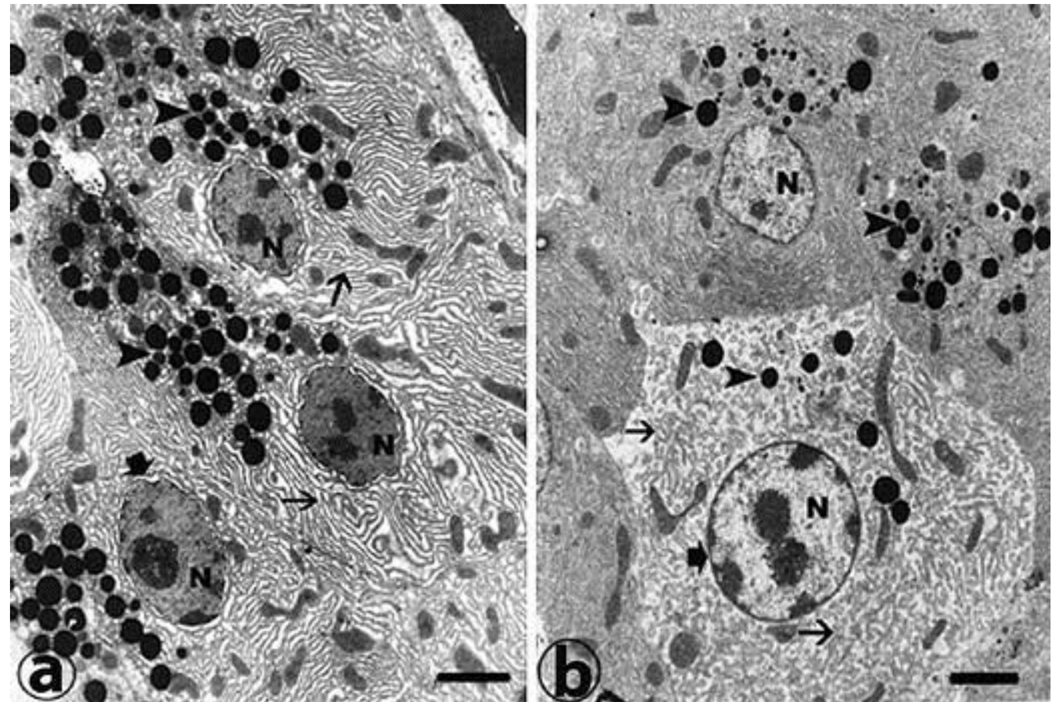


Electron micrograph of sections of acinar cells. **a** Control group, **b** treated with 2.5 ppm aflatoxin group. Nucleus (*N*), zymogen granules (*z*), mitochondria (*m*), free ribosomes and polisomes (*arrows*

head), rough endoplasmic reticulum vesicules (*arrows*) (magnification × 14,280). *Bar* 0.7 μm **Simsek *et al.* (2007)**



Electron micrograph of sections of capillaries in the exocrine pancreas. **a** Control group, **b** treated with 2.5 ppm aflatoxin group. Capillary wall (*arrows*), endothelial cells (*e*), lumen of capillary (*L*), zymogen granules (*arrow heads*), nucleus of the acinar cell (*N*) (magnification × 3,400). *Bar* 3 μm **Simsek *et al.* (2007)**



Electron micrograph of sections of acinar cells. **a** Control group, **b** treated with 5 ppm aflatoxin group. Nucleus (*N*), rough endoplasmic reticulum (*arrows*), zymogen granules (*arrow heads*), nuclear membrane of the acinar cells (*bold arrow*) (magnification × 5,000). *Bar* 2 μm **Simsek *et al.* (2007)**

Verma *et al.* (2007) studied the effect of dietary **aflatoxin B_1** (AF) at levels of 0.5, 1 and 2 mg kg^{-1}, **ochratoxin** A (OA) at levels of 1, 2 and 4 mg kg^{-1} and their

corresponding combinations on protein and energy utilisation as well as energy partitioning in white leghorn laying hens. Protein retention was adversely affected at all levels of AF and OA either singly or in combination, though the effect was more evident with OA and AF + OA. Minimum protein retention was recorded in hens fed the combination of toxins at their highest levels (2 mg kg^{-1} AF + 4 mg kg^{-1} OA). Aflatoxin at 1 and 2 mg kg^{-1} and OA and AF + OA at all levels caused a significant reduction in **metabolisable energy (ME)** value of the diets. The minimum ME value was recorded for the diet containing both toxins at their highest levels (2 mg kg^{-1} AF + 4 mg kg^{-1}OA). A significant depression in egg energy deposition was observed with dietary inclusion of 1 and 2 mg kg^{-1} AF, 2 and 4 mg kg^{-1} OA and all levels of AF + OA in period I. In period II the reduction in egg energy deposition was significant at all levels of toxins either singly or in combination. Body energy deposition was adversely affected in hens fed the highest levels of AF (2 mg kg^{-1}) and OA (4 mg kg^{-1}) and all levels of AF + OA in period I. However, in period II a significant decrease in body energy deposition was observed at all levels of toxins except 1 mg kg^{-1} OA. A significant increase in maintenance energy ($ME_m/W^{0.75}$ day^{-1}) requirement was recorded in hens fed 2 mg kg^{-1}AF, 4 mg kg^{-1} OA and all levels of AF + OA. It is suggested that AF and OA either singly or in combination affect not only protein and energy utilisation in laying hens but also energy partitioning i.e. egg and body energy deposition and maintenance energy requirement. However, the combination of toxins (AF + OA) has more severe adverse effects on all parameters than the individual toxins because of their synergistic toxicity effect.

Diaz *et al.* (2008) assessed how relatively low levels of aflatoxin consumption in feed may affect the **growth rate** of chickens. In general, multiple independent investigations have shown that such aflatoxin consumption affects growth in a hormetic-like biphasic manner with a low dose stimulation and a high dose inhibition. Such observations were then generalized to other toxic agents and animal models, suggesting that low doses of stressor agents induce adaptive responses as reflected in accelerated growth rates. The implications of such hormetic dose responses are briefly discussed.

Ebrahimi and Shahsavandi (2008) evaluated **chickens** fed 200 ppb aflatoxin from 10 days of age for their **immune response** to a modified live infectious laryngotracheitis vaccine. Vaccination was administered at age 4 and 12 weeks. Antibody titers to the vaccine were reduced in chickens given dietary aflatoxin. After 7 weeks, aflatoxin feeding was continued for one month in a treated group and was withdrawn in another. Serology indicated significant differences between the two treated groups relative to whether aflatoxin was fed or not. Significant reduction in body weights, antibody titers and elevated SGOT and SGPT levels were found in chickens treated with aflatoxin. The impact of aflatoxin on reduced body weight, decreased SGOT and SGPT levels and lower antibody titers was shown to be significant in the treated group fed on a ration of aflatoxin until throughout the experiment.

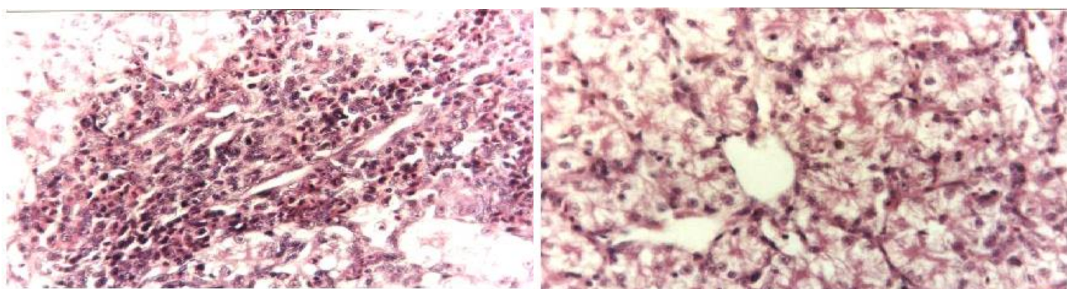
Gowda *et al.* (2008) conducted a 3-wk feeding study to evaluate the efficacy of turmeric (***Curcuma longa***) **powder (TMP)**, containing a known level of curcumin, and a **hydrated sodium calcium aluminosilicate (HSCAS**; Improved Milbond–TX, IMTX, an adsorbent, Milwhite Inc., Houston, TX) to ameliorate the adverse effects of aflatoxin B_1 (AFB_1) in **broiler chicks**. Four pen replicates of 5 chicks each were assigned to each of 7 dietary treatments, which included the basal diet not containing TMP, HSCAS, or AFB_1 (control); basal diet supplemented with 0.5% food grade

TMP that contained 1.48% total curcuminoids (74 mg/kg); basal diet supplemented with 0.5% HSCAS; basal diet supplemented with 1.0 mg/kg AFB_1; basal diet supplemented with 0.5% TMP and 1.0 mg/kg AFB_1; basal diet supplemented with 0.5% HSCAS and 1.0 mg/kgAFB_1; and basal diet supplemented with 0.5% TMP, 0.5% HSCAS, and 1.0 mg/kg AFB_1. The addition of TMP to the AFB_1 diet significantly ($P < 0.05$) improved the weight gain of chicks, and the addition of HSCAS to the AFB_1 diet significantly ($P < 0.05$) improved feed intake and weight gain, and reduced relative liver weight. The addition of TMP or HSCAS and TMP with HSCAS ameliorated the adverse effects of AFB_1 on some of the serum chemistry parameters (total protein, albumin, cholesterol, calcium). Further, decreased antioxidant functions in terms of level of peroxides, superoxide dismutase activity, and total antioxidant concentration in liver homogenate due to AFB1 were also alleviated by the inclusion of TMP, HSCAS, or both. The reduction in the severity of hepatic microscopic lesions due to supplementation of the AFB_1 diet with TMP and HSCAS demonstrated the protective action of the antioxidant and adsorbent used in the present study.

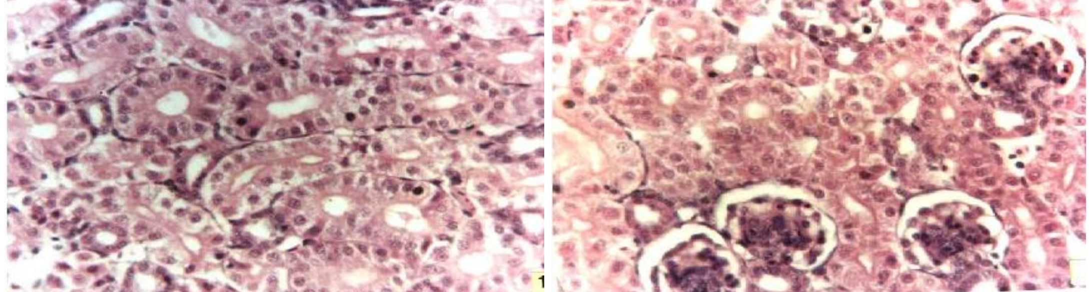
Guarisco *et al.* (2008) have shown that dietary **BHT** protected against clinical signs of aflatoxicosis in **turkeys**, a species that is very susceptible to this mycotoxin. In this study, the effect of BHT on AFB1 metabolism and other cytochrome P450 (CYP)-related enzyme activities in turkey liver microsomes was examined to discern possible mechanisms of BHT-mediated protection againstaflatoxicosis. Ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), prototype activities for CYP1A1 and 1A2, respectively, were decreased in the BHT fed (4000 ppm) animals, while oxidation of nifedipine, a prototype activity for CYP3A4, was increased. However, BHT added to microsomal incubations inhibited these CYP activities in a concentration-related manner. Importantly, BHT inhibited conversion of AFB1 to the reactive intermediate AFB1-8,-9-epoxide (AFBO), exhibiting Michaelis-Menton competitive inhibition kinetics (Ki=0.81 microM). Likewise, microsomes prepared from turkeys fed BHT were significantly less active in AFBO formation compared to those from controlbirds. When turkeys were fed BHT for up to 40 days, residual BHT was present in liver, breast meat, thigh meat and abdominal fat in concentrations substantially below U.S. FDA guidelines for this antioxidant, but in concentrations greater than the Ki, likely sufficient to inhibit bioactivation of AFB1in vivo. BHT-induced hydropic degeneration in the livers of BHT fed animals was significantly greater in birds that remained on BHT treatment for up to 30 days, but this lesion diminished in animals fed for 40 days or when returned to a control diet. The data indicated that the observed chemopreventive properties of BHT in turkeys may be due, at least in part, to its ability to inhibit hepatic AFB1 epoxidation and also that the BHT-induced hydropic degeneration is reversible and does not appear to cause long-term effects.

Ahmed *et al.* (2009) conducted an experiement to study the protective role of herbo-mineral toxin binder product in induced aflatoxicosis in broilers on the basis of histopathological studies. Total sixty day old broiler chicks of either sex were randomly divided into three groups, each comprising of twenty birds and two replicates. Healthy birds of group I were supplemented with standard basal ration as per NRC (1994), group II birds were fed with standard feed mixed with aflatoxin B1@1ppm, group III birds were fed with mycotoxicated feed of 1 ppm aflatoxin B1 and medicated with Toxiroak (M/s Ayurvet Ltd, Baddi, India) @ 0.125%. Fourty percent of birds were sacrificed at sixth week for necropsy examination.

Microscopically, congestion of liver parenchyma, cytoplasmic vacoulation/fatty change of hepatocytes and renal tubules, necrosis, mononuclear cell infiltration was observed in aflatoxicated group II. Milder form of pathological lesions in treatment groups birds reveal pallor discoloration of liver and hepatomegaly, splenomegaly and mild lesion in kidneys. Present study revealed that supplementation of herbomineral toxin binder product could ameliorate aflatoxicity in broilers.



Section of liver showing massive fatty changes, biliary hyperplasia and heterophil infiltration in 1 ppm AFB1, Section of liver showing mild fatty changesand infiltration of fewer heterophils in group III



Section of kidney showing little lymphoid cell infiltration in interstitial spaces, mild fatty changes in untreated group II, Section of kidney showing **Ahmed *et al.* (2009)**

Applegate *et al.* (2009) conducted a 2-wk feeding study with a crude AFLA culture with laying hens to measure **endogenous losses and digestive functionality** of the intestine. Hy-Line W36 hens were fed 1 of 4 experimental diets containing a crude AFLA culture from 20 to 22 wk of age. Diets were analyzed to contain 0, 0.6, 1.2, or 2.5 mg/kg of AFLA B(1). Dietary AFLA concentration had no effect on BW, egg production, or feed intake. Intestinal crypt depth (but not villus length) increased linearly with increasing AFLA concentration. Similarly, specific activity of the intestinal disaccharidase, maltase, increased quadratically by feeding up to 1.2 mg/kg of AFLA and declined at 2.5 mg/kg of AFLA (P <or= 0.022). Although there was no effect of AFLA on goblet cell number, density, or crude mucin excretion (P > 0.05), sialic acid excretion increased quadradically such that it was increased 12% when 0.6 and 1.2 mg/kg of AFLA was fed versus the control (P <or= 0.025). Digestibility of DM and N per hen per day were unaffected by AFLA. Feeding of 0.6 and 1.2 mg/kg reduced the apparent digestible and AME(n) of the hen by 10 and 4%, respectively (P <or= 0.025). Because intestinal characteristics (intestinal morphology, sialic acid production, and apparent digestible energy) were altered by AFLA feeding, it can be surmised that AFLA can have a direct or indirect effect, or both, on functionality of the gastrointestinal tract.

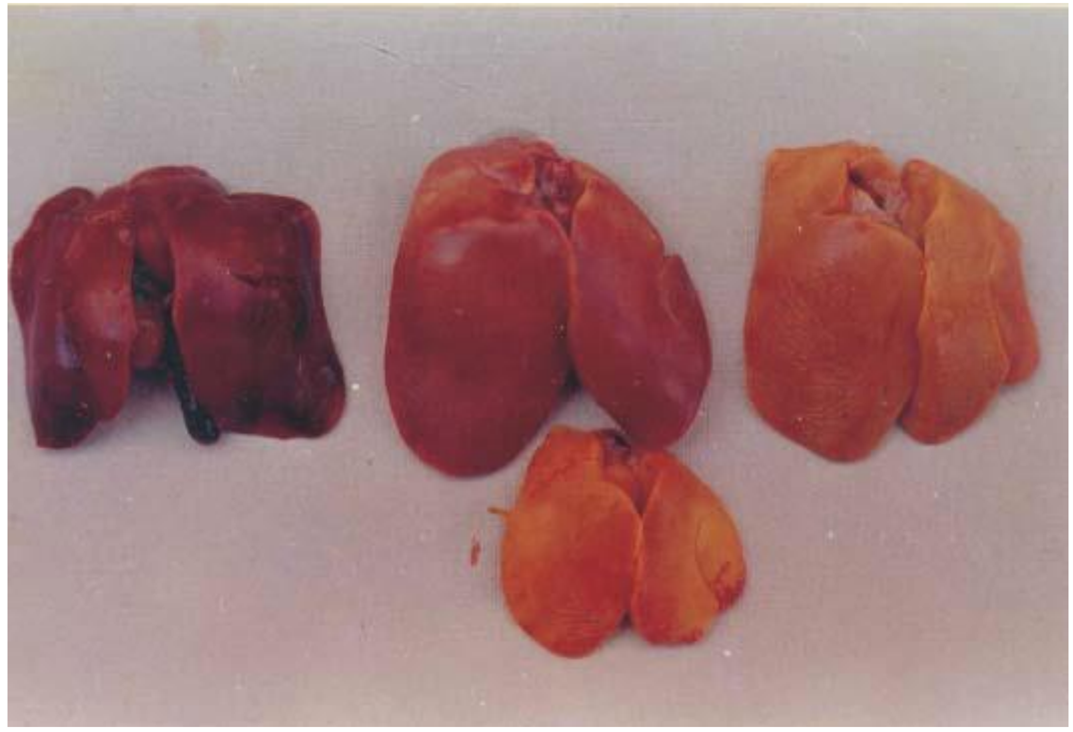
Denli *et al.* (2009) performed a study to evaluate the ability of **AflaDetox** (Adiveter, Agro-Reus, Reus, Tarragona, Spain) in counteracting the deleterious effects of aflatoxin B(1) (AFB(1)) in **broiler chicks**. A total of 120 Ross 308 one-day-old male broiler chicks were assigned to 8 treatments for 42 d. The experiment had a 2 x 4 factorial arrangement of treatments involving 0 and 1 mg of AFB(1)/kg feed and 0, 1,

2, and 5 g of AflaDetox/kg feed. Chicks were fed on the ground during the first 7 d and in cages (3 chicks/cage; 5 cages/treatment) from 7 to 42 d. Growth performance was measured from d 7 to 42 and whole-tract digestibility of gross energy and protein on d 40 to 41. Serum biochemical parameters, organ weights, histopathological examination of liver, and AFB(1) residues in liver and breast muscle tissues were determined on d 42. Aflatoxin B(1) significantly decreased the BW gain, feed intake, and impaired feed conversion rate ($P < 0.05$). The addition of AflaDetox in the contaminated diets significantly diminished the inhibitory effects of dietary AFB(1) ($P < 0.05$) on the growth performance with no differences compared to the control diet. Feeding AFB(1) alone decreased serum protein concentration, increased the serum activity of alkaline phosphatase, and caused significant increases in the relative weights of livers. Treatment with AflaDetox significantly alleviated the negative effects of AFB(1) on these parameters ($P < 0.05$) with no effect on uncontaminated diets. Liver tissue of broilers receiving AFB(1) alone had perilobular inflammation and vacuolar degeneration of hepatocytes as compared with the tissue from the control group ($P < 0.05$). Residues of AFB(1) were detected in the liver tissues of broilers fed on the AFB(1) diet (0.166 microg/kg). Supplementation of AflaDetox reduced the incidence and severity of the hepatic histopathology changes associated withaflatoxicosis and the amount of AFB(1) residue in liver. In conclusion, our results showed that addition of AflaDetox may reduce the adverse effects produced by the presence of AFB(1) in broiler chickens diets.

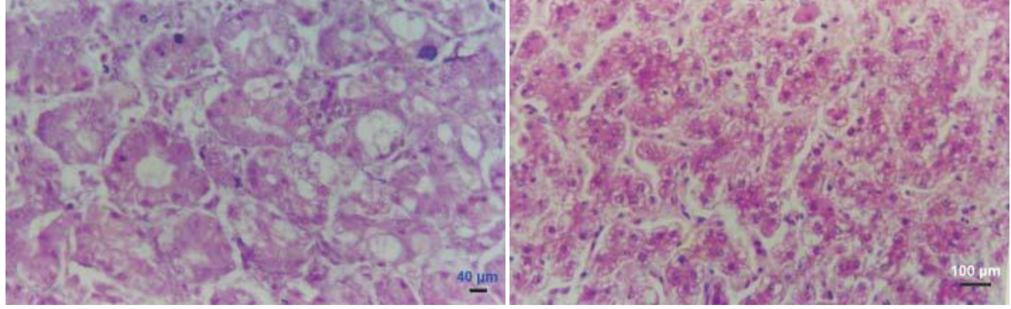
Hashem *et al.* (2009) conducted a study to evaluate the **prophylactic efficacy of protexin (probiotic), inulin (prebiotic) and both (synbiotics),** when included in a diet containing aflatoxins and fed to growing broiler chicks (from 1 to 21 days old). The criteria of the evaluation included body weight gain, haematological profile and biochemistry, in addition to associated lesions in chicks. A total of 160 Hubbard male day-old broiler chicks were separated into eight groups that all received different diets (additional aflatoxins, protexin, inulin and symbiotic). The birds were weighed and sacrificed at the age of 21 days. Compared to the controls, aflatoxins alone significantly ($p<0.05$) decreased body weight gain in one group. No difference in body weight gain was found in three groups, indicating apparent protection against the deleterious effects caused by aflatoxins. The weight gain of chicks fed on the diet containing synbiotics alone or synbiotics and aflatoxins was significantly ($p<0.05$) greater than that of chicks on a diet containing the other treatments. The birds the second group showed significant ($p<0.05$) reduction in the haematological parameters in comparison with the controls. The biochemical analysis showed a considerable ($p<0.05$) increase in the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), uric acid and creatinine levels, with a reduction in the serum total proteins, albumin and globulins. The addition of protexin, inulin, or both, diminished the adverse effects of aflatoxins. Finally, it was concluded that the protexin, inulin and synergism of both are effective in the amelioration of the toxic effects of aflatoxins that may be present in poultry rations at levels up to 4 mg/kg diet. Synbiotics (protexin and inulin) are more effective than the protexin and the inulin alone which are variable in the alleviation of toxic effects caused by aflatoxins.

Kumar and Balachandran (2009) induced an experimental mycotoxicoses into **broiler chickens** by feeding 1 ppm aflatoxin (AF) and 20 ppm cyclopiazonic acid (CPA) from 0 to 28 days of age to evaluate the **gross and histopathological changes**. Grossly, AF and AF-CPA fed birds showed enlargement, yellowish discoloration of

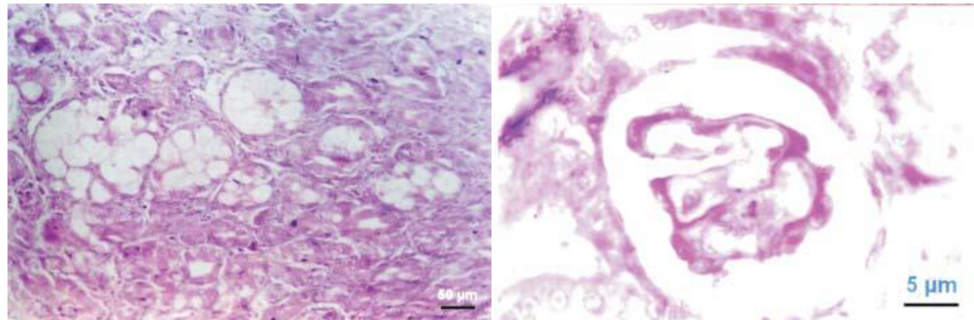
the liver while the CPA fed birds showed enlargement and congestion. The CPA and AF-CPA fed birds showed thickening of crop and necrosis and thickening of proventricular mucosa. Histopathologically, degenerative and necrotic changes were observed in the liver, kidneys, intestine, pancreas, heart, pectoral muscle, spleen and bursa of Fabricius of all toxin fed birds. Besides, hyperplastic changes were also observed in the crop, proventriculus and gizzard in the CPA fed birds. The lesions were more marked in the AF-CPA group. The study revealed that AF and CPA in combination could act cumulatively and adversely affect the health of broiler chicken.



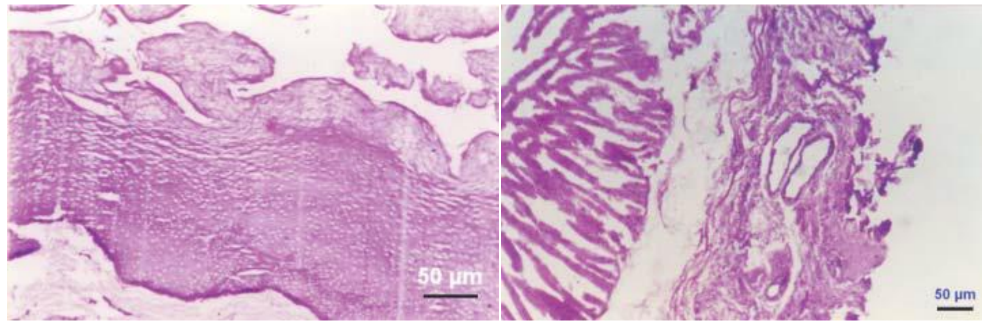
Liver CPA toxicosis. Congestion (upper left), aflatoxicosis-paleness and yellow discoloration (middle and upper right), AF-CPA toxicosis - yellow discoloration (bottom). **Kumar and Balachandran (2009)**



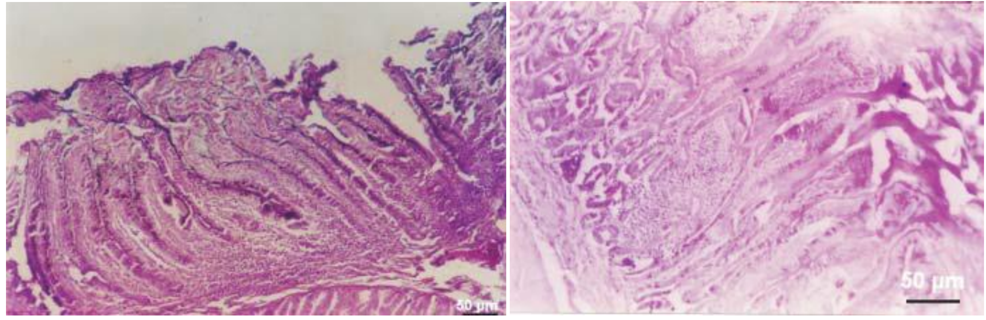
Aflatoxicosis. Liver showing acinar arrangement of regenerating hepatocytes. H&E, scale bar = 40 µm. CPA toxicosis. Liver showing microvesicular fatty degeneration of hepatocytes. H&E, scale bar = 100 µm. **Kumar and Balachandran (2009)**



AF-CPA toxicosis. Liver showing macrovesicular fatty degeneration and fatty cyst formation in the regenerating hepatocytes. H&E, scale bar = 50 µm. AF-CPA toxicosis. Kidney showing thickening of glomerular basement membrane and collapse of glomerulus .H&E, scale bar = 5 µm. **Kumar and Balachandran (2009)**



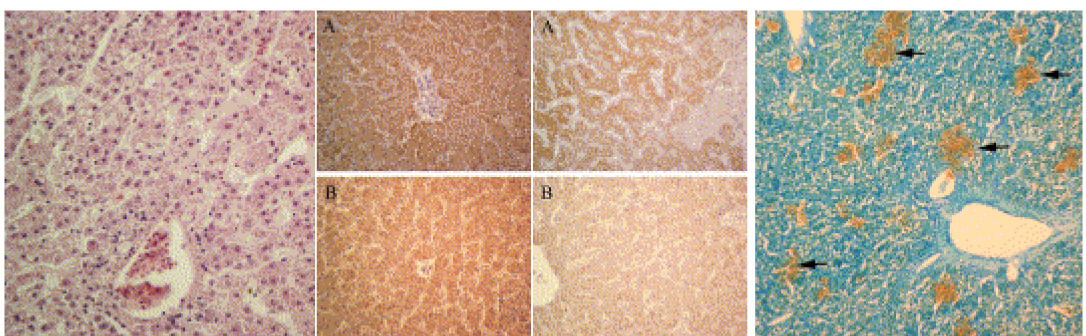
AF-CPA toxicosis. Crop mucosa showing epithelial hyperplasia and vacuolar degeneration. H&E, scale bar = 50 µm. Aflatoxicosis. Proventriculus showing partial necrosis of mucosa, dilated crypts and submucosal edema. H&E, scale bar = 50 µm. **Kumar and Balachandran (2009)**



CPA toxicosis. Proventriculus hyperplasia of mucosa with heavy infi ltration of lymphocytes. H&E, scale bar = 50 µm. AF-CPA toxicosis. Gizzard showing defective keratinoid membrane formation. H&E, scale bar = 50 µm.
Kumar and Balachandran (2009)

Ozen *et al*. (2009) investigated the effectiveness of melatonin on aflatoxicosis in chicks. Ross PM3 **breed chicks** were divided into groups of 10 and given conventional feed. One of the groups was kept as a control (C), and the others were given 150ppb aflatoxin (AF1), 300ppb aflatoxin (AF2), 150ppb aflatoxin plus 10mg/kg/bwt melatonin (AF1+M), 300ppb aflatoxin plus 10mg/kg/bwt **melatonin** (AF2+M), 10mg/kg/bwt melatonin (M), and 1% ethanol (E). After 21 day-treatment period, the chicks were sacrificed, liver and kidney tissues were collected, processed for immuno-histochemical staining, in situ TUNEL method, and biochemical analyses. Vacuolar degeneration, necrosis, bile duct hyperplasia in liver, and mild tubular degeneration in kidney were detected in AF groups. Pathological changes were markedly reduced in AF+M groups, and a microscopic view similar to group C was observed. Increased immunoreactivity against inducible nitric oxide synthase (iNOS) and nitrotyrosine was detected in AF groups compared to weak immunoreactivity in group C. **Immunoreactivity** in AF+M groups was markedly

reduced compared to AF groups and was similar to group C in liver and kidney. Many apoptotic cells were detected in the livers of AF groups, whereas there were no apoptotic cells in AF+M groups. While reduced glutathione (GSH) levels in liver and kidney of AF groups were greatly reduced, malondialdehyde (MDA) levels increased. With melatonin co-administration, the levels of GSH and MDA approached to the values of group C. These results indicated that nitrosative tissue degeneration caused by aflatoxin could be greatly reduced by melatonin supplementation in chicks.



Varga *et al.* (2009) reported that aflatoxins are decaketide-derived secondary metabolites which are produced by a complex biosynthetic pathway. Aflatoxins are among the economically most important mycotoxins. Aflatoxin B_1 exhibits hepatocarcinogenic and hepatotoxic properties, and is frequently referred to as the most potent naturally occurring carcinogen. Acute aflatoxicosis epidemics occur in several parts of Asia and Africa leading to the death of several hundred people. Aflatoxin production has incorrectly been claimed for a long list of*Aspergillus* species and also for species assigned to other fungal genera. Recent data indicate that aflatoxins are produced by 13 species assigned to three sections of the genus *Aspergillus*: section *Flavi* (*A. flavus, A. pseudotamarii, A. parasiticus, A. nomius, A. bombycis, A. parvisclerotigenus, A. minisclerotigenes, A. arachidicola*), section *Nidulantes* (*Emericella astellata, E. venezuelensis, E. olivicola*) and section*Ochraceorosei* (*A. ochraceoroseus, A. rambellii*). Several species claimed to produce aflatoxins have been synonymised with other aflatoxin producers, including *A. toxicarius* (=*A. parasiticus*), *A. flavus* var. *columnaris*(=*A. flavus*) or A. *zhaoqingensis* (=*A. nomius*). Compounds with related structures include sterigmatocystin, an intermediate of aflatoxin biosynthesis produced by several Aspergilli and species assigned to other genera, and dothistromin produced by a range of non-*Aspergillus* species. In this review, we wish to give an overview of aflatoxin production including the list of species incorrectly identified as aflatoxin producers, and provide short descriptions of the 'true' aflatoxin producing species.

Diaz *et al.* (2010) conducted a study to identify the cytochrome P450 (CYP, CYP450) enzyme orthologs involved in the bioactivation of aflatoxin B(1) (AFB(1)) into the highly toxic metabolite known as aflatoxin-8,9-epoxide (AFBO) in quail and chicken hepatic microsomes. The strategies used included the use of specific CYP450 inhibitors and the correlation of prototype substrate activities with AFBO production. Additionally, the presence of the enzymes was qualitatively determined using an immunoblotting technique. The results showed that both quail and chicken microsomes have CYP1A1, CYP1A2, CYP2A6, and CYP3A4 enzymatic activity. A strong relationship between CYP1A1 and CYP2A6 activities and AFB(1) bioactivation was found in both species. Inhibition studies provided more evidence for the role of CYP2A6 in the bioactivation of AFB(1). The immunoblot results showed

clear bands for the CYP2A6 and CYP3A4 orthologs in both species. The results of the present study indicate that CYP2A6 and, to a lesser extent, CYP1A1 are responsible for the bioactivation of AFB(1) into AFBO in both quail and chicken hepatic microsomes.

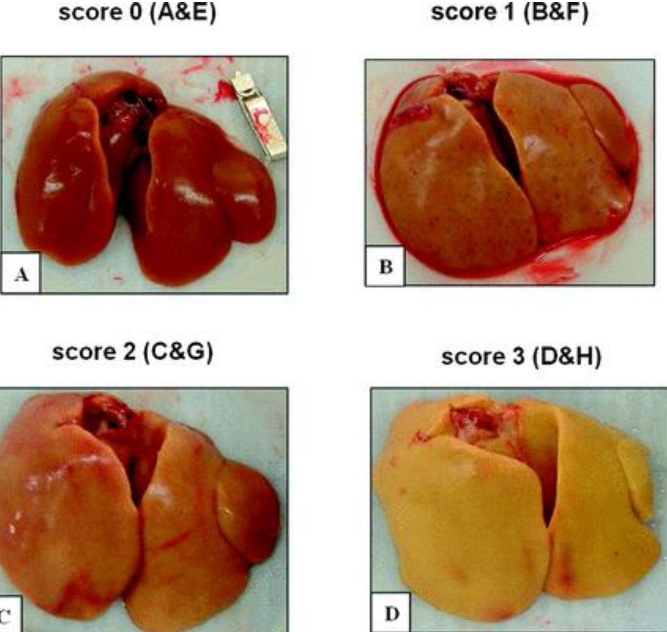
Hussain *et al.* (2010) described the effect of dietary levels of aflatoxin B1 (AFB1) and age of the birds upon the **residue level in liver and muscles of broiler chicks**. In three different experiments broiler chicks of 7, 14 and 28 days of age were kept for 7 days on contaminated rations having 1600, 3200 and 6400 µg/kg AFB1. AFB1 residues were detected earlier in younger birds and those fed high AFB1 dietary levels. The highest residue levels in liver and muscles of young chicks fed 6400 µg/kg AFB1 was 6.97±0.08 and 3.27±0.05 ng/g, respectively. Maximum residue concentration was high in birds of young age and those kept on high AFB1 ration. After withdrawal of AF contaminated rations, residues clearance was slow and AFB1 was detectable in liver and muscles of birds for longer duration in younger birds and those fed high AFB1 dietary levels. AFB1 residues in poultry tissues may buildup to high levels in areas with no regulatory limits on AFB1 levels of poultry feed and may pose a risk to consumers health.

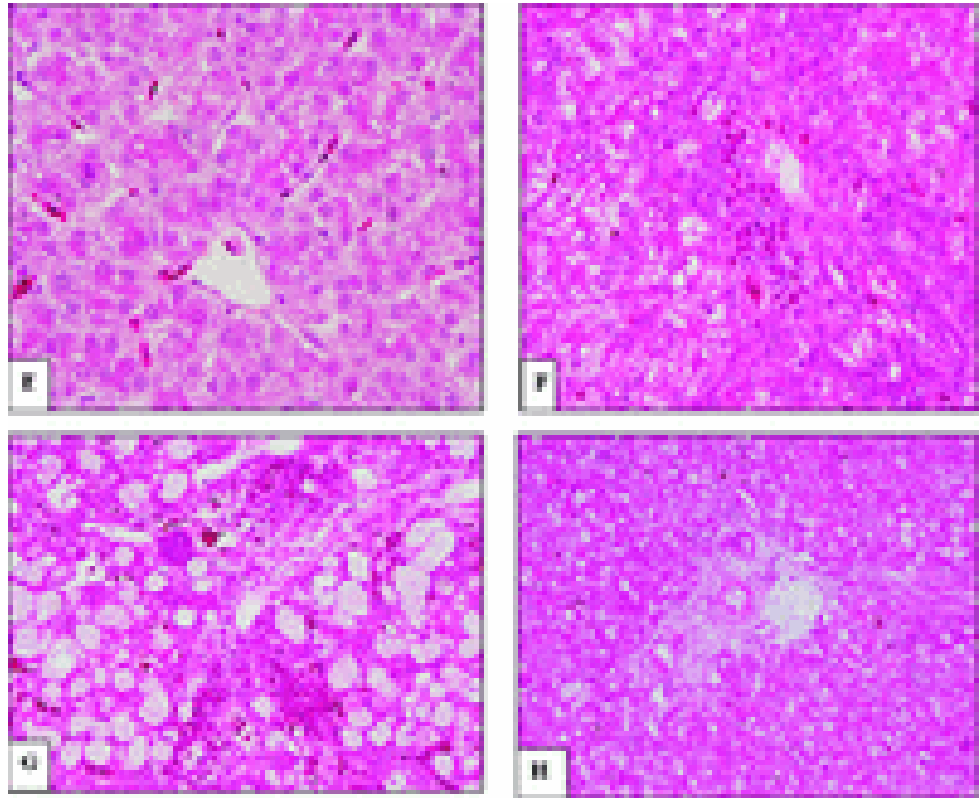
Kana *et al.* (2010) performed a study to evaluate the effect of including **plant charcoal from *Canarium schweinfurthii* (charcoal A) and maize cob (charcoal B**) in the diet on aflatoxin B_1 toxicosis in **broiler chickens**. Three-weeks-old male chicks (Hybro) were randomly divided into 8 groups of 20 individual birds each individually caged in a completely randomised design. The birds in group 1 received diet C- without aflatoxin B_1 and considered as negative control, while the chickens in group 2 were fed with diet C+ (positive control) containing 22.02 ppb of aflatoxin B_1 produced in peanut meal by *Aspergillus flavus*. The chickens in groups 3 to 8 were fed diets containing 22.02 ppb of aflatoxin B_1 and supplemented with either 0.20, 0.40, or 0.60% of charcoal A ($A_{0.20}$, $A_{0.40}$ and $A_{0.60}$ respectively) or charcoal B ($B_{0.20}$, $B_{0.40}$, and $B_{0.60}$ respectively). The result indicated that feeding 0.20, 0.40 and 0.60% of charcoal A and 0.60% of charcoal B significantly (p<0.05) increased feed consumption as compared with C+. Birds fed 0.20, 0.40 and 0.60% of charcoal A had significantly (p<0.05) higher final body weight as compared with C+. When compared with C+, birds fed 0.40 and 0.60% of charcoal B had significantly (p<0.05) higher body weight, average weight gain and intestine length. Feed conversion ratio, intestine circumference, carcass yield, relative weight of legs, heart and abdominal fat were not affected either by aflatoxin B_1 or charcoal. Both charcoal A and B depressed (p<0.05) liver weight and increased intestine density as compared with C+. It was concluded that 0.20% of *Canarium schweinfurthii* charcoal and 0.60% of maize charcoal could be used as feed additives to absorb aflatoxin B_1 and promote growth performance of broiler chickens.

Yunus *et al.* (2010) conducted a study to evaluate if aflatoxin B(1) (AFB(1)) has the capacity to affect the **electrophysiological variables and active glucose uptake** in jejunal epithelium of **chicken**. For this purpose, intestinal segments from the middle jejunum of broilers (35 to 39 d old) were incubated in Ussing chambers in the presence of 0 (vehicle control), 1.25, 2.50, and 3.75 microg of AFB(1)/mL of buffer. After 40 and 60 min of incubation with AFB(1), d-glucose (20 mmol/L) and carbamylcholine (200 micromol/L; an analog of acetylcholine and inducer of apical Cl(-) secretion) were respectively added to the incubation medium. Addition of 3.75 microg of AFB(1) caused an increase (P < 0.04) in short-circuit current (I(sc)) and

transmural potential difference (V(t)) between 12 to 27 min postexposure as compared with the control. Glucose-induced DeltaI(sc) and percentage of DeltaV(t) were reduced (P < 0.04) at 2.5 and 3.75 microg of AFB(1)/mL, respectively, as compared with the control. The carbamylcholine-induced DeltaI(sc) and DeltaV(t) were both lower (P < 0.05) at 3.75 microg of AFB(1)/mL as compared with the control (-0.05 microA/cm(2), 0.1 mV vs. 1.1 microA/cm(2), and 0.6 mV, respectively). These observations indicate that acute exposure to AFB(1) may increase apical anion secretion in the jejunal epithelium of chicken. The negative effect of this increased anion secretion on active glucose uptake was, however, not prominent and may be considered as moderate or progressive in nature.

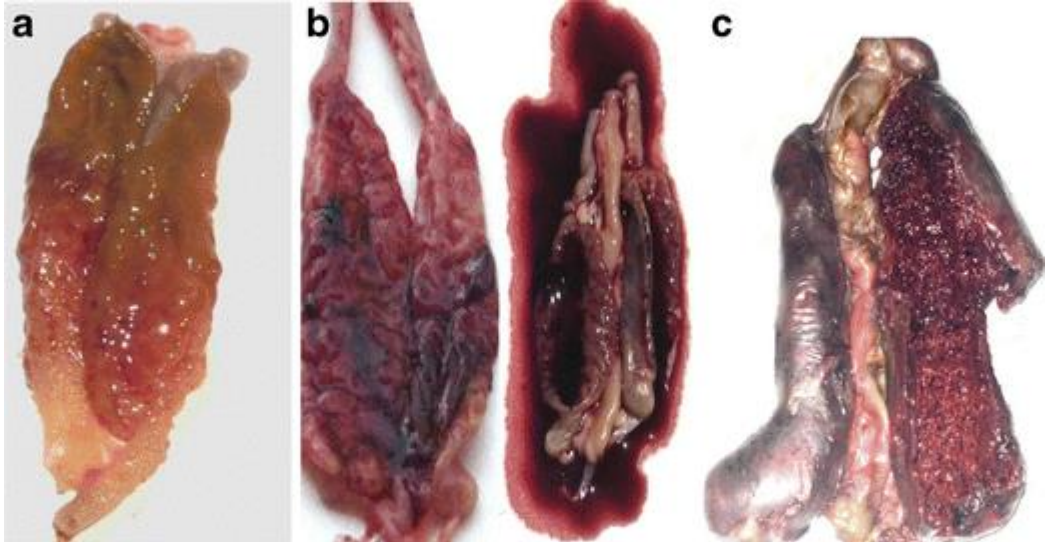
Zhao *et al.* (2010) determined the efficacy of 2 types of adsorbents [hydrated sodium calcium aluminosilicates (HSCAS) vs. a combination of clay and yeast cell wall] in preventing aflatoxicosis in broilers. A total of 275 one-day-old birds were randomly divided into 11 treatments, with 5 replicate pens per treatment and 5 chicks per pen. The 11 treatments included 3 diets without any adsorbent containing either 0, 1, or 2 mg/kg of aflatoxin B1 (AFB1) plus 8 additional treatments employing 2 dietary levels of AFB1 (1 or 2 mg/kg), 2 different adsorbents [Solis (SO) and MTB-100 (MTB)], and 2 different levels of each absorbent (0.1 and 0.2%) in a 2×2×2 factorial arrangement. Solis is a mixture of different HSCAS and MTB is a combination of clay and yeast cell wall. Feed and water were provided ad libitum throughout the 21-d study period. Body weight gain and feed intake were depressed and relative liver weight was increased in chicks fed AFB1 compared with the positive control (P<0.05). Severe liver damage was observed in chicks fed 2 mg/kg of AFB1 with lesions consistent with aflatoxicosis, including fatty liver and vacuolar degeneration. Serum glucose, albumin, total protein, Ca, P, and alkaline phosphatase concentrations were reduced by AFB1 (P<0.05). The addition of either SO or MTB ameliorated the negative effects of 1 mg/kg of AFB1 on growth performance and liver damage (P<0.05). However, supplemental MTB failed to diminish the negative effects of 2 mg/kg of AFB1, whereas SO was more effective compared with MTB at 2 mg/kg of AFB1 (P<0.05). These data indicated that the HSCAS product effectively ameliorated the negative effect of AFB1 on growth performance and liver damage, whereas the yeast cell wall product was less effective especially at the higher AFB1 concentration



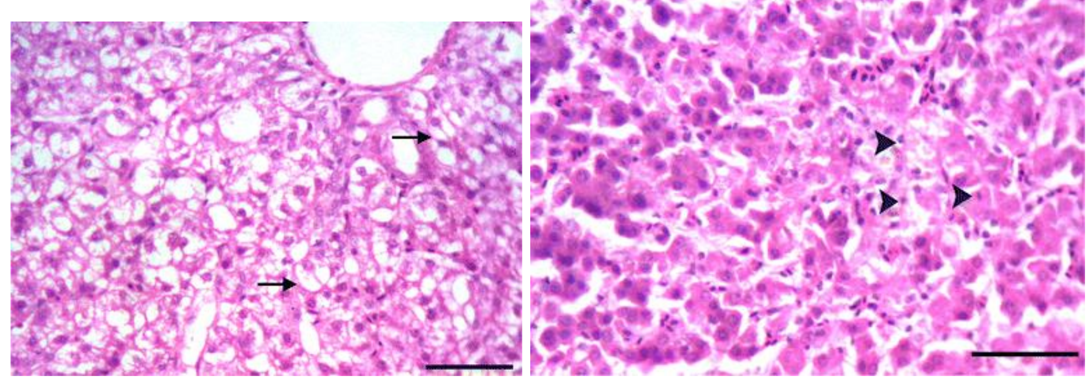


Effects of aflatoxin on gross (panels A, B, C, and D) and histological appearance (panels E, F, G, and H) of livers in broilers. Gross and histological appearances of livers exhibiting lesion scores of 0, 1, 2, and 3 are shown with 0 = no changes, liver unremarkable; 1 = mild aflatoxicosis lesions; 2 = moderate aflatoxicosis lesions; and 3 = severe aflatoxicosis lesions. Note that with increased score, the severity of vacuolar degeneration and fatty infiltration increases. Histological sections were stained with hematoxylin and eosin, **Zhao *et al.* (2010)**

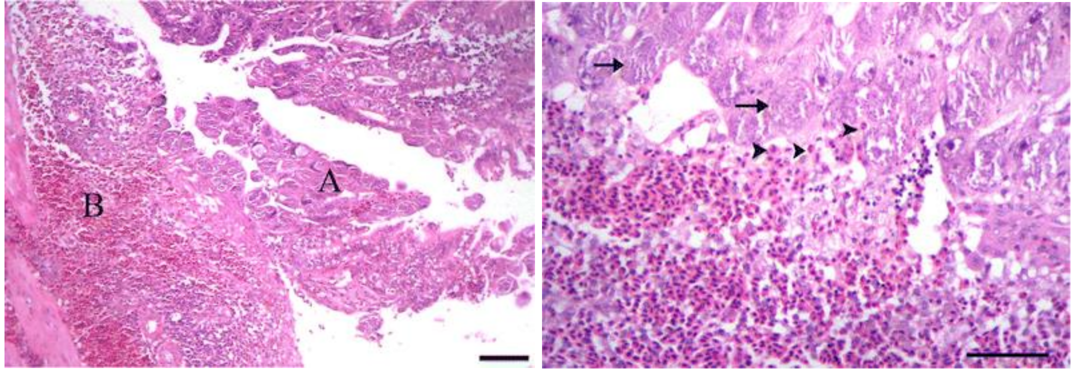
Ellakany *et al.* (2011) evaluated the adverse effects of an interaction between low levels of dietary aflatoxins (AF) and Eimeria tenella infection on broiler chicks. A set of 1-day-old chicks were raised for 35 days in the following groups: a control group, a group fed AF, a group fed AF and inoculated with E. tenella (AF + E.ten), and a group inoculated with E. tenella alone. AF in the contaminated diet were given at 200 ppb starting from the seventh day after hatching while E. tenella was inoculated at a dose of 5 × 10(4) sporulated oocysts per chick at the 14th day after hatching. Worsened performance traits and high mortality were all observed in the treated birds, particularly the AF + E.ten group. Lesion scores and oocyst outputs were not different within groups. Chickens fed with AF had significantly increased serum ALT and ALP activities as well as decreased albumin content. They also showed hepatomegaly, hepatocytic vacuolation and necrosis, an atrophied bursa of Fabricius, and a thymus with tissue depletion. E. tenella-infected broilers displayed a significant reduction in packed cell volume, hemoglobin content and lymphocyte percentage, and showed hemorrhagic typhlitis. The deficits in hepatic function and hematologic parameters as well as the gross pathological, and histopathological changes, were more common and more severe in the group that was exposed to both aflatoxicosis and coccidiosis than in the groups exposed to either treatment alone. Thus, the combination of aflatoxicosis and E. tenella infection may influence the course of coccidial infection due to additive effects.



Photograph of ceca from broiler chickens inoculated with *E. tenella* (5×10^4 sporulated oocyst/chick) (groups III and IV). Gross cecal lesions can be seen. **a** Score 2: noticeable blood in the cecal contents with thickened cecal wall; **b** Score 3: blood or cecal cores and severely thickened cecal wall; **c** Score 4: severely distended cecal wall with bloody cores. **Ellakany *et al.* (2011)**

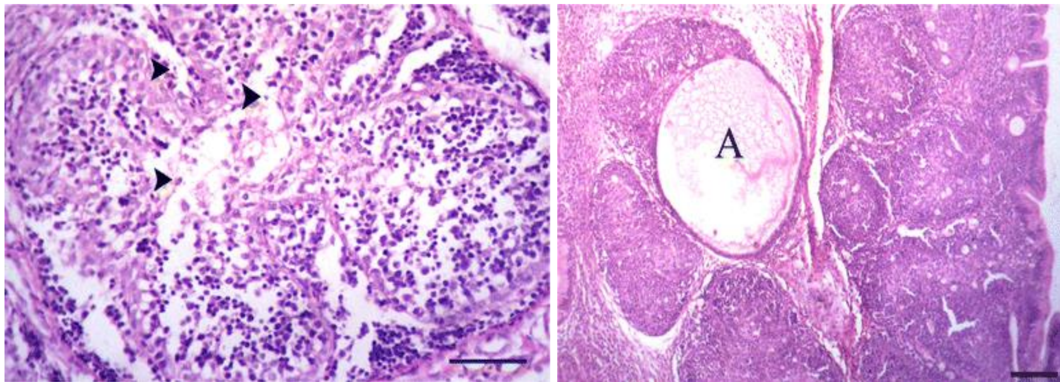


liver from broiler chickens fed an AF-contaminated diet (200 ppb) and inoculated with*E. tenella* (5×10^4 sporulated oocyst/chick) (group III, AF + *E.ten*). Severe diffuse hepatocytic vacuolation (*arrows*) can be seen (H & E, *bar* = 50 μm), liver from broiler chickens fed an AF-contaminated diet (200 ppb) and inoculated with*E. tenella* (5×10^4 sporulated oocyst/chick) (group III, AF + *E.ten*). Focal areas of coagulative necrosis represented by pyknotic and karyorrhectic hepatocytes (*arrow heads*) can be seen (H & E, *bar* = 50 μm) **Ellakany *et al.* (2011)**

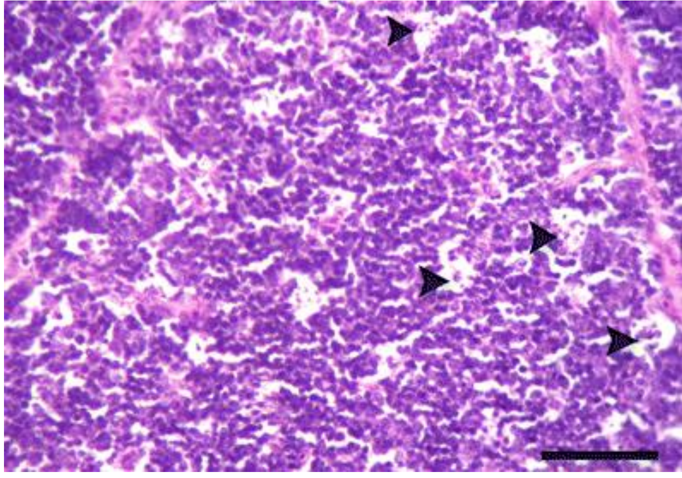


cecum from a broiler chicken fed an AF-contaminated diet (200 ppb) and inoculated with *E. tenella* (5×10^4 sporulated oocyst/chick) (group III, AF + *E.ten*). Severe necrosis and desquamation of the mucosal epithelium can be seen, with replacement by schizonts (**a**) next to the submucosal hemorrhage (**b**) (H & E, *bar* = 100 μm), cecum from a broiler chicken fed an AF-contaminated diet (200 ppb) and inoculated with *E. tenella* (5×10^4 sporulated oocyst/chick) (group III, AF + *E.ten*).

Numerous intracellular schizonts containing banana-shaped merozoites (*arrows*) can be seen with severe submucosal hemorrhage and infiltration of numerous heterophils (*arrow heads*) (H & E, *bar* = 50 μm) **Ellakany *et al.* (2011)**



bursa of Fabricius from a broiler chicken fed an AF-contaminated diet (200 ppb) and inoculated with *E. tenella* (5×10^4 sporulated oocyst/chick) (group III, AF + *E.ten*). Severe diffuse lymphocytic cell necrosis and depletion (*arrow heads*) can be seen (H & E, *bar* = 50 μm), bursa of Fabricius from a broiler chicken fed an AF-contaminated diet (200 ppb) and inoculated with *E. tenella* (5×10^4 sporulated oocyst/chick) (group III, AF + *E.ten*). A large cystic cavitation in a bursal follicle containing faint eosinophilic necrotic debris (**a**) can be seen (H & E, *bar* = 100 μm) **Ellakany *et al.* (2011)**

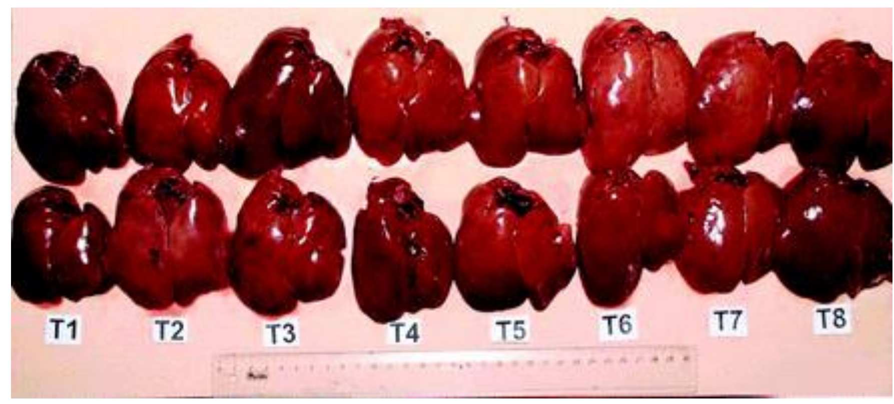


Photomicrograph of a thymus from a broiler chicken fed an AF-contaminated diet (200 ppb) and inoculated with *E. tenella* (5×10^4 sporulated oocyst/chick) (group III, AF + *E.ten*). Apoptotic changes with a resulting "starry-sky" appearance (*arrow heads*) can be seen (H & E, *bar* = 50 μm) **Ellakany *et al.* (2011)**

Luciano Polonelli *et al*. (2011) studied the potential of an aflatoxin B1 (AnAFB1) conjugated to keyhole limpet hemocyanin (KLH) as a vaccine (AnAFB1-KLH) in controlling the carry-over of the aflatoxin B1 (AFB1) metabolite aflatoxin M1 (AFM1) in cow milk is reported. AnAFB1-KLH was used for immunization of cows proving to induce a long lasting titer of anti-AFB1 IgG antibodies (Abs) which were cross reactive with AFB1, AFG1, and AFG2. The elicited anti- AFB1 Abs were able to hinder the secretion of AFM1 into the milk of cows continuously fed with AFB1.

Magnoli *et al.* (2011a) investigated the influence of Na-B (0.3%) and monensin (MON, 100 mg/kg), alone or in combination. The dietary treatments were as follows: treatment (T) 1: basal diet (B); T2: B + MON; T3: B + Na-B; T4: B + Na-B + MON; T5: B + AFB_1; T6: B + AFB_1 + Na-B + MON; T7: B + AFB_1 + MON; T8: B + AFB_1

+ Na-B. Birds were fed dietary treatments for 28 d (d 18 to 46). No significant differences ($P < 0.05$) were observed among treatments with respect to broiler performance, biochemical parameters, or relative liver weights. With the exception of T8, all livers showed histopathological alterations, with accumulation of fat vacuoles. The normal appearance of livers from T8 showed the protective effect of Na-B against aflatoxicosis. The residual AFB_1 levels in livers from T5 to T8 ranged from 0.2 to 1.0 ng/g and were higher in livers from T6 ($P < 0.05$). Results of this study indicated a competition between AFB_1 and MON for adsorption sites on Na-B when feed contains low levels of the toxin, indicating a nonselective adsorption capacity of this particular Na-B. In addition, significant levels of AFB_1 in livers indicated that this determination is an important technique not only for diagnosis of aflatoxicosis in broilers, but also for quality control of avian products.



Representative livers from broilers (46 d old) fed different treatments (T). T1: basal diet (B); T2: B + monensin (MON); T3: B + sodium bentonite (Na-B); T4: B + MON + Na-B; T5: B + aflatoxin B_1 (AFB_1); T6: B + AFB_1 + Na-B + MON; T7: B + AFB_1 + MON; T8: B + AFB_1 + Na-B. AFB_1 (50 µg/kg), Na-B (0.3%), MON (100 mg/kg) **Magnoli *et al.* (2011a)**

Photomicrographs (optical microscopy) of hematoxylin and eosin-stained broiler liver sections from different treatments. A) Basal diet (B); B) B + monensin (MON); C) B + sodium bentonite

(Na-B); D) B + MON + Na-B; E) B + aflatoxin B_1 (AFB_1); F) B + AFB_1 + MON + Na-B; G) B + AFB_1 + MON; H) B + AFB_1 + Na-B. AFB_1 (50 µg/kg), Na-B (0.3%), MON (100 mg/kg). Bar equals 5 µm. Photomicrographs (high-resolution optical microscopy) of toluidine blue-stained broiler liver sections from different treatments. A) Basal diet (B); B) B + monensin (MON); C) B + sodium bentonite (Na-B); D) B + MON + Na-B; E) B + aflatoxin B_1(AFB_1); F) B + AFB_1 + MON + Na-B; G) B + AFB_1 + MON; H) B + AFB_1 + Na-B. AFB_1 (50 µg/kg), Na-B (0.3%), MON (100 mg/kg). Bar equals 2 and 5 µm **Magnoli *et al.* (2011a)**

Magnoli *et al.* (2011b) investigated the use of sodium bentonite (Na-B) from a mine in the province of Mendoza, Argentina, as a sequestering agent to prevent the effects of 100 µg/kg of dietary aflatoxin B(1) (AFB(1)). In vitro studies demonstrated that the above Na-B was a good candidate to prevent aflatoxicosis. They also showed that MON competes with AFB(1) for the adsorption sites on the clay surface and effectively displaces the toxin when it is in low concentration. Even though the levels of MON in diets, approximately 55 mg/kg, are high enough to not be significantly changed as a consequence of the adsorption, they can further affect the ability of the clays to bind low levels of AFB(1). An in vivo experiment carried out with poultry showed that 100 µg/kg of AFB(1) does not significantly change productive or biochemical parameters. However, liver histopathology not only confirmed the ability of this particular Na-B to prevent aflatoxicosis but also the decrease of this capacity in the presence of 55 mg/kg of MON. This is the first report stressing this fact and further research should be performed to check if this behavior is a characteristic of the assayed Na-B or of this type of clay. On the other hand, the presence of MON should also be taken into account when assaying the potential AFB(1) binding ability of a given bentonite.

Rangsaz and hangaran (2011) evaluated the effect of ethanolic turmeric extract (ETE; Curcuma longa) on overall performance including body weight (BW), body weight gain (BWG), feed intake and feed conversion ratio (FCR) weekly and cumulative for a period of 4 weeks with 300 commercial broiler chicks (Ross strain). These chicks were randomly divided into four groups with three replicates of 15 chicks in each replicate. In group A, chickens were fed a basal diet, in group B, chickens were fed a basal diet plus 3 ppm productive aflatoxin. In group C, chickens consumed a basal diet plus 0.05% ETE and in group D, chickens received a basal diet with 0.05% ETE plus 3 ppm productive aflatoxin. Aflatoxin production by Aspergillus parasiticus (PTTC NO:1850) in maize was according to the Shotwell method. The results revealed that there were no significant differences in BW, BWG and FCR between groups fed turmeric at 0.05% and the control group. The supplement of ETE in a diet containing 3 ppm aflatoxin can significantly improve performance indices compared with the group that consumed aflatoxin alone. In conclusion, our results suggest that turmeric extract (Curcuma longa) can provide protection against the negative effects of aflatoxin on performance of broiler chickens.

Rawal *et al.* (2011) mentioned that the extreme sensitivity of turkeys to aflatoxin B(1) (AFB(1)) is associated with efficient epoxidation by hepatic cytochromes P450 (P450) 1A5 and 3A37 to exo-aflatoxin B(1)-8,9-epoxide (exo-AFBO). The combined presence of 1A5 and 3A37, which obey different kinetic models, both of which metabolize AFB(1) to the exo-AFBO and to detoxification products aflatoxin M(1) (AFM(1)) and aflatoxin Q(1) (AFQ(1)), respectively, complicates the kinetic analysis of AFB(1) in turkey liver microsomes (TLMs). Antisera directed against 1A5 and

3A37, thereby individually removing the catalytic contribution of these enzymes, were used to identify the P450 responsible for epoxidating AFB(1) in TLMs. In control TLMs, AFB(1) was converted to exo-AFBO in addition to AFM(1) and AFQ(1) confirming the presence of functional 1A5 and 3A37. Pretreatment with anti-1A5 inhibited exo-AFBO formation, especially at low, submicromolar (~0.1μM), while anti-3A37, resulted in inhibition of exo-AFBO formation, but at higher (>50μM) AFB(1) concentrations. Metabolism in immunoinhibited TLMs resembled that of individual enzymes: 1A5 produced exo-AFBO and AFM(1), conforming to Michaelis-Menten, while 3A37 produced exo-AFBO and AFQ(1) following the kinetic Hill equation. At 0.1μM AFB(1), close to concentrations in livers of exposed animals, 1A5 contributed to 98% of the total exo-AFBO formation. At this concentration, 1A5 accounted for a higher activation:detoxification (50:1, exo-AFBO: AFM(1)) compared to 3A37 (0.15: 1, exo-AFBO: AFQ(1)), suggesting that 1A5 is high, while 3A4 is the low affinity enzyme in turkey liver. The data support the conclusion that P450 1A5 is the dominant enzyme responsible for AFB(1) bioactivation and metabolism at environmentally-relevant AFB(1) concentrations in turkey liver.

Yunus *et al.* (2011a) mentioned that aflatoxin B(1) is a common contaminant of poultry feeds in tropical and subtropical climates. Research during the last five decades has well established the negative effects of the mycotoxin on health of poultry. However, the last ten years of relevant data have accentuated the potential of low levels of aflatoxin B(1) to deteriorate broiler performance. In this regard, any attempt to establish a dose-effect relationship between aflatoxin B(1) level and broiler performance is also complicated due to differences in types of broilers and length of exposure to the mycotoxin in different studies. Contrary to the prevalent notion regarding literature saturation with respect to aflatoxicosis of chicken, many areas of aflatoxicosis still need to be explored. Literature regarding effects of the mycotoxin on the gastrointestinal tract in this regard is particular scanty and non-conclusive. In addition to these issues, the metabolism of aflatoxin B(1) and recently proposed hypotheses regarding biphasic effects of the mycotoxin in broilers are briefly discussed.

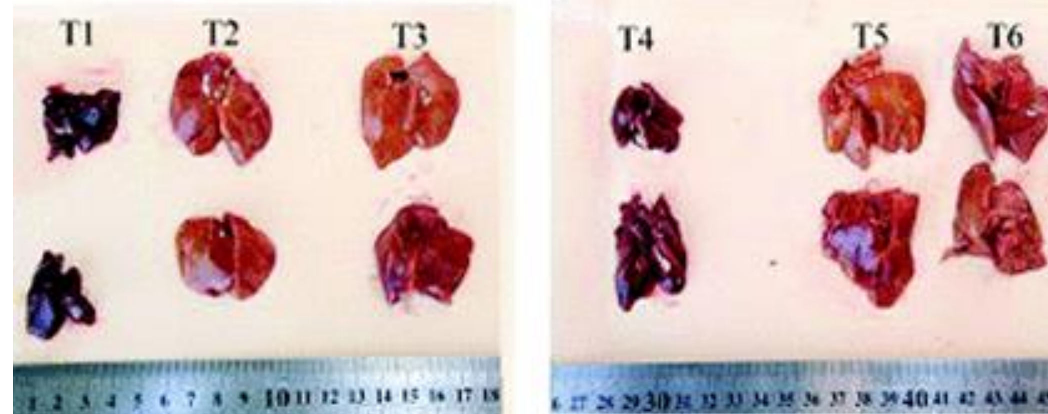
Yunus *et al.* (2011b) conducted a trial to study some morphological, digestive, and electrophysiological variables of the small intestine during chronic exposure of broilers to aflatoxin B(1) (AFB(1)). Ross 308 male chicks (7 d old) were randomly allotted to control (no AFB(1)), low AFB(1) (0.07 mg of AFB(1)/kg), or high AFB(1) (0.75 mg of AFB(1)/kg) diet. The high AFB(1) diet resulted in reduced ($P \leq 0.002$) bird performance during the first 4 wk of exposure, whereas the low AFB(1) diet temporarily reduced ($P = 0.034$) the bird performance during wk 3 of exposure. During wk 4 of exposure, a linear ($P \leq 0.013$) decrease in the unit weight of both the duodenum and jejunum was observed with increasing levels of AFB(1). This reduction in unit weight appeared to progress from the proximal (duodenum) to the distal (jejunum) small intestine with increase in the length of exposure and was not accompanied by modulation of electrophysiological variables in jejunal epithelium. Response from amiloride, a specific blocker of epithelial sodium channel, was also similar among jejunal epithelia of birds under different treatments. Interestingly, a compensatory linear ($P \leq 0.002$) increase in the length of the duodenum and jejunum under high AFB(1) diets was noted to occur during wk 4 of exposure. Thus, retention of DM and nitrogen was not negatively affected by the AFB(1) diets. These data

indicate that the intestine in broilers may adapt to an ongoing dietary challenge to AFB(1).

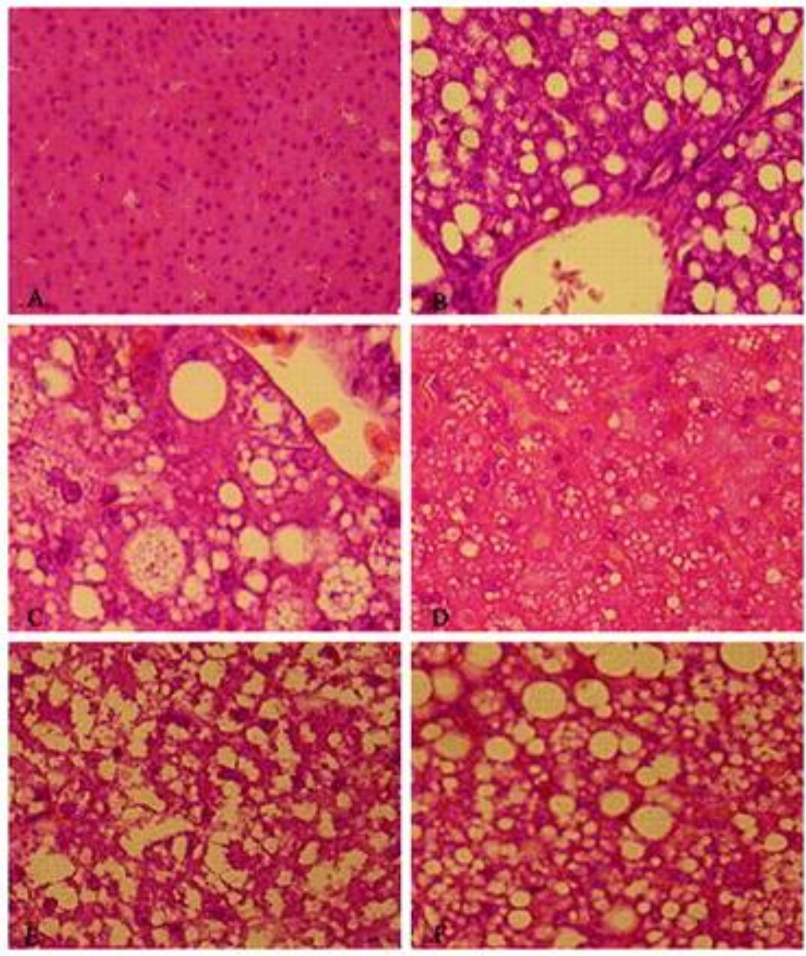
Kasmani *et al.* (2012) performed 2 experiments to screen bacilli isolated from quails for their aflatoxin removal potential and to assess the efficiency of their amelioration of experimental aflatoxicosis. Nonhemolytic bacilli were selected for in vitro aflatoxin B1 (AFB1) removal and conventional probiotic tests. The isolate with the highest scores was selected for assessment in field experiments and was identified as Berevibacillus laterosporus (Bl). In the second experiment, 125 male Japanese quails (21 d old) were divided into 5 groups with 5 replications to compare the toxin removal efficiency of Bl with that of a commercial toxin binder, improved Millbond-TX (IMTX). The experimental groups were as follows: Control (without any feed additive or AFB1); AFB1 (2.5 mg/kg); AFB1+Bl (2.5 mg/kg+10(8) cfu/mL); AFB1+IMTX (2.5 mg/kg+2.5 g/kg); and Bl (10(8) cfu/mL). The greatest BW gain and slaughter and carcass weights were found in the Bl group and the lowest values were observed in the AFB1 group ($P<0.05$). Feeding AFB1 alone to the chicks resulted in a significant decrease in serum albumin, total protein, and glucose and cholesterol levels but a significant increase in serum uric acid, urea, creatinin and phosphorus ($P<0.05$). Treatment of birds on AFB1 with Bl restored these to their original levels ($P<0.05$). AFB1+Bl-fedbirds had serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline phosphatase enzyme activity similar to control birds ($P<0.05$). Antibody titer against Newcastle disease virus was found to be lowest in the AFB1 group but highest in the Bl group ($P<0.05$). Antibody production against sheep red blood cells was lower in the AFB1 group compared with the AFB1+Bl group ($P<0.05$). Berevibacillus laterosporus supplementation of the AFB1 diet restored the skin response to 2,4-dinitro 1-chlorobenzene to levels comparable with control birds($P<0.05$). It can be concluded that selected indigenous Bl is a promising probiotic with AFB1 removal potential.

Magnoli *et al.* (2012) conducted a study to evaluate the effects of feed contamination with AFB(1) in combination with **corticosteron**e treatment in drinking water (a model to induce physiological stress in birds) on **selected performance indices**: BW, feed conversion, egg production, and macroscopic and microscopic liver alterations. At 5 wk of age, **quails** were randomly assigned to 1 of 6 dietary treatment groups that resulted from the combination of the presence or absence of corticosterone in drinking water (5 mg/L) with the presence or absence of AFB(1) contamination (0, 100, or 500 µg/kg). The quails remained in these treatments from 5 to 11 wk of age. There were 6 replicates per treatment, each containing 2 males and 2 females. Contamination with 100 µg of AFB(1) per kilogram of feed induced no changes in BW, feed conversion, and egg production parameters. Quail fed with 500 µg of AFB(1) per kilogram of feed showed significant decreases in BW and feed consumption compared with their control counterparts. Corticosterone in combination with 500 µg of AFB(1) per kilogram of feed intensified the negative effects observed on BW and feed consumption and also had negative effects on feed conversion rate and egg production parameters, suggesting that the adverse effects of contamination with AFB(1) are intensified in situations of chronic stress. Quail treated with 500 µg of AFB(1) per kilogram showed hepatocytes with degree 1 and 2 lesions, and all quail treated with 500 µg of AFB(1) per kilogram of feed in combination with corticosterone showed degree 2 liver lesions (i.e., hepatocytes with fatty macro and microvacuoles and necrosis). This result is also consistent with the hypothesis that chronic stress exacerbates the effect of AFB(1) contamination. In conclusion, this study suggests

that the negative effects of AFB(1) contamination are increased when overlapped with chronic stressful stimulation.



Representative livers from birds (77 d old) fed different treatments. T1 = control; T2 = aflatoxin B_1 (AFB_1; 100 μg/kg); T3 = AFB_1 (500 μg/kg); T4 = corticosterone (CORT); T5 = CORT + AFB_1 (100 μg/kg); and T6 = CORT + AFB_1 (500 μg/kg **Magnoli *et al.* (2012)**



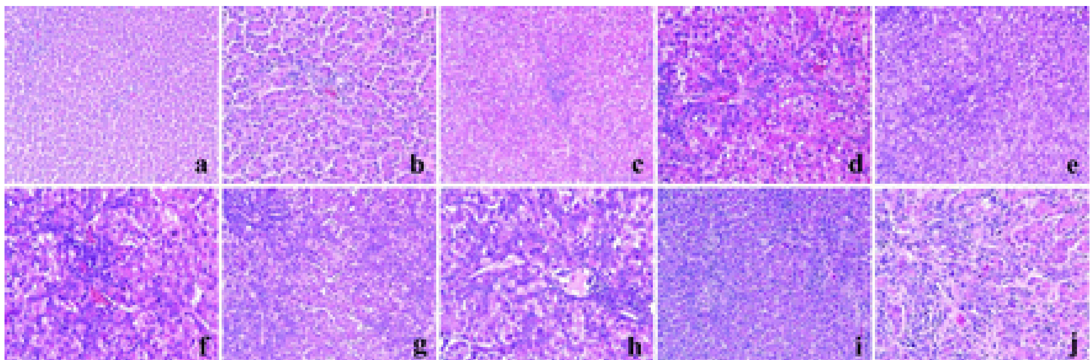
Photomicrographs of hematoxylin and eosin-stained broiler liver sections from different treatments. A) T1 = control; B) T2 = aflatoxin B_1 (AFB_1; 100 μg/kg); C) T3 = AFB_1 (500 μg/kg); D) T4 = corticosterone (CORT); E) T5 = CORT + AFB_1 (100 μg/kg); and F) T6 = CORT + AFB_1 (500 μg/kg). **Magnoli *et al.* (2012)**

Nazar ***et al.*** **(2012)** evaluated the potential effects of the combined administration of aflatoxin B(1) (AFB(1)) and **corticosterone** on **biochemica**l (concentration of globulins, proteins, and albumin) and *immunological* (inflammatory response and heterophil:lymphocyte ratio) parameters of **Japanese quail**. Potential sex effects on those parameters were also considered. The provision of corticosterone in drinking water is a method used for mimicking the effects of chronic stress inavian species. At 35 d of age, 24 mixed-sex groups of 4 animals (2 males and 2 females) were housed in cages and assigned to 1 of 4 treatments: plain drinking water and laying diet, corticosterone administration in drinking water, feed contamination with AFB(1) (100 μg/kg of feed), or corticosterone plus AFB(1) administration. There were 6 cages per treatment. No significant effect of sex in any of the parameters analyzed was detected. Hypoproteinemia, hypoalbuminemia, and hypoglobulinemia were observed in animals treated with corticosterone or contaminated feed. These responses were exacerbated when the factors were combined. The immunodepressive effect of corticosterone administration was confirmed, and a higher effect was noticed when combined with the aflatoxin contamination. Aflatoxin contamination affected birds' physiology similar to a chronic stressor stimulation because it elevates the heterophil:lymphocyte ratio. This study suggests that the effects of the AFB(1) contamination are further increased when overlapped with a chronic stressful stimulation and emphasizes the importance of controlling potential stressor combinations during animal rearing to preserve not only the animal's health status but also their welfare.

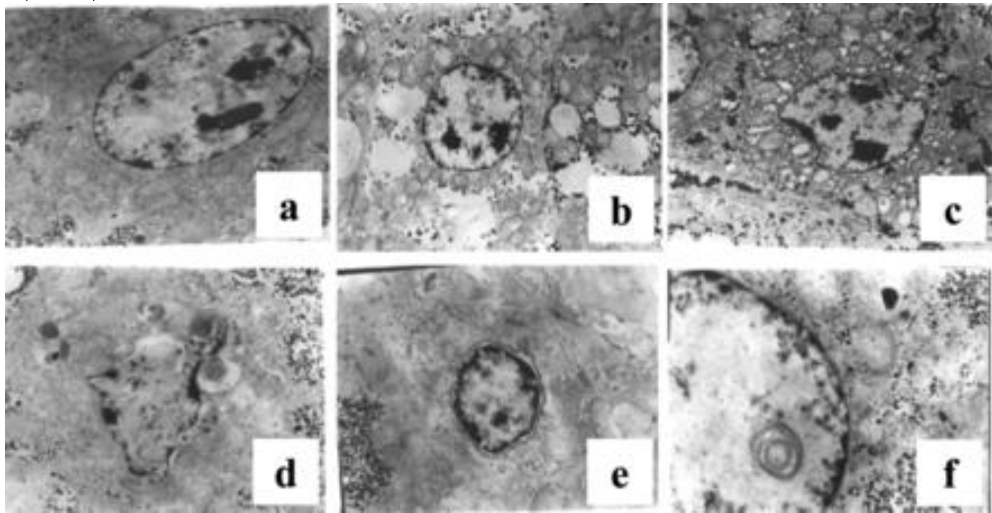
Pizzolitto ***et al.*** **(2012)** evaluated the aflatoxin B_1 (AFB_1) removal capacity, the tolerance to salivary and gastrointestinal conditions, autoaggregation and coaggregation with **Saccharomyces cerevisiae** strains isolated from broiler feces.. Only four of twelve isolated strains were identified as Saccharomyces cerevisiae using molecular techniques. The results obtained in AFB_1 binding studies indicated that the amount of AFB_1 removed was both strain and mycotoxin-concentration dependent. Therefore, a theoretical model was applied in order to select the most efficient strain to remove AFB_1 in a wide range of mycotoxin concentration. The results indicated that S. cerevisiae 08 and S. cerevisiae 01 strains were the most efficient microorganisms in the mycotoxin removal. Viability on simulated salivary and gastrointestinal conditions was investigated and S. cerevisiae 08 strain showed the best results, achieving 98% of total survival whereas S. cerevisiae 01 reached only 75%. Autoaggregation and coaggregation assays showed S. cerevisiae 08 as the most appropriate strain, mainly because it was the unique strain able to coaggregate with the four bacterial pathogens assayed. Consequently, S. cerevisiae 08 is the best candidate for future in vivo studies useful to prevent aflatoxicosis. Further quantitative in vitro and in vivo studies are required to evaluate the real impact of yeast-binding activity on the bioavailability of AFB_1 in poultry. However, this study could be useful in selecting efficient strains in terms of AFB_1 binding and provide an important contribution to research into microorganisms with potential probiotic effects on the host.

Yang ***et al.*** **(2012)** carried out a study to evaluate the effects of feeding corn naturally contaminated with aflatoxin B(1) (AFB(1)) and aflatoxin B(2) (AFB(2)) on serum biochemical parameters, hepatic antioxidant enzyme activities, and pathological lesions of broilers. In total, 1,200 Cobb male broilers were randomly allocated into 5 treatments, with 8 replicates per treatment and 30 birds per replicate, in a 42-d experiment. The dietary treatments were as follows: control, 25, 50, 75, and 100%

contaminated corn groups. Results showed that serum aspartate aminotransferase activity in the 75 and 100% contaminated groups were higher than that in the control group on d 21 ($P < 0.05$). Decreased content of hepatic total protein and increased activities of hepatic glutathione reductase and glutathione-S-transferase were observed as the percentage of contaminated corn increased ($P < 0.05$). The activity of superoxide dismutase and the content of hepatic malondialdehyde increased when the broilers were fed with more than 50% contaminated corn ($P < 0.05$). A reduction in glutathione peroxidase level was observed in the AFB(1)- and AFB(2)-contaminated groups on d 21 ($P < 0.05$). The average pathological lesion scores and apoptosis rate of liver cells increased as the concentration of dietary AFB(1) and AFB(2) increased. Ultrastructural changes were found in the livers of broilers fed 100% contaminated corn. In conclusion, diets containing AFB(1) and AFB(2) could induce pathological lesions in the livers, slightly change the serum biochemical parameters, and damage the hepatic antioxidant functions when the inclusion of AFB(1)- and AFB(2)-contaminated corn reached or exceeded 50%.



Morphology of chicken livers from the control and aflatoxin B_1-contaminated groups on d 21 and d 42. (a) Control (0 points), hematoxylin and eosin (H.E) 200×; (b) control, cytoplasm is homogeneous and less connective tissue is present in the portal areas (0 points), H.E 400×; (c) slight hyperplastic bile duct epithelium is present in some portal areas (1 points), H.E 200×; (d) bile duct epithelia with funicular hyperplasia are present in the portal areas (1 points), H.E 400×; (e) hyperplastic bile duct epithelia are observed in the portal areas, and the regions are approximately half of hepatic lobule (2 points), H.E 200×; (f) bile duct epithelia with funicular hyperplasia are present (2 points), H.E 400×; (g) hyperplasia of the bile duct epithelium is involved in the whole hepatic lobule (3 points), H.E 200×; (h) hyperplastic bile duct epithelia are involved in major areas (3 points), H.E 400×; (i) bile duct epithelium with diffuse hyperplasia is present and hepatic tissues are damaged (4 points), H.E 200×; (j) massive hyperplasia of the bile duct epithelium is observed in portal areas (4 points), H.E 400×. **Yang *et al.* (2012)**



Ultrastructural changes of hepatocytes from broilers that were fed a control diet or a 100% aflatoxin B_1-contaminated diet for 42 d. (a) Control, with a normal nucleus and organelles; (b–f) 100% aflatoxin B_1-contaminated group; (b) fatty degeneration, lipid droplets with variable sizes appeared in the cytoplasm (6,000×); (c) swelling of the endoplasmic reticulum, irregular nuclei (8,000×); (d) many more secondary lysosomes are present in cytoplasm (10,000×); (e) swelling of nuclear membrane (12,000×); (f) circular chromatin (17,000×) **Yang *et al.* (2012)**

Abidin *et al.* (2013) determined the levels of aflatoxin B1 (AFB1) in the poultry finished feed samples collected from different poultry farms and local markets of Lahore, Pakistan. This study was conducted from July 2009 to June 2012 with each year divided into three periods i.e. July-October (hot and humid), November-February (winter) and March-June (moderate). During each period 80 samples were analyzed by competitive direct-Enzyme Linked Immuno-sorbent assay (CD-ELISA) constituting a total of 720 samples throughout the study. The levels of AFB1 in poultry feed samples were highest during rainy seasons (48.2±20.0, 51.6±22.6 and 46.0±19.8 µg/kg) followed by Mar-Jun (29.9±10.4, 27.2±9.72 and 28.8±13.1 µg/kg) and Nov-Feb (19.7±6.30, 16.3±6.76 and 17.1±6.20 µg/kg). The levels were below maximum tolerable levels (MTL) for poultry as recommended by US-Food and Drug Administration (FDA) i.e. 20µg/kg during winter seasons only. The highest level during this study was 119.2µg/kg in Jul-Oct (2010-11). Percentage of samples below MTL was minimum during rainy season and at the peak during winter season confirming a high production of AFB1 in stored feed during rainyseason compared to other seasons. Poultry feed becomes highly contaminated with AFB1 during rainy season due to high humidity and hot atmosphere which gives best favorable conditions for the growth of different storage fungi. This is the first most extensive study of levels of AFB1 from poultry finished feed samples collected from different areas of Lahore (Pakistan).

Fan *et al.* (2013) conducted a study to investigate the toxic effects of aflatoxins and the efficacy of ***Bacillus subtilis*** ANSB060 for the amelioration of aflatoxicosis in broiler chickens. Six replicates of ten **broilers** each were assigned to one of seven dietary treatments, which were labeled C0 (basal diet); M0 (basal diet containing moldy peanut meal); C500 and C1000 (C0+500 or 1000 g/t aflatoxin biodegradation preparations, composed mainly of ANSB060); and M500, M1000 and M2000 (M0+500, 1000 or 2000 g/t aflatoxin biodegradation preparations). The concentrations of aflatoxin B_1, B_2, G_1 and G_2 in the moldy diets (M0, M500, M100 and M2000) fluctuated around 70.7±1.3, 11.0±1.5, 6.5±0.8 and 2.0±0.3 µg/kg, respectively. The results showed that the M0 diet caused a significant decrease in average daily weight gain and increased feed requirements, with a gain ratio increasing from d 8 to 42, deterioration in meat quality and aflatoxin residues in broilers' livers as compared with the C0 diet. The addition of ANSB060 to the aflatoxin-contaminated diets offset these negative effects, leading to the conclusion that ANSB060 has a protective effect on growth performance and meat quality while reducing the amount of aflatoxin residues in the livers of broilers fed naturally moldy peanut meal.

Gholami-Ahangaran and Zia-Jahromi (2013) evaluated the effect of one **commercial nanocompound, Nanocid** (Nano Nasb Pars Co., Iran) in reduction of aflatoxin effects on the **growth and performance** indices in broiler chickens suffering from experimentalaflatoxicosis. For this, a total of 300 one-day-old broiler

chicks (Ross strain) were randomly divided into 4 groups with 3 replicates of 15 chicks in each separated pen during the 28-day experiment. Treatment groups including group A: chickens fed basal diet, group B: chickens fed 3 ppm productive aflatoxin in basal diet, group C: chickens fed basal diet plus 2500 ppm Nanocid, and group D: chickens fed 3 ppm productive aflatoxin and 2500 ppm Nanocid, in basal diet. Data on body weight, body weight gain (BWG), feed intake, and feed conversion ratio (FCR) were recorded at weekly intervals. Also cumulative data were assessed. Results showed, although supplement of Nanocid to conventional diet had no effect on performance but addition of Nanocid to diet containing 3 ppm aflatoxin increased significantly the cumulative BWG, cumulative feed consumption and decreased FCR in the last 2 weeks of experimental period. The improvement in these performance indices by supplement of Nanocid to diet containing aflatoxin showed the ability of Nanocid to diminish the inhibitory effects of aflatoxin.

Marchioro *et al.* (2013) evaluated, on a weekly basis, the effects of aflatoxins on the activity of digestive enzymes (alpha-amylase, lipase, and trypsin) in the pancreas as well as on the performance and histology of pancreas in broiler chickens over the course of 42 days. One thousand and eighty 1-day-old male Cobb broilers were divided into four treatments with 18 replicates and 15 birds per replicate (i.e., 270 broilersper treatment). Treatments were established according to the amount of aflatoxins added to the diet, as follows: T1 = 0 mg of aflatoxins per kilogram of feed (mg/kg); T2 = 0.7 mg/kg; T3 = 1.7 mg/kg; and T4 = 2.8 mg/kg. Pancreas sample collection was performed from one bird out of each replicate at 7, 14, 21, 28, 35, and 42 days of experiment, which yielded a total of 18 samples per treatment on each collection. Each sample was homogenized in distilled water, frozen in liquid nitrogen, lyophilized, and stored at -20 C until analysis. Performance parameters (body weight, feed consumption, and feed conversion rate) were measured at 21, 35, and 42 days of experiment. At the end of the experiment (42 days), six birds from each treatment were randomly chosen for histologic evaluation of the pancreas. The presence of aflatoxins in the diet induced a negative effect on all performance parameters. The pancreatic activity of lipase and alpha-amylase were significantly increased in treatments T3 and T4, while the specific activity of trypsin was only affected during treatment T4. In addition, several histologic changes were observed in the pancreas of birds receiving aflatoxin-contaminated feed. Aflatoxins present in the feed determined an increase in the activity of pancreatic enzymes in broilers, affecting the digestibility of the diet, thereby leading to losses in performance and productivity

Neeff *et al.* (2013) performed a study to determine the binding capacity of a **hydrated sodium calcium aluminosilicate (HSCAS)** for aflatoxin B(1) (AFB(1)), and the efficacy of the HSCAS to reduce the concentrations of residual AFB(1) and its metabolites in the liver and kidney of **broilers** fed AFB(1). One hundred 1-d-old male broilers (Ross 708) were maintained in chick batteries and allowed ad libitum access to feed and water. A completely randomized design was used with 5 replicate pens of 5 chicks assigned to each of 4 dietary treatments from hatch to 21 d. Dietary treatments included the following: A) basal diet (BD), with no HSCAS or AFB(1), B) BD supplemented with 0.5% HSCAS only, C) BD supplemented with 2.5 mg of AFB(1)/kg of feed, and D) BD supplemented with 2.5 mg of AFB(1)/kg of feed and 0.5% HSCAS. On d 21, 5 chicks from each treatment were anesthetized with carbon dioxide, killed by cervical dislocation, and samples of liver and kidney were collected for analysis of AFB(1) residues. The percentage of AFB(1) bound for each concentration of adsorbent (100, 10, 1, 0.5, 0.25, and 0.05 mg/10 mL) was 100, 91.1,

81.8, 75.4, 40.1, and 8.8%, respectively. Concentrations of aflatoxin residues (AFB(1), aflatoxicol, aflatoxins B(2) and G(1)) were lower ($P < 0.05$) in livers and kidneys of birds fed AFB(1) plus HSCAS (diet D), when compared with birds fed AFB(1) alone (diet C). However, histopathology data from the in vivo study indicated that HSCAS did not prevent lesions associated with aflatoxicosis. The decrease in the bioavailability of AFB(1) caused by the HSCAS reduced aflatoxin residues in liver and kidney, but not enough to completely prevent the toxic effects of AFB(1) in broilers.

Pizzolitto *et al.* (2013) performed a study to evaluate the ability of ***S. cerevisiae*** CECT 1891 in counteracting the deleterious effects of AFB1 in broiler chicks. Experimental aflatoxicosis was induced in 6-d-old **broilers** by feeding them 1.2 mg of AFB1/kg of feed for 3 wk, and the yeast strain was administrated in feed (10(10) cells/kg), in the drinking water (5 × 10(9) cells/L), or a combination of both treatments. A total of 160 chicks were randomly divided into 8 treatments (4 repetitions per treatment). Growth performance was measured weekly from d 7 to 28, and serum biochemical parameters, weights, and histopathological examination of livers were determined at d 28. The AFB1 significantly decreased the BW gain, feed intake, and impaired feed conversion rate. Moreover, AFB1 treatment decreased serum protein concentration and increased liver damage. The addition of S. cerevisiae strain to drinking water, to diets contaminated with AFB1, showed a positive protection effect on the relative weight of the liver, histopathology, and biochemical parameters. Furthermore, dietary addition of the yeast strain to drinking water alleviated the negative effects of AFB1 on growth performance parameters. In conclusion, this study suggests that in feed contaminated with AFB1, the use of S. cerevisiae is an alternative method to reduce the adverse effects of aflatoxicosis. Thus, apart from its excellent nutritional value, yeast can also be used as a mycotoxin adsorbent.

Pourelmi (2013) tested aflatoxin (AF) (0.5ppm) in an in vivo study forming 2 dietary treatments each with three replicates on a total of 336 on broiler chicks up to six weeks. Results showed that chicks receiving AF contaminated feed had suppressed body weight and improved feed consumption. The serum antibody titers against ND and IBD vaccination were significantly depressed by AF. The serum concentration of total protein, uric acid and albumin were not affected in AF fed supplemented group. The activity of serum GGT significantly increased in AF fed group. Compared with control, activity of serum ALT was not affected in AF or control supplemented groups.

Siloto *et al.* (2013) conducted an experiment to determine the effects of AF (1 mg/kg of feed) and FU (25 mg/kg of feed), singly or in combination, on the lipid metabolism in commercial layers and investigate the efficacy of a **commercial binder** (2 kg/t of feed) on reducing the toxic effects of these mycotoxins. A total of 168 Hisex Brown **layer hens**, 37 wk of age, were randomized into a 3 × 2 + 1 factorial arrangement (3 diets with no binder containing AF, FU, and AF+FU; 3 diets with binder containing AF, FU, and AF+FU; and a control diet with no mycotoxins and binders), totaling 7 treatments. The hens contaminated with AF showed the characteristic effects of aflatoxicosis, such as a yellow liver, resulting from the accumulation of liver fat, lower values of plasma very low-density lipoprotein and triglycerides, and higher relative weight of the kidneys and liver. Hepatotoxic and nephrotoxic effects of FU were not observed in this study. On the other hand, the FU caused a reduction in small intestine length and an increase in abdominal fat deposition. The glucan-based binder

prevented some of the deleterious effects of these mycotoxins, particularly the effects of AF on hepatic lipid metabolism, kidney relative weight, and FU in the small intestine.

Chen *et al.* (2014) carried out a study to evaluate the efficiency of a **hydrated sodium calcium aluminosilicate (HSCAS) adsorb**ent to ameliorate the adverse effects of 0.5 to 2 mg of aflatoxin B1 (AFB1)/kg in **broiler chicks**. The study consisted of 8 dietary treatments, including 4 concentrations of AFB1 (0, 0.5, 1, and 2 mg/kg) with or without HSCAS (0.5%) fed to 8 replicate cages per diet (6 males chicks per cage) from 0 to 21 d of age. Cumulative feed intake, BW gain ($P < 0.0001$), and G:F ($P = 0.004$) of birds fed the 2 mg of AFB1/kg of diet were significantly lower in comparison with birds fed 0 to 1 mg of AFB1/kg. Relative liver weight was increased in the 2 mg of AFB1/kg group ($P < 0.0001$). Dietary HSCAS improved cumulative BW gain (main effect $P = 0.06$), particularly from 14 to 21 d of age ($P = 0.037$). Dietary HSCAS also reversed the increase in relative liver weight for birds fed AFB1 ($P = 0.019$). Dietary AFB1 negatively affected major serum parameters (albumin, total protein, globulin, phosphorus, glucose, alkaline phosphatase, and creatine phosphokinase), whereas supplementation with HSCAS partially alleviated the affected serum biochemistry. In addition, serum complement activity and liver gene expression were negatively affected by 2 mg of AFB1/kg. The HSCAS supplement increased the liver expression of catalase and superoxide dismutase ($P < 0.05$). Results from this study indicated that dietary supplementation with HSCAS can effectively improve BW gain and partially ameliorate aflatoxicosis for broiler chicks fed AFB1-contaminated feeds.

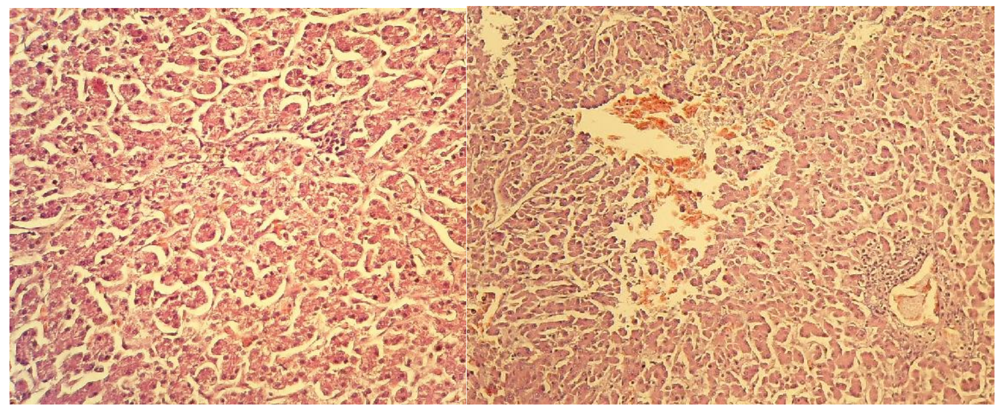
Gholami-Ahangaran and Zia-Jahromi (2014) designed an experiment to investigate the positive effects of **commercial nanosilver** compound on blood parameters in experimentalaflatoxicosis in **broiler chickens**. For this, 270 one-day-old broiler chickens were randomly divided into six treatment groups with three replicates. The experimental groups were group A: chickens fed with basal diet; group B: chickens fed with 3 ppm productive aflatoxin in basal diet; groups of C, D, E and F received Mycoad (2.5 g/kg diet), Mycoad (2.5 g/kg diet) + productive aflatoxin (3 ppm), Nanocid (2500 ppm), and Nanocid (2500 ppm) + productive aflatoxin (3 ppm) in basal diet, respectively. Results revealed that some of the blood parameters such as mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration lymphocytes, neutrophils, basophils, monocytes, and eosinophils percentage were not affected in this experiment; whereas, hemoglobin percentage and white blood cell (WBC) count in all the groups fed with 3 ppm aflatoxin except nanocid + aflatoxin decreased significantly ($p < 0.05$). There are no significant differences between the groups that received nanocid + aflatoxin and mycoad + aflatoxin in hemoglobin percentage and WBC count parameters. The red blood cell count and hematocrit in chickens received aflatoxin were significantly lower than other groups ($p < 0.05$). Therefore, this study suggests that nanocid similar as mycoad can be useful in reducing the adverse effects of aflatoxin on blood parameters in chickens affected with aflatoxicosis

Magnoli *et al.* (2014) carried out a study to determine if the competitive adsorption of **tryptophan** (Trp) and aflatoxin B_1 (AFB_1) could potentially affect the ability of a **sodium bentonite (NaB)** to prevent aflatoxicosis in monogastric animals. The adsorption of Trp and AFB_1 on this adsorbent is fast and could be operating on the same time-scale making competition feasible. In vitro competitive adsorption experiments under simulated gastrointestinal conditions were performed. A high affinity of the clay for Trp and NaB was observed. The effect of an excess of KCl to mimic the ionic strength of the physiological conditions were also investigated. A six-times decrease in the Trp surface excess at saturation was observed. A similar behaviour was previously found for AFB_1 adsorption. Taking into account the amount of Trp adsorbed by the clay and the usual adsorbent supplementation level in diets, a decrease in Trp bioavailability is not expected to occur. Tryptophan adsorption isotherms on NaB were 'S'-shaped and were adjusted by the Frumkin-Fowler-Guggenheim model. The reversibility of the adsorption processes was investigated in order to check a potential decrease in the ability of NaB to protect birds against chronic aflatoxicoses. Adsorption processes were completely reversible for Trp, while almost irreversible for AFB_1. In spite of the high affinity of the NaB for Trp, probably due to the reversible character of Trp adsorption, no changes in the AFB_1 adsorption isotherm were observed when an excess of the amino acid was added to the adsorption medium. As a consequence of the preferential and irreversible AFB_1 adsorption and the reversible weak binding of Trp to the NaB, no changes in the aflatoxin sorption ability of the clay are expected to occur in the gastrointestinal tract of birds.

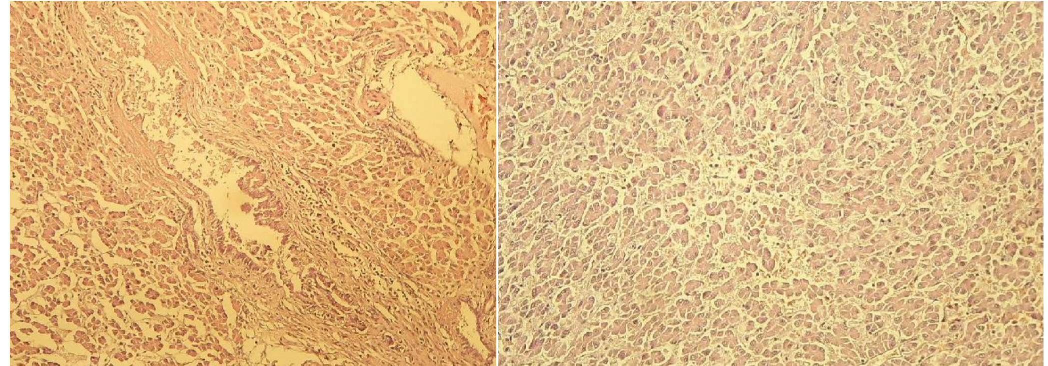
Rawal *et al.* (2014) conducted a study to determine whether **probiotic Lactobacillus,** shown to be protective in animal and clinical studies, would likewise confer protection in turkeys, which were treated for 11 days with either AFB1 (AFB; 1 ppm in diet), probiotic (PB; 1 × 10(11) CFU/ml; oral, daily), probiotic + AFB1 (PBAFB), or PBS control (CNTL). The AFB1 induced drop in body and liver weights were restored to normal in CNTL and PBAFB groups. Hepatotoxicity markers were not significantly reduced by probiotic treatment. Major histocompatibility complex (MHC) genes BG1 and BG4, which are differentially expressed in liver and spleens, were not significantly affected by treatments. These data indicate modest protection, but the relatively high dietary AFB1 treatment, and the extreme sensitivity of this species may reveal limits of probiotic-based protection strategies.

Valchev *et al.* (2014) carried out an experiment to evaluate the toxic effects of AFB1 through follow-up of changes in blood activities of **aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (γGT), lactate dehydrogenase (LDH), alkaline phosphatase (AP) and liver morphology**. Also, the possibility for effective alleviation or prevention of toxic effects of AFB1 by feed supplementation with the **mycosorbent Mycotox** NG was evaluated. The experiments were conducted with 50 7-day-old Cobb broiler chickens allotted to one control and 4 experimental groups. The chickens were orally treated with 1 g/kg Mycotox NG, 0.5 mg/kg AFB1, 0.8 mg/kg AFB1 и 0.5 mg/kg AFB1 + 1 g/kg Mycotox NG over 42 days. Blood samples for analysis were collected on days 21 and 42. Blood chemistry revealed that the groups receiving only AFB1 showed increased activities of studied enzymes and total bilirubin concentrations. Total protein,

albumin, cholesterol, triglycerides and blood glucose were lower than respective control values. Histopathological changes consisted in various degree of dystrophy depending on the amount of ingested toxin. The addition of mycosorbent to the feed of group V reduced partially the deleterious impact of AFB1 as could be seen from blood biochemical changes and the lower frequency and severity of liver lesions.



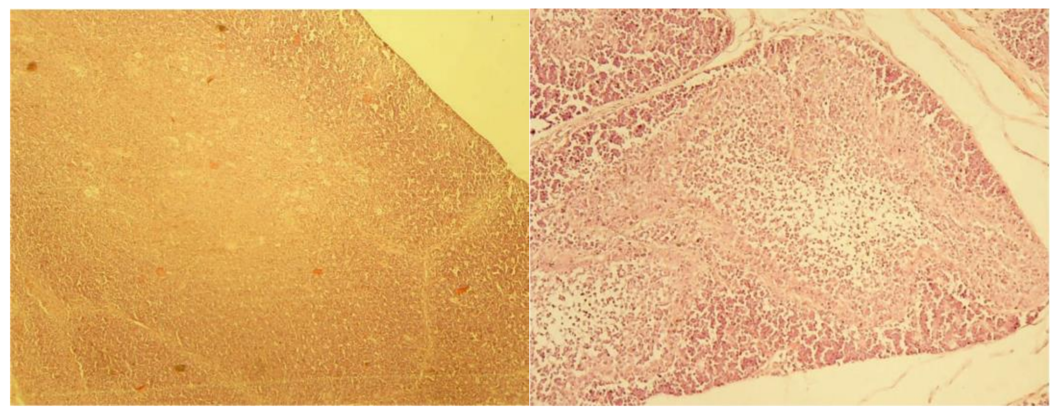
Liver of a chicken treated with 0.5 mg/kg AFB1. Granular and fatty dystrophy of the cytoplasm of hepatocytes. H/E, bar=20 µm. Liver of a chicken treated with 0.8 mg/kg AFB1. Hepatocellular necroses and pericapillary proliferations in the parenchyma of the organ. H/E, bar=20 µm. **Valchev *et al.* (2014a)**



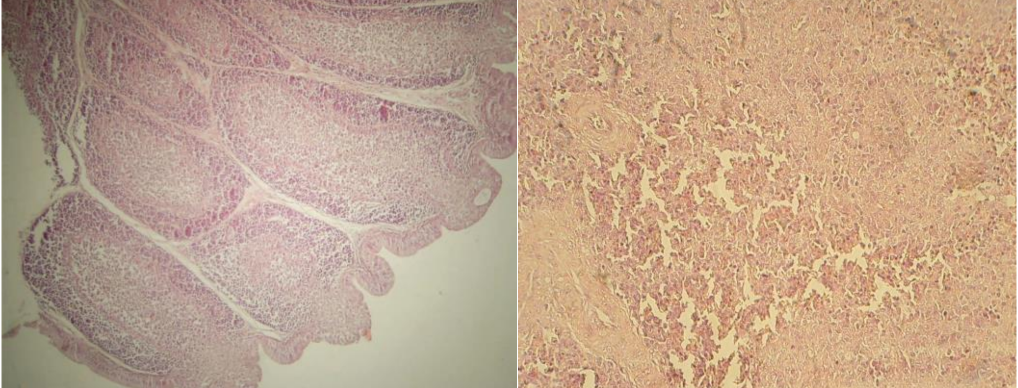
Liver of a chicken treated with 0.8 mg/kg AFB1. Bile duct hyperplasia. H/E, bar=20 µm. Liver of a chicken treated with 0.5 mg/kg AFB1 and Mycotox NG. Hyperaemia and activation of the capillary endothelium. H/E, bar=20 µm. **Valchev *et al.* (2014a)**

Valchev *et al.* (2014b) performed an experiment to investigate the toxic effects of aflatoxin B (AFB) on **production traits (weight gain, feed intake and feed** 1 1 conversion), antibody titre (antihaemagglutinins) after vaccination against Newcastle disease, relative weights of immunocompetent organs (thymus, spleen, bursa of Fabricius) and changes in their morphology. Also, the study aimed at testing the possibility for prevention of toxic effects of AFB by supplementation of poultry feed with the **mycosorbent (Mycotox NG**). The experiments were conducted with five groups of ten 7-day-old Cobb broiler chickens in each. The formed groups were: group I – control, fed a standard compound feed; group II – experimental, whose feed was supplemented with 1 g/kg Mycotox NG, group III – experimental, receiving 0.5 mg/kg AFB ; group IV – experimental, receiving 0.8 mg/kg AFB and group V – experimental, supplemented with 0.5 mg/kg AFB 1 1 1 and 1 g/kg Mycotox NG. The experiment's duration was 42 days. Production traits were evaluated on days 21, 35 and 49 days of age. The differences in relative weights of immune organs between control and experimental groups were determined after the end of the trial. Antibody titres after vaccination against Newcastle disease were determined in blood samples

collected from v. metatarsalis medialis on days 21 and 42. Lower weight gain, feed intake, increased feed conversion ratio and relative spleen weight were established in experimental groups III and IV. At the same time, relative weights of the thymus and bursa of Fabricius were statistically significantly lower than those in controls. Lower antibody titres were demonstrated in groups III and IV vs untreated birds. Atrophy and degenerative changes were observed in immunocompetent organs of birds from groups III and IV. The supplementation of the feed with 1 g/kg MycotoxNG resulted in statistically significant reduction of the deleterious effects of AFB on production traits, relative weights of immunocompetent organs and histological 1 changes. Also, dietary supplementation of group V with mycosorbent protected birds against the inhibiting effect of AFB on antibody formation against 1 Newcastle disease.



Dystrophy and reduction of cells in the thymic cortex in a chicken from group IV. H/E. Bar 20 ,µm, Dystrophic changes and cell rarefaction in lymph follicles of the bursa of Fabricius in a chicken from group V. H/E. Bar 20 µm **Valchev *et al.* (2014b)**



Strong reduction of cells in the central part of lymph follicles and interfollicular swelling of the bursa of Fabricius in a chicken from group V. H/E. Bar 15 µm, Cell dystrophy – karyolysis and karyopyknosis in the spleen of the chicken from group V. H/E. Bar 20 µm **Valchev *et al.* (2014b)**

Bovo *et al.* (2015) carried out a study to determine the aflatoxin B1 (AFB1) binding capacity of **a beer fermentation residue (BFR) containing Saccharomyces cerevisiae** cells, and the efficacy of BFR to ameliorate the toxic effects of AFB1 on **performance**, serum biochemistry, and histology of broilers. The BFR was collected from a microbrewery, and the yeast cells were counted, dried, and milled before it was used in the study. In vitro evaluation of the BFR was conducted using different concentrations of AFB1 (2.0, 4.0, 8.0, 16.0, and 32.0 µg AFB1/mL) and 100 mg/10 mL of BFR at pH 3.0 or 6.0. Two hundred 1-day-old male broilers (Ross 308) were assigned to chick batteries and allowed ad libitum access to feed and water. A completely randomized design was used with 5 replicate pens of 5 chicks assigned

to each of 4 dietary treatments from hatch to 21 d, which included: 1) basal diet (BD), with no BFR or AFB1; 2) BD supplemented with 1% BFR; 3) BD supplemented with 2 mg AFB1/kg of feed; and 4) BD supplemented with 2 mg AFB1/kg feed and 1% BFR. Performance variables were determined weekly, while serum analyses were performed on d 14 and 21. At the end of the study, chicks were anesthetized with carbon dioxide, euthanized by cervical dislocation, and the kidney, liver, and bursa of Fabricius were removed for determination of relative weights, and for histological evaluation. In vitro assays showed that the higher the initial AFB1 concentration in solution, the greater the AFB1 amount adsorbed by BFR at both pHs tested. Feed intake, BW gain, and concentrations of albumin, total protein, and globulin increased ($P < 0.05$) in broilers fed BFR+AFB1 (Diet 4), when compared to the birds receiving only AFB1 (Diet 2). Although BFR was not able to reduce or prevent the effects of AFB1 on relative weights of kidneys and liver, it reduced the severity of histological changes in the liver and kidney caused by AFB1.

Fowler *et al.* (2015) evaluated, growth and relative organ weights along with a **residue analysis for aflatoxin B_1 in liver tissue** collected from broiler chickens consuming dietary aflatoxin (0, 600, 1200, and 1800 µg/kg) both with and without 0.2% of a **calcium bentonite clay additive** (TX4). After one week, only the combined measure of a broiler productivity index was significantly affected by 1800 µg/kg aflatoxin. However, once birds had consumed treatment diets for two weeks, body weights and relative kidney weights were affected by the lowest concentration. Then, during the third week, body weights, feed conversion, and the productivity index were affected by the 600 µg/kg level. Results also showed that 0.2% TX4 was effective at reducing the accumulation of aflatoxin B_1 residues in the liver and improving livability in birds fed aflatoxin. The time required to clear all residues from the liver was less than one week. With evidence that the liver's ability to process aflatoxin becomes relatively efficient within three weeks, this would imply that an alternative strategy for handling aflatoxin contamination in feed could be to allow a short, punctuated exposure to a higher level, so long as that exposure is followed by at least a week of a withdrawal period on a clean diet free of aflatoxin.

Hsin-Bai *et al.* (2015) investigated the inhibitory effect of 2 generally regarded as safe (GRAS), natural plant compounds, namely carvacrol (CR) and *trans*-cinnamaldehyde (TC), on *A. flavus* and *A. parasiticus* growth and AF production in potato dextrose broth (PDB) and in poultry feed. In broth culture, PDB supplemented with CR (0%, 0.02%, 0.04% and 0.08%) or TC (0%, 0.005%, 0.01% and 0.02%) was inoculated with *A. flavus* or *A. parasiticus* (6 log CFU/mL), and mold counts and AF production were determined on days 0, 1, 3, and 5. Similarly, 200 g portions of poultry feed supplemented with CR or TC (0%, 0.4%, 0.8%, and 1.0%) were inoculated with each mold, and their counts and AF concentrations in the feed were determined at 0, 1, 2, 3, 4, 8, and 12 weeks of storage. Moreover, the effect of CR and TC on the expression of AF synthesis genes in *A. flavus* and *A. parasiticus* (*aflC, nor1, norA*, and*ver1*) was determined using real-time quantitative PCR (RT-qPCR). All experiments had duplicate samples and were replicated 3 times. Results indicated that CR and TC reduced *A. flavus* and *A. parasiticus* growth and AF production in broth culture and chicken feed ($P < 0.05$). All tested concentrations of CR and TC decreased

AF production in broth culture and chicken feed by at least 60% when compared to controls ($P < 0.05$). In addition, CR and TC down-regulated the expression of major genes associated with AF synthesis in the molds ($P < 0.05$). Results suggest the potential use of CR and TC as feed additives to control AF contamination in poultry feed.

Kumar *et al*. (2015) conducted a study to evaluate the efficacy of citrus fruit oil (CFO; 2.5 g kg(-1)) on the clinicopathological changes in broilers fed with diets containing 1 ppm of aflatoxin (AF). A total of 160 Ross 308 broiler chicks of 1-day-old were procured from a commercial hatchery, divided randomly on 7th day of age into four groups with two replicates of 20 birds each and fed with basal diet (group A), basal diet + CFO (group B), basal diet + AF (group C) and CFO + basal diet + AF (group D). The gross and histopathological changes in the liver, kidney, spleen, thymus and bursa of Fabricius were investigated and relative organ weights were calculated. Slight to moderate hydropic degeneration, fatty change with the formation of cyst in some cases, periportal necrosis, infiltration of heterophils and mononuclear cells and bile duct hyperplasia were observed in chicks fed with 1 ppm AF-containing diet. The addition of CFO to AF-containing diet moderately decreased the magnitude and severity of lesions (hydropic degeneration and bile duct hyperplasia) in the liver. The supplementation of CFO to the basal diet did not produce any adverse effects in birds.

Monson *et al.* (2015) investigated the molecular mechanisms of AFB1 immunotoxicity and the ability of a Lactobacillus-based probiotic to protect against aflatoxicosis in the domestic turkey (Meleagris gallopavo). The spleen transcriptome was examined by RNA sequencing (RNA-seq) of 12 individuals representing four treatment groups. Sequences (6.9 Gb) were de novo assembled to produce over 270,000 predicted transcripts and transcript fragments. Differential expression analysis identified 982 transcripts with statistical significance in at least one comparison between treatment groups. Transcripts with known immune functions comprised 27.6 % of significant expression changes in the AFB1-exposed group. Short exposure to AFB1 suppressed innate immune transcripts, especially from antimicrobial genes, but increased the expression of transcripts from E3 ubiquitin-protein ligase CBL-B and multiple interleukin-2 response genes. Up-regulation of transcripts from lymphotactin, granzyme A, and perforin 1 could indicate either increased cytotoxic potential or activation-induced cell death in the spleen during aflatoxicosis. Supplementation with probiotics was found to ameliorate AFB1-induced expression changes for multiple transcripts from antimicrobial and IL-2-response genes. However, probiotics had an overall suppressive effect on immune-related transcripts.

Sridhar *et al*. (2015) performed a study to evaluate the possible protective effects of resveratrol against the adverse effects of AFB1 in broiler birds. A feeding trial of 42 days of duration was undertaken in a completely randomized design with five dietary treatments: G1-AFB1(1.0 ppm); G2-CTR (basal diet alone); G3-AFB1(1.0 ppm)+Resv 0.5%; G4-AFB1(1.0 ppm)+Resv 1%; and G5-Resv 1%. Gain in body weight (BWG) and feed intake (FI) was observed to be highest ($p < 0.05$) in the AFB1birds followed by the control group. Feed conversion ratio was lowest in G2-CTR birds and failed to record any significant variation ($p > 0.05$) between groups as well as within groups. Birds fed resveratrol at both 0.5% and 1.0% levels in combination with AFB1 as well as alone along with basal diet had lower BWG and FI

between the fourth and fifth week and also at the fifth week ($p < 0.05$). No variation ($p > 0.05$) was obtained in the FCR of AFB1 and resveratrol group of broiler birds. AFB1 feeding significantly increased the activities of aspartate-(AST) and alanine-(ALT) amino transferase, superoxide dismutase (SOD) and catalase (CAT) activities ($p < 0.05$) but lowered glucose, cholesterol and triglyceride levels in serum. Supplementation of resveratrol helped in increasing the activities of the oxidative enzymes and in improving the plasma total antioxidant capacity (TAOC) and total protein (TP) significantly ($p < 0.05$) and protein values. The livers of AFB1 group showed degeneration of hepatocytes, bile duct hyperplasia and microgranuloma formation. In resveratrol supplemented birds, the severity and degree of the liver lesions was far less. Apoptotic proteins failed to show any variation in expression between AFB1, control and resveratrol group of birds. The inclusion of resveratrol in broiler diets enhanced antioxidant status of birds indicating the protective effect of resveratrol against AFB1-induced toxicity. So, we advice use of resveratrol as a feed additive to control aflatoxicosis in poultry farms.

Sun *et al.* (2015) conducted 2 experiments to screen microorganisms with aflatoxin B1 (AFB1) removal potential from soils and to evaluate their ability in reducing the toxic effects of AFB1 in ducklings. In experiment 1, we screened 11 isolates that showed the AFB1 biodegradation ability, and the one exhibited the highest AFB1 removal ability (97%) was characterized and identified as Cellulosimicrobium funkei (C. funkei). In experiment 2, 80 day-old Cherry Valley ducklings were divided into four groups with four replicates of five birds each and were used in a 2 by 2 factorial trial design, in which the main factors included administration of AFB1 versus solvent and C. funkei versus solvent for 2 weeks. The AFB1 treatment significantly decreased the body weight gain, feed intake and impaired feed conversion ratio. AFB1 also decreased serum albumin and total protein concentration, while it increased activities of alanine aminotransferase and aspartate aminotransferase and liver damage in the ducklings. Supplementation of C. funkei alleviated the adverse effects of AFB1 on growth performance, and provided protective effects on the serum biochemical indicators, and decreased hepatic injury in the ducklings. Conclusively, our results suggest that the novel isolated C. funkei strain could be used to mitigate the negative effects of aflatoxicosis in ducklings.

Chen *et al.* (2016) conducted a 20-day trial to determine the impact of aflatoxin B_1 (AFB_1) and dietary protein concentration on performance, nutrient digestibility, and gut health in broiler chicks. The 6 dietary treatments were arranged in a 2×3 factorial with 3 crude protein (CP) concentrations (16, 22, and 26%) with or without 1.5 mg/kg AFB_1. Each diet was fed to 6 replicate cages (6 chicks per cage) from zero to 20 d of age. Endogenous N and amino acid loss were estimated from birds fed a N-free diet with or without 1.5 mg/kg AFB_1. A significant interaction between AFB_1 and CP concentration was observed for growth performance, where reduction of BW gain, feed intake, gain:feed ratio, and breast muscle weight by AFB_1 were most profound in birds fed the 16%-CP diet, and were completely eliminated when birds were fed the 26%-CP diet (AFB_1 by CP interaction; $P \leq 0.023$). Similarly, AFB_1 reduced serum albumin, total protein, and globulin concentrations in birds fed 16 and 22% CP diets, but not in those fed the 26%-CP (AFB_1 by CP interaction; $P \leq 0.071$). Gut permeability was increased in birds fed AFB_1-contamiated diets as measured by serum lactulose/rhamnose ratio (main effect; $P = 0.04$). Additionally, AFB_1 tended to

increase endogenous N loss (P = 0.09), and significantly reduced apparent ileal digestible energy and standardized ileal N and amino acid digestibility in birds fed the 16%-CP diet, while birds fed higher dietary CP were not affected ($AFB_1$by CP interaction; P ≤ 0.01). Further, AFB_1 increased the translation initiation factor 4E-binding protein (4EBP1), claudin1, and multiple jejunal amino acid transporters expression (main effect; P ≤ 0.04). Results from this study indicate that a 1.5 mg AFB_1/kg diet significantly impairs growth, major serum biochemistry measures, gut barrier, endogenous loss, and energy and amino acid digestibility. Aflatoxicosis can be augmented by low dietary CP, while higher dietary CP completely eliminated the impairment of performance, serum proteins, and nutrient digestibility from aflatoxicosis in zero to 20 d broiler chicks.

Galarza-Seeber *et al.* (2016) conducted 2 experiments in broilers to evaluate the effect of three concentrations of Aflatoxin B1 (AFB1; 2, 1.5, or 1 ppm) on gastrointestinal leakage and liver bacterial translocation (BT). In experiment 1, 240 day-of-hatch male broilers were allocated in two groups, each group had six replicates of 20 chickens (*n* = 120/group): Control feed or feed + 2 ppm AFB1. In experiment 2, 240 day-of-hatch male broilers were allocated in three groups, each group had five replicates of 16 chickens (*n* = 80/group): Control feed; feed + 1 ppm AFB1; or feed + 1.5 ppm AFB1. In both experiments, chickens were fed starter (days 1–7) and grower diets (days 8–21) *ad libitum* and performance parameters were evaluated every week. At day 21, all chicks received an oral gavage dose of FITC-d (4.16 mg/kg) 2.5 h before collecting blood samples to evaluate gastrointestinal leakage of FITC-d. In experiment 2, a hematologic analysis was also performed. Liver sections were aseptically collected and cultured using TSA plates to determine BT. Cecal contents were collected to determine total colony-forming units per gram of Gram-negative bacteria, lactic acid bacteria (LAB), or anaerobes by plating on selective media. In experiment 2, liver, spleen, and bursa of Fabricius were removed to determine organ weight ratio, and also intestinal samples were obtained for morphometric analysis. Performance parameters, organ weight ratio, and morphometric measurements were significantly different between Control and AFB1 groups in both experiments. Gut leakage of FITC-d was not affected by the three concentrations of AFB1 evaluated ($P > 0.05$). Interestingly, a significant reduction in BT was observed in chickens that received 2 and 1 ppm AFB1. An increase ($P < 0.05$) in total aerobic bacteria, total Gram negatives, and total LAB were observed in chickens fed with 2 and 1.5 ppm of AFB1 when compared with Control and 1 ppm chickens. The integrity of gut epithelial barrier was not compromised after exposure to the mycotoxin.

Hussain *et al.* (2016) conducted a study to assess tissue residues of aflatoxin B1 (AFB1), and alterations in select clinical chemistry variables in serum during chronic aflatoxicosis in broiler chicks fed different dietary levels of AFB1. Six groups of broiler chickens were fed diets containing between 0 and 800 ppb of AFB1 for 28 days. Groups of birdswere terminated on days 0, 5, 13, 15, 20, and 28, and AFB1 levels were determined by HPLC in liver and muscle. Serum activities of ALT and ALP, and total protein and albumin concentrations were determined. No AFB1 residues were detected in liver after 50 ppb AFB1, and muscle after 50 and 100 ppb AFB1 feeding. Residues above the permissible threshold (> 2.0 ng/g) were only detected in liver tissues of groups fed 400 ppb and 800 ppb AFB1 in feed. The ALT and ALP activities in treated groups were significantly higher, and total protein and albumin concentrations were significantly lower in all treated groups compared to

controls. Continuous feeding of AFB1 to broiler chicken at levels of 50 and 100 ppb for 28 days did not reveal measurable AFB1 residues in muscle tissues. Serum values of ALT, ALP, total protein, and albumin may serve as markers for chronic aflatoxicosis in affected poultry.

Mohaghegh *et al.* (2016) evaluated the effect of esterified glucomannan (E-GM) on performance, immunity, blood haematological and serum biochemical parameters in broilers exposed to diets naturally contaminated with mycotoxins. A total of 630 one-day-old male broiler chicks (Ross 308) were randomly assigned to 9 treatments and 5 replicates of 14 chicks based on factorial (3 × 3) arrangement in completely randomized design. The dietary treatments included 3 levels of substituting naturally contaminated corn (0%, 50% and 100%), three levels of E-GM (0%, 0.05% and 0.1%) and their interaction. Body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were evaluated from 7 to 49 days of age. Haematology, serum biochemical and enzyme activities were assessed. Antibody titre against Newcastle disease virus and infectious bursal disease was measured to evaluate the humoral immunity. In comparison to diets with no contamination, 50% and 100% naturally contaminated corn significantly decreased FI, BWG and FCR ($P < .05$). Supplementing 0.05% and 0.1% E-GM considerably improved the decreased BWG and FI ($P < .05$). However, only 0.1% binder ameliorated the negative impact of mycotoxins on FCR ($P < .05$). Replacement of contaminated corn remarkably diminished humoral immunity of chickens and increased liver enzyme activities which ameliorated by supplementing 0.05% and 0.1% of binder inclusion ($P < .05$). Results indicated that supplementing E-GM particularly at 0.1% level efficiently reversed the adverse effects of mycotoxins on broiler chickens.

Shang *et al.* (2016) investigated the effects of feeds naturally contaminated with mycotoxins on growth performance, serum biochemical parameters, carcass traits, and splenic heat shock protein 70 (Hsp70) mRNA expression levels in broiler chickens. The efficacy of yeast cell wall (YCW) adsorbent in preventing mycotoxicosis was also evaluated. Three hundred 1-d-old Arbor Acres broiler chicks were randomly allotted to 3 treatments in completely randomized design for 42 d. Each treatment group had 5 replicate pens with 20 birds. The treatments were as follows: i) basal diet (control), ii) naturally contaminated diet (NCD), and iii) NCD+0.2% YCW adsorbent (NCDD). The NCD decreased average daily gain (ADG) ($p<0.01$) of 0 to 21 d, 22 to 42 d, and 0 to 42 d, and increased feed conversion ratio ($p<0.01$) of 22 to 42 d and 0 to 42 d. Both the breast meat percentage and thigh meat percentage of the NCD group were significantly higher ($p<0.01$) than that of the control group on d 21. The NCD group showed significantly increased levels of triglycerides ($p<0.05$) and cholesterol ($p<0.05$) on both d 21 and d 42 compared to the control group. However, the NCD significantly reduced ($p<0.01$) the high-density lipoprotein (HDL) on d 42 compared to controls. Compared with the NCD, supplementation with YCW significantly improved ($p<0.01$) the ADG of 0 to 21 d and 0 to 42 d, and increased ($p<0.01$) concentrations of HDL on d 42, and on d 21, and triglycerides ($p<0.05$) on d 21 and d 42. Supplementation with YCW reduced ($p<0.01$) the breast meat percentage, the thigh meat percentage, the concentrations of cholesterol ($p<0.01$) and the low-density lipoprotein ($p<0.05$) on d 21, and improved ($p<0.01$) the splenic Hsp70 mRNA expression levels compared with the NCD group. The results of this study indicated that feeding NCD for 42 d had adverse effects on broiler chickens, and that YCW might be beneficial in counteracting the effects of mycotoxins.

Refertences

1. Abidin ZU, A Khatoon, MA Qureshi and TM Butt, 2013. Determination of aflatoxin B1 in finished poultry feed samples collected from different poultry farms and markets of Lahore, Pakistan. Inter J Vet Sci, 2(1): 28-31. www.ijvets.com
2. Ahmed, M.A.E., Ravikanth, K.2 , Rekhe, D.S.2 , Maini. Histopathological alterations in Aflatoxicity and its amelioration with herbomineral toxin binder in broilers. Veterinary World, Vol.2(10):390-392, 2009
3. Allcroft, R., and Carnaghan, R.B.A. 1963a. Toxic products in groundnuts. Biological effects. Chemistry and Industry (London) 2 : 50-53.
4. Allcroft, R., and Carnaghan, R.B.A. 1963b. Groundnut toxicity : An examination for toxin in human food products from animals fed toxic groundnut meal. Veterinary Record 75: 259-263. Co-":
5. Allcroft, R., and Loosmore, R.M. 1963. Toxic effects associated with the feeding of groundnuts. Pages 175-178 in Proceedings of the XVIith World Veterinary Congress, Hanover, 1963.
6. Amaya-Farfan J. Aflatoxin B1-induced hepatic steatosis: role of carbonyl compounds and active diols on steatogenesis. Lancet. 1999 Feb 27;353(9154):747-8.
7. Amer AM, Fahim EM, Ibrahim RK. Effect of aflatoxicosis on the kinetic behaviour of ceftiofur in chickens. Res Vet Sci. 1998 Sep-Oct;65(2):115-8.
8. Applegate T.J., Schatzmayr G., Pricket K., Troche C., Jiang Z. Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. Poult. Sci. 2009;88:1235–1241
9. Archibald, R.Mc.G., Smith, H.J., and Smith, J.D. 1962. Brazilian groundnut toxicosis in Canadian broiler chickens. Canadian Veterinary Journal 3(10): 322-325.
10. Asao T., Büchi G., Abdel-Kadar M.M., Chang S.B., Wick E.L., Wogan G.N. Structures of aflatoxins $B_1$and G_1. J. Am. Chem. Soc. 1965;87:882–886.
11. Asuzu, I.U. and Shetty, S.N. (1986): "Acute aflatoxicosis in broiler chicken in Nsuka, Nigeria." Trop. Vet., 4: 79-80.
12. Asplin, F.D., and Carnaghan, R.B.A. 1961. The toxicity of certain groundnut meals for poultiy with special reference to their effect on ducklings and chickens. Veterinary Record 73(46): 1215-1219.
13. Bailey RH[1], Kubena LF, Harvey RB, Buckley SA, Rottinghaus GE. Efficacy of various inorganic sorbents to reduce the toxicity of aflatoxin and T-2 toxin in broiler chickens. Poult Sci. 1998 Nov;77(11):1623-30.
14. Batra, P.; Pruthi, A.K. and Sadana, J.R. (1991): "Effect of AFB1 of the efficacy of turkey herpes virus vaccine against Marek's disease." Res. Vet. Sci., 51: 115-119.
15. Bintvihok A, Kositcharoenkul S. Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon. 2006 Jan;47(1):41-6. Epub 2005 Nov 18.
16. Blount, W.F. 1960a. A new turkey disease problem in England characterized by heavy mortality. Quaterly Poultry Bulletin 27: 1-3. D
17. Blount, W.P. 1960b. Disease of turkey poults (Letter). Veterinary Record 72(38): 786.
18. Blount, W.P. 1961a. Turkey "X" disease and the labelling of poultry foods (Letter). Veterinary Record 73(9): 227.
19. Blount, W.P. 1961b. Turkey "X" disease. Turkeys (Journal of British Turkey Federation) 9(2): 52,55-58,61
20. Bovo F, Franco LT, Kobashigawa E, Rottinghaus GE, Ledoux DR, Oliveira CA· Efficacy of beer fermentation residue containing Saccharomyces cerevisiae cells for ameliorating aflatoxicosis in broilers. Poult Sci. 2015 May;94(5):934-42.
21. Bryden, W.L.; Lioyd, A.B. and Cumming, R.B. (1980): "Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis." Aust. Vet. J., 56: 176-180.

22. Calnek, B.C.; Barnes, H.J.; MCDougald, L.R. and Saif, Y.M. (1997): "Diseases of poultry." 10th ed., Pp. 951-979. MosbyWolfe, Iowa state Univ. press, Ames, Iowa, USA.
23. Campbell, M.L.Jr.; May, J.D.; Huff, W.E. and Doerr, J.A. (1983): "Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis." Poult. Sci., 62: 2138- 2144.
24. Carnaghan, I-B.A. 1961. The toxicity of certain groundnut meals to poultry (Letter). Veterinary Record 73(29): 726-727.
25. Carnaghan, .B.A., and Sargeant, K. 1961. The toxicity of certain groundnut meals to poultry. Veterinary Record 73: 726- 727.
26. Çelik I., Oğuz H., Demet Ö., Dönmez H.H., Boydak M., Sur E. Efficacy of polyvinylpolypyrrolidone in reducing the immunotoxicity of aflatoxin in growing broilers. Br. Poult. Sci. 2000;41:430–439
27. Chen X, Horn N, Applegate TJ. Efficiency of hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of graded levels of aflatoxin B1 in broiler chicks. Poult Sci. 2014 Aug;93(8):2037-47.
28. Chen X, Naehrer K, Applegate TJ Interactive effects of dietary protein concentration and aflatoxin B1 on performance, nutrient digestibility, and gut health in broiler chicks. Poult Sci. 2016 Mar 4. pii: pew022. [Epub ahead of print]
29. Cheng YH, Shen TF, Pang VF, Chen BJ. Effects of aflatoxin and carotenoids on growth performance and immune response in mule ducklings. Comp Biochem Physiol C Toxicol Pharmacol. 2001 Jan;128(1):19-26.
30. Chotinski D., Profirov I., Voĭnova R., Borisova L. SH group content and hydrolase activity of the small intestine mucosa in chickens fed a mixture containing aflatoxin B1. Vet. Med. Nauki. 1987;24:48–51
31. CHU, F. S. AND I. UENO. Production of Antibody Against Aflatoxin B1. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1977, p. 1125-1128
32. Citil M, Gunes V, Atakisi O, Ozcan A, Tuzcu M, Dogan A. Protective effect of L-carnitine against oxidative damage caused by experimental chronic aflatoxicosis in quail (Coturnix coturnix). Acta Vet Hung. 2005;53(3):319-24.
33. Corrier, D.E. (1991): "Mycotoxicosis: Mechanism of immunosuppression." Vet. Immunol. Immuno Pathol., 30: 73-87.
34. Dafalla, R.; Hassan, Y.M. and Adam, S.E.I. (1987a): "Fatty and hemorrhagic liver and kidney syndrome in breeding hens caused by AFB1 and heat stress in the Sudan." Vet. Hum. Toxicol., 29: 222-226.
35. Dafalla, R.; Yagi, A.I. and Adam, S.E.I. (1987b): "Experimental aflatoxicosis in Hybro-type chicks: Sequential changes in growth and serum constituents and histopathological changes." Vet. Human Toxicol., 29: 222-225
36. Dalvi RR, McGowan C. Experimental induction of chronic aflatoxicosis in chickens by purified aflatoxin B1 and its reversal by activated charcoal, phenobarbital, and reduced glutathione. Poult Sci. 1984 Mar;63(3):485-91..
37. de Iongh, H., Beerthuis, R.K., Vles, R.O., Barrett, C.B., and Ord, W.O. 1962. Investigation of the factor in groundnut meal responsible for "turkey X disease". Biochimica et Biophysica Acta 65 : 548-551.
38. Denli M, Blandon JC, Guynot ME, Salado S, Perez JF. Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B(1). Poult Sci. 2009 Jul;88(7):1444-51.
39. Dersjant-Li Y., Verstegen M.W.A., Gerrits W.J.J. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. Nutr. Res. Rev. 2003;16:223–239.
40. Derzsy, D., Msziros, J., Prokopovitsch, L., and T6th-Baranyi, I. 1962. [Virus hepatitis and toxic liver damage in ducks.]. A kacsdk virusos ds toxikus mijgyulladsa. Magyar Ailatorvosok Lapja 17(2): 49-53.

41. Diaz G.J., Calabrese E., Blain R. Aflatoxicosis in chickens (*Gallus gallus*): An example of hormesis?Poult. Sci. 2008;87:727–732

42. Diaz G., Murcia H.W., Cepeda S.M. Cytochrome P450 enzymes involved in the metabolism of aflatoxin B_1 in chickens and quail. Poult. Sci. 2010;89:2461–2469.
43. Dozier W.A., III, Kidd M.T., Corzo A. Dietary amino acid responses of broiler chickens. J. Appl. Poult. Res. 2008;17:157–167. doi: 10.3382/japr.2007-00071.
44. Ebrahimi MM, Shahsavandi S. Evaluation of antibody levels during simultaneous aflatoxicosis and vaccination against infectious laryngotracheitis in pullets. Biologicals. 2008 Sep;36(5):327-9.
45. Edds, G.T.; Nair, N.P. and Simpson, C.F. (1973): "Effect of AFB1 on resistance in poultry against cecal coccidiosis and Marek's disease." Am. J. Vet. Res., 34: 819-826.
46. Edrington T., Kubena L., Harvey R., Rottinghaus G. Influence of a superactivated charcoal on the toxic effects of aflatoxin or T-2 toxin in growing broilers. Poult. Sci. 1997;76:1205–1211
47. Ellakany HF, Abuakkada SS, Oda SS, El-Sayed YS. Influence of low levels of dietary aflatoxins on Eimeria tenella infections in broilers. rop Anim Health Prod. 2011 Jan;43(1):249-57.
48. Fan Y, Zhao L, Ma Q, Li X, Shi H, Zhou T, Zhang J, Ji C. Effects of Bacillus subtilis ANSB060 on growth performance, meat quality and aflatoxin residues in broilers fed moldy peanut meal naturally contaminated with aflatoxins. Food Chem Toxicol. 2013 Sep;59:748-53.

49. Fernandez A., Verde M.T., Gascon M., Ramos J.J., Gomez J. Aflatoxin and its metabolites in tissues from laying hens and broiler chickens fed a contaminated diet. J. Sci. Food Agric. 1994;65:407–414.
50. Fowler J, Li W, Bailey C. Effects of a Calcium Bentonite Clay in Diets Containing Aflatoxin when Measuring Liver Residues of Aflatoxin B_1 in Starter Broiler Chicks. Toxins (Basel). 2015 Aug 26;7(9):3455-64..
51. Galarza-Seeber R, Latorre JD, Bielke LR, Kuttappan VA, Wolfenden AD, Hernandez-Velasco X, Merino-Guzman R, Vicente JL, Donoghue A, Cross D, Hargis BM and Tellez G (2016) Leaky Gut and Mycotoxins: Aflatoxin B1 Does Not Increase Gut Permeability in Broiler Chickens. *Front. Vet. Sci.* 3:10. doi: 10.3389/fvets.2016.00010
52. Gardiner, M.R., and Oldroyd, B. 1965. Avian aflatoxicosis. Australian Veterinary Journal 41: 272-276.
53. Gathumbi JK, Usleber E, Ngatia TA, Kangethe EK, Märtlbauer E. Application of immunoaffinity chromatography and enzyme immunoassay in rapid detection of aflatoxin B1 in chicken liver tissues. Poult Sci. 2003 Apr;82(4):585-90.
54. Gholami-Ahangaran M, Zia-Jahromi N. Nanosilver effects on growth parameters in experimental aflatoxicosis in broiler chickens. Toxicol Ind Health. 2013 Mar;29(2):121-5.
55. Gholami-Ahangaran M, Zia-Jahromi N. Effect of nanosilver on blood parameters in chickens having aflatoxicosis. Toxicol Ind Health. 2014 Mar;30(2):192-6.
56. Giambrone J.J., Partadiredja M., Eidson C.S., Kleven S.H., Wyatt R.D. Interaction of aflatoxin with infectious bursal disease virus infection in young chickens. Avian Dis. 1978;22:431–439.
57. Giambrone, J.J.; Ewert, D.L.; Wyatt, R.D. and Eidson, C.S. (1978a): "Effect of aflatoxin on the humoral and cell-mediated immune systems of chicken." Am. J. Vet. Res., 39: 305.
58. Giambrone, J.J.; Partadiredja, M.; Eidson, C.S.; Kleven, S.H. and Wyatt, R.D. (1978b): "Interaction of aflatoxin with infectious bursal disease virus infection in young chickens." Avian Dis., 22: 431. Gibson, E.A.. and Harris, A.H. 1961. Disease of turkey poults (Letter). Veterinary Record 73: 150.
59. Gibson, W.W.C. 1961. Turkey "X"disease and the labelling ofpoultry foods. Veterinary Record 73: 150-151.

60. Gonzalo J. Diaz and Hansen W. Murcia. Biotransformation of Aflatoxin B1and Its Relationship with the Differential Toxicological Response to Aflatoxin in Commercial Poultry Species Aflatoxins and Aflatoxicosis in Human and Animals. www.intechopen.com

61. Gopal, T., Zaki, S., Narayanaswamy, M., and Premlata, S. 1969. Aflatoxicosis in fowls. Indian Veterinary Journal 46: 348-349.
62. Gowda, N. K. S., D. R. Ledoux, G. E. Rottinghaus, A. J. Bermudez, and Y. C. Chen Efficacy of Turmeric (*Curcuma longa*), Containing a Known Level of Curcumin, and a Hydrated Sodium Calcium Aluminosilicate to Ameliorate the Adverse Effects of Aflatoxin in Broiler ChicksPoultry Sciencet (2008) 87 (6): 1125-1130
63. Guarisco JA, Hall JO, Coulombe RA Jr. Mechanisms of butylated hydroxytoluene chemoprevention of aflatoxicosis--inhibition of aflatoxin B1 metabolism. Toxicol Appl Pharmacol. 2008 Mar 15;227(3):339-46.
64. Hall, JA, JO, Coulombe RA Jr. Butylated hydroxytoluene chemoprevention of aflatoxicosis - effects on aflatoxin B(1) bioavailability, hepatic DNA adduct formation, and biliary excretion. Food Chem Toxicol. 2008 Dec;46(12):3727-31.
65. Hamilton, P.B., Tung, H.T.; Harris, J.R. and Donaldson, W.E. (1972): "The effect of dietary fat on aflatoxins in turkeys." Poult. Sci., 51: 165-170.
66. Hart, L. 1965. Avian aflatoxicosis. Australian Veterinary Journal 41: 395-396.
67. Hashem MA, Mohamed MH. Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt..Vet Ital. 2009 Apr-Jun;45(2):323-37.
68. Havenstein G.B., Ferket P.R., Qureshi M.A. Growth, livability, and feed conversion of 1991 vs. 1957 broilers when fed “typical” 1957 and 2001 broiler diets. Poult. Sci. 2003;82:1500–1508.
69. Havenstein G.B., Ferket P.R., Scheideler S.E., Larson B.T. Growth, livability, and feed conversion of 1991 *vs.* 1957 broilers when fed “typical” 1957 and 1991 broiler diets. Poult. Sci. 1994;73:1785–1794
70. Hegazy, S.M.; Azzam, A. and Gabal, M.A. (1991): "Interaction of naturally occurring aflatoxins in poultry feed and immunization against fowl cholera." Poult. Sci., 70: 2425-2428.
71. Hetzel, D.J.S.; Hoffmann, D.; Van De Ven, J. and Soeripto, S. (1984): "Mortality rate and liver histopathology in four breeds of ducks following long term exposure to low levels of aflatoxins." Sing. Vet. J., 8: 6-14.
72. Hsin-Bai Yin, Chi-Hung Chen, Anup Kollanoor-Johny, Michael J. Darre,and Kumar Venkitanarayanan. Controlling *Aspergillus flavus* and *Aspergillus parasiticus* growth and aflatoxin production in poultry feed using carvacrol and *trans*-cinnamaldehyde. Poultry Science (September 2015) 94 (9): 2183-2190 first published online July 27, 2015doi:10.3382/ps/pev207
73. Huff, W.E.; Wyatt, R.D. and Hamilton, P.B. (1975): "Effects of dietary aflatoxin on certain egg yolk parameters." Poult. Sci., 54: 2014-2018.

74. Hussain Z., Khan M.Z., Khan A., Javed I., Saleemi M.K., Mahmood S., Asi M.R. Residues of aflatoxin B_1 in broiler meat: Effect of age and dietary aflatoxin B_1 levels. Food Chem. Toxicol. 2010;48:3304–3307.
75. Hussain Z, Rehman HU, Manzoor S, Tahir S, Mukhtar M. Determination of liver and muscle aflatoxin B1 residues and select serum chemistry variables during chronicaflatoxicosis in broiler chickens. Vet Clin Pathol. 2016 Apr 4. doi: 10.1111/vcp.12336. [Epub ahead of print]
76. Ibrahim IK, Shareef AM, Al-Joubory KM. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Res Vet Sci. 2000 Oct;69(2):119-22.

77. Jakhar KK, Sadana JR. Sequential pathology of experimental aflatoxicosis in quail and the effect of selenium supplementation in modifying the disease process. Mycopathologia. 2004 Jan;157(1):99-109.
78. Jones, F.T.; Hagler, W.H. and Hamilton, P.B. (1982): "Association of low levels of aflatoxin in feeds with productivity losses in commercial broiler operations." Poult. Sci., 61: 861-868.
79. Kana, J.R.; Teguia, A.; Tchoumboue, J. Effect of dietary plant charcoal from *Canarium schweinfurthii* Engl. and maize cob on aflatoxin B_1 toxicosis in broiler chickens. *Adv. Anim. Biosci.* 2010, *1*, 462–463.
80. Karaman M, Basmacioglu H, Ortatatli M, Oguz H. Evaluation of the detoxifying effect of yeast glucomannan on aflatoxicosis in broilers as assessed by gross examination and histopathology. Br Poult Sci. 2005 Jun;46(3):394-400.
81. Kasmani, B. F, Karimi Torshizi MA, Allameh A, Shariatmadari F. A novel aflatoxin-binding Bacillus probiotic: Performance, serum biochemistry, and immunological parameters in Japanese quail. Poult Sci. 2012 Aug;91(8):1846-53.
82. Kiran MM, Demet O, Ortatath M, Oğuz H. The preventive effect of polyvinylpolypyrrolidone on aflatoxicosis in broilers. Avian Pathol. 1998;27(3):250-5.
83. Klein PJ, Van Vleet TR, Hall JO, Coulombe RA Jr. Dietary butylated hydroxytoluene protects against aflatoxicosis in Turkeys. Toxicol Appl Pharmacol. 2002 Jul 1;182(1):11-9.
84. Klein PJ, Van Vleet TR, Hall JO, Coulombe RA Jr. Effects of dietary butylated hydroxytoluene on aflatoxin B1-relevant metabolic enzymes in turkeys. Food Chem Toxicol. 2003 May;41(5):671-8.
85. Kumar R., Balachandran C. Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid. Vet. Arhiv. 2009;79:31–40.
86. Kumar DS, Rao S, Satyanarayana ML, Kumar PG, Anitha N. Amelioration of hepatotoxicity induced by aflatoxin using citrus fruit oil in broilers (Gallus domesticus). Toxicol Ind Health. 2015 Nov;31(11):974-81.
87. Lamont, M.H. (1979): "Cases of suspected mycotoxicosis as reported by veterinary investigation centers." Proc. Mycotoxins Anim. Dis., 3: 38-39.
88. LANCASTER, M. C. F. P. JENKINS & J. MCL. PHILP.Toxicity associated with Certain Samples of Groundnuts. *Nature* 192, 1095 - 1096 (16 December 1961); doi:10.1038/1921095a0
89. Lawson B, MacDonald S, Howard T, Macgregor SK, Cunningham AA. Exposure of garden birds to aflatoxins in Britain. Sci Total Environ. 2006 May 15;361(1-3):124-31..
90. Leeson, S.; Diaz, G.J. and Summers, J.D. (1995): "Poultry metabolic disorders and mycotixns." Pp. 249-298, University Books, Guelph, Ontario, Canada.
91. Ledoux, D.; Rottinghaus, G.; Bermudez, A.; Alonso-Debolt, M. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. *Poult. Sci.* 1999, *78*, 204–210.
92. Mabee M.S., Chipley J.R. Tissue distribution and metabolism of aflatoxin B 1-14 C in Broiler chickens.Appl. Microbiol. 1973;25:763–769
93. Magnoli AP, Monge MP, Nazar FN, Magnoli CE, Cavaglieri LR, Bagnis G, Dalcero AM, Marin RH. Combined effects of aflatoxin B1 and corticosterone treatment on selected performance indices and liver histopathology in Japanese quail. Poult Sci. 2012 Feb;91(2):354-61.
94. Magnoli AP, Monge MP, Miazzo RD, Cavaglieri LR, Magnoli CE, Merkis CI, Cristofolini AL, Dalcero AM, Chiacchiera SM. Effect of low levels of aflatoxin B_1 on performance, biochemical parameters, and aflatoxin B_1 in broiler liver tissues in the presence of monensin and sodium bentonite. Poult Sci. 2011a Jan;90(1):48-58.

95. Magnoli AP, Texeira M, Rosa CA, Miazzo RD, Cavaglieri LR, Magnoli CE, Dalcero AM, Chiacchiera SM. Sodium bentonite and monensin under chronic aflatoxicosis in broiler chickens. Poult Sci. 2011b Feb;90(2):352-7.
96. Magnoli AP, Copia P, Monge MP, Magnoli CE, Dalcero AM, Chiacchiera SM. Negligible effects of tryptophan on the aflatoxin adsorption of sodium bentonite. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2014;31(12):2063-70.
97. Marchioro A, Mallmann AO, Diel A, Dilkin P, Rauber RH, Blazquez FJ, Oliveira MG, Mallmann CA. Effects of aflatoxins on performance and exocrine pancreas of broiler chickens. Avian Dis. 2013 Jun;57(2):280-4.
98. McKenzie KS, Kubena LF, Denvir AJ, Rogers TD, Hitchens GD, Bailey RH, Harvey RB, Buckley SA, Phillips TD. Aflatoxicosis in turkey poults is prevented by treatment of naturally contaminated corn with ozone generated by electrolysis. Poult Sci. 1998 Aug;77(8):1094-102.
99. Miazzo, R., M. F. Peralta, C. Magnoli, M. Salvano, S. Ferrero, S.M. Chiacchiera,
100. . Carvalho, E. C. Q , C. A. R. Rosa, and A. Dalcero Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin Poultry Science (2005) 84 (1): 1-8
101. Miazzo R., Rosa C., de Queiroz C.E., Magnoli C., Chiacchiera S., Palacio G., Saenz M., Kikot A., Basaldella E., Dalcero A. Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks.Poult. Sci. 2000;79:1–6.
102. Micco C., Miraglia M., Onori R., Brera C., Mantovani A., Ioppolo A., Stasolla D. Long-term administration of low doses of mycotoxins to poultry. 1. Residues of aflatoxin B_1 and its metabolites in broilers and laying hens. Food Addit. Contam. 1988;5:303–308
103. Mohaghegh, A., Mohammad Chamani, Mahmoud Shivazad, Ali Asghar Sadeghi & Nazar Afzali (2016): Effect of esterified glucomannan on broilers exposed to natural mycotoxin-contaminated diets, Journal of Applied Animal Research, DOI: 10.1080/09712119.2016.1174122
104. Monson MS, Settlage RE, Mendoza KM, Rawal S, El-Nezami HS, Coulombe RA, Reed KM. Modulation of the spleen transcriptome in domestic turkey (Meleagris gallopavo) in response to aflatoxin B1 and probiotics. Immunogenetics. 2015 Mar;67(3):163-78.
105. Nazar FN, Magnoli AP, Dalcero AM, Marin RH. Effect of feed contamination with aflatoxin B1 and administration of exogenous corticosterone on Japanese quail biochemical and immunological parameters. Poult Sci. 2012 Jan;91(1):47-54.
106. Neeff DV, Ledoux DR, Rottinghaus GE, Bermudez AJ, Dakovic A, Murarolli RA, Oliveira CA. In vitro and in vivo efficacy of a hydrated sodium calcium aluminosilicate to bind and reduce aflatoxin residues in tissues of broiler chicks fed aflatoxin B1. Poult Sci. 2013 Jan;92(1):131-7.
107. Nesbitt B.F., O'Kelly J., Sargeant K., Sheridan A. Toxic metabolites of *Aspergillus flavus*. Nature.1962;195:1062–1063
108. Nesheim, M.C. and Ivy, C.A. (1971): "Effect of aflatoxin on egg production and liver fat in laying hens." Cornell Nutr. Conf. Buffalo, N.Y. 1971, Pp. 126-129
109. Okotie-Eboh GO, Kubena LF, Chinnah AD, Bailey CA. Effects of beta-carotene and canthaxanthin on aflatoxicosis in broilers. Poult Sci. 1997 Oct;76(10):1337-41.
110. Okoye, J.O.A.; Asuzu, I.U. and Gugnani, J.C. (1988): "Paralysis and lameness associated with aflatoxicosis in broilers." Avian Pathol., 17: 731-734.
111. OGUZ,H., H.H. HADIMLI, V. KURTOGLU and O. ERGANIS. Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. Revue Méd. Vét., 2003, 154, 7, 483-486
112. Oğuz H, Kurtoğlu V. Effect of clinoptilolite on performance of broiler chickens during experimental aflatoxicosis. Br Poult Sci. 2000 Sep;41(4):512-7.

113. Oğuz H, Keçeci T, Birdane YO, Onder F, Kurtoğlu V. Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens duringaflatoxicosis. Res Vet Sci. 2000 Aug;69(1):89-93.
114. Ortatatli M, Oğuz H. Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. Res Vet Sci. 2001 Aug;71(1):59-66.
115. Ortatatli M[1], Ciftci MK, Tuzcu M, Kaya A. The effects of aflatoxin on the reproductive system of roosters. Res Vet Sci. 2002 Feb;72(1):29-36.
116. Osborne D.J., Hamilton P.B. Decreased pancreatic digestive enzymes during aflatoxicosis. Poult. Sci.1981;60:1818–1821
117. Otim MO, Mukiibi-Muka G, Christensen H, Bisgaard M. Aflatoxicosis, infectious bursal disease and immune response to Newcastle disease vaccination in rural chickens. Avian Pathol. 2005 Aug;34(4):319-23.
118. Ozen H, Karaman M, Ciğremiş Y, Tuzcu M, Ozcan K, Erdağ D. Effectiveness of melatonin on aflatoxicosis in chicks. Res Vet Sci. 2009 Jun;86(3):485-9..
119. Parlat SS[1], Yildiz AO, Oguz H. Effect of clinoptilolite on performance of Japanese quail (Coturnix coturnix japonica) during experimentalaflatoxicosis. Br Poult Sci. 1999 Sep;40(4):495-500.
120. Parlat SS, Ozcan M, Oguz H. Biological suppression of aflatoxicosis in Japanese quail (Coturnix coturnix japonica) by dietary addition of yeast (Saccharomyces cerevisiae). Res Vet Sci. 2001 Dec;71(3):207-11.
121. Patterson D.S.P. Aflatoxin and related compounds: Introduction. In: Wyllie T.D., Morehouse L.G., editors.Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, an Encyclopaedic Handbook. 1st. Vol. 1. Marcel Dekker Inc.; New York, NY, USA: 1977. pp. 131–135
122. Pizzolitto RP, Armando MR, Salvano MA, Dalcero AM, Rosa CA. Evaluation of Saccharomyces cerevisiae as an antiaflatoxicogenic agent in broiler feedstuffs. Poult Sci. 2013 Jun;92(6):1655-63.

123. Patterson D.S.P. Aflatoxin and related compounds: Introduction. In: Wyllie T.D., Morehouse L.G., editors.Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, an Encyclopaedic Handbook. 1st. Vol. 1. Marcel Dekker Inc.; New York, NY, USA: 1977. pp. 131–135.
124. Pimpukdee K, Kubena LF, Bailey CA, Huebner HJ, Afriyie-Gyawu E, Phillips TD. Aflatoxin-induced toxicity and depletion of hepatic vitamin A in young broiler chicks: protection of chicks in the presence of low levels of NovaSil PLUS in the diet. Poult Sci. 2004 May;83(5):737-44.
125. Pizzolitto RP, Armando MR, Combina M, Cavaglieri LR, Dalcero AM, Salvano MA. Evaluation of Saccharomyces cerevisiae strains as probiotic agent with aflatoxin B_1 adsorption ability for use in poultry feedstuffs. J Environ Sci Health B. 2012;47(10):933-41.
126. Pourelmi, M. R. Performance depression due to ingestion of aflatoxin in poultry. Annals of Biological Research, 2013, 4 (3):73-75
127. Qureshi,MA, J Brake, PB Hamilton, WM Hagler, Jr, and S Nesheim Dietary exposure of broiler breeders to aflatoxin results in immune dysfunction in progeny chicksPoultry Science (1998) 77 (6): 812-819
128. Quezada T, Cuéllar H, Jaramillo-Juárez F, Valdivia AG, Reyes JL. Effects of aflatoxin B(1) on the liver and kidney of broiler chickens during development. Comp Biochem Physiol C Toxicol Pharmacol. 2000 Mar;125(3):265-72.

129. Raju M.V.L.N., Devegowda G. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin) Br. Poult. Sci. 2000;41:640–650.

130. Raju MV, Rama Rao SV, Radhika K, Panda AK. Effect of amount and source of supplemental dietary vegetable oil on broiler chickens exposed to aflatoxicosis. Br Poult Sci. 2005 Oct;46(5):587-94
131. Rangsaz N, Ahangaran MG. Evaluation of turmeric extract on performance indices impressed by induced aflatoxicosis in broiler chickens. Toxicol Ind Health. 2011 Nov;27(10):956-60.
132. Rao, V.N. and Joshi, H.C. (1993): "Effect of certain drugs on acute induced aflatoxicosis in chicken (4 mg AFB1/ kg b.wt.)." Ind. Vet. J., 70: 344-347.
133. Rao J.R., Sharma N.N., Iyer P.K., Sharma A.K. Interaction between *Eimeria uzura* infection and aflatoxicosis in Japanese quail (*Coturnix coturnix* japonica). Vet. Parasitol. 1990;35:259–267.
134. Rao J.R., Sharma N.N., Johri T.S. Influence of dietary aflatoxin on *Eimeria uzura* infection in Japanese quail (*Coturnix coturnix japonica*) Vet. Parasitol. 1995;56:17–22.
135. Rawal S, Bauer MM, Mendoza KM, El-Nezami H, Hall JR, Kim JE, Stevens JR, Reed KM, Coulombe RA Jr . Aflatoxicosis chemoprevention by probiotic Lactobacillius and lack of effect on the major histocompatibility complex. Res Vet Sci. 2014 Oct;97(2):274-81.
136. Rawal S, Coulombe RA Jr. Metabolism of aflatoxin B1 in turkey liver microsomes: the relative roles of cytochromes P450 1A5 and 3A37. Toxicol Appl Pharmacol. 2011 Aug 1;254(3):349-54.
137. Richardson K.E., Hamilton P.B. Enhanced production of pancreatic digestive enzymes during aflatoxicosis in egg-type chickens. Poult. Sci. 1987;66:640–644
138. Rosa CA, Miazzo R, Magnoli C, Salvano M, Chiacchiera SM, Ferrero S, Saenz M, Carvalho EC, Dalcero A. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poult Sci. 2001 Feb;80(2):139-44.
139. Ruff M.D. Influence of dietary aflatoxin on the severity of *Eimeria acervulina* infection in broiler chickens. Avian Dis. 1978;22:471–480.
140. SARGEANT, K., ANN SHERIDAN, J. O'KELLY & R. B. A. CARNAGHAN. Toxicity associated with Certain Samples of Groundnuts. *Nature* 192, 1096 - 1097 (16 December 1961a); doi:10.1038/1921096a0
141. Sargeant, K, O'Kelly, J., Carnaghan, R.B.A., and Allcroft, R. (1961b) The assay of a toxic principle in certain groundnut meals. Veterinary Record. 73: 1219-1223.
142. Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R.B.A. 1961c. Toxicity associated with certain samples of groundnuts. Nature 192: 1096-1097.
143. Sawhney, D.S.; Vadehra, D.V. and Baker, R.C. (1973a): "Aflatoxicosis in the laying Japanese quail." Poult. Sci., 52: 485-493.
144. Sawhney, D.S.; Vadehra, D.V. and Baker, R.C. (1973b): "The metabolism of 14C aflatoxins in laying hens." Poult. Sci., 52: 1302-1309.
145. Sehu A, Cakir S, Cengiz O, Eşsiz D. MYCOTOX and aflatoxicosis in quails. Br Poult Sci. 2005 Aug;46(4):520-4.
146. SCHEIDELER, S.. Effects of Various Types of Aluminosilicates and Aflatoxin B_1 on Aflatoxin Toxicity, Chick Performance, and Mineral Status.Poultry Science (1993) 72 (2):282-288
147. Shang, Q. H., Z. B. Yang, W. R. Yang, Z. Li, G. G. Zhang, and S. Z. Jiang. Toxicity of Mycotoxins from Contaminated Corn with or without Yeast Cell Wall Adsorbent on Broiler Chickens. Asian Australas. J. Anim. Sci. Vol. 29, No. 5 : 674-680 May 2016
148. Sharma, R.P. (1993): "Immunotoxicity of mycotoxins." J. Dairy Sci., 76: 892-897.
149. Shivachandra S.B., Sah R.L., Singh S.D., Kataria J.M., Manimaran K. Immunosuppression in broiler chicks fed aflatoxin and inoculated with fowl

adenovirus serotype-4 (FAV-4) associated with hydropericardium syndrome. Vet. Res. Commum. 2003;27:39–51
150. Siloto EV, Oliveira EF, Sartori JR, Fascina VB, Martins BA, Ledoux DR, Rottinghaus GE, Sartori DR. Lipid metabolism of commercial layers fed diets containing aflatoxin, fumonisin, and a binder. Poult Sci. 2013 Aug;92(8):2077-83.
151. Siller, W.G. and Ostler, D.C. (1961): "The histopathology of an entero-hepatic syndrome of turkey poults." Vet. Rec., 73: 134-138.
152. Simsek, N. Ergun. L.,Essiz, D., Alabay, B. The effects of experimental aflatoxicosis on the exocrine pancreas in quails (Coturnix coturnix japonica) Article · Sep 2007 · Archive für Toxikologie, 81:583–588
153. Smith, K.M. 1960. "Disease" of turkey poults (Letter). Veterinary Record 72(32): 652.
154. Smith, J.E. and Hamilton, P.B. (1970): "Aflatoxicosis in the broiler chicken." Poult. Sci., 49: 207.
155. Sridhar M, Suganthi RU, Thammiaha V. Effect of dietary resveratrol in ameliorating aflatoxin B1-induced changes in broiler birds. J Anim Physiol Anim Nutr (Berl). 2015 Dec;99(6):1094-104.
156. Stevens, AJ., Sanders, C.N., Spence, J.B., and Newnham, A.G. 1960. Investigations into "disease" of turkey poults (Letter). Veterinary Record 72(31): 627-628.
157. Stewart, R.G.; Skeeles, J.K.; Wyatt, R.D.; Brown, J.; Page, R.K.; Russell, I.D. and Lukert, P.D. (1985): "The effect of aflatoxin on complement activity in broiler chickens" Poult. Sci., 64: 616-619.
158. Sun LH, Zhang NY, Sun RR, Gao X, Gu C, Krumm CS, Qi DS. A novel strain of Cellulosimicrobium funkei can biologically detoxify aflatoxin B1 in ducklings. Microb Biotechnol. 2015 May;8(3):490-8.
159. Swarbrick, 0. 1960. Disease of turkey poults (Letter). Veterinary Record 72(33): 671.
160. Tedesco D., Steidler S., Galletti S., Tameni M., Sonzogni O., Ravarotto L. Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B_1 in broiler chicks. Poult. Sci. 2004;83:1839–1843
161. Thaxton, J.P.; Tung, H.T. and Hamilton, P.B. (1974): "Immunosuppression in chickens by aflatoxin" Poult. Sci., 53: 721.
162. Yang J, Bai F, Zhang K, Bai S, Peng X, Ding X, Li Y, Zhang J, Zhao L. Effects of feeding corn naturally contaminated with aflatoxin B1 and B2 on hepatic functions of broilers. Poult Sci. 2012 Nov;91(11):2792-801.
163. Yunus A.W., Awad W.A., Kröger A., Zentek J., Böhm J. *In vitro* aflatoxin B_1 exposure decreases response to carbamycholine in the hehunal epithelium of broilers. Poult. Sci. 2010;89:1372–1378
164. Yunus A.W., Ghareeb K., Abd-El-Fattah A.A.M., Twaruzek M., Böhm B. Gross intestinal adaptations in relation to broiler performance during a chronic aflatoxin exposure. Poult Sci. 2011b Aug;90(8):1683-9.
165. Yunus AW, Razzazi-Fazeli E, Bohm J. Aflatoxin B(1) in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. Toxins (Basel). 2011 Jun;3(6):566-90.
166. VALCHEV ,I., D. KANAKOV , TS. HRISTOV , L. LAZAROV , R. BINEV , N. GROZEVA2 & Y. NIKOLOV. INVESTIGATIONS ON THE LIVER FUNCTION OF BROILER CHICKENS WITH EXPERIMENTAL AFLATOXICOSIS . Bulg. J. Vet. Med. 2014a ONLINE FIRST ISSN 1311-1477; online at http://tru.uni-sz.bg/bjvm/bjvm.htm Original article

167. Valchev , I. Zarkov , N. Grozeva , Y. Nikolov Effects of aflatoxin B1 on production traits, humoral immune response and immunocompetent organs in broiler chickens . AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 6, No 3, pp , 2014b

168. Valdivia A., Martinez A., Damian F., Quezada T., Ortiz R., Martinez C., Llamas J., Rodriguez M., Yamamoto L., Jaramillo F., et al. Efficacy of *N*-acetylcysteine to reduce the effects of aflatoxin B1 intoxication in broiler chickens. Poult. Sci. 2001;80:727–734.
169. Van Vleet PJ,, TR, Hall JO, Coulombe RA Jr. Dietary butylated hydroxytoluene protects against aflatoxicosis in Turkeys. Toxicol Appl Pharmacol. 2002 Jul 1;182(1):11-9.
170. Van der Zijden A.S.M., Koelensmid W.A.A.B., Boldingh J., Barrett C.B., Ord W.O., Philp J. Isolation in crystalline form of a toxin responsible for turkey X disease. Nature. 1962;195:1060–1062.
171. Varga,J., J. Frisvad, R. Samson. A reappraisal of fungi producing aflatoxins World Mycotoxin Journal: 2 (3) - Pages: 263 – 277 Published Online: April 28, 2009

172. Verma J., Johri T.S., Swain B.K., Ameena S. Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. Br. Poult. Sci. 2004;45:512–518.
173. Verma J., Johri T.S., Swain B.K. Effect of aflatoxin, ochratoxin and their combination on protein and energy utilisation in white leghorn laying hens. J. Sci. Food Agric. 2007;87:760–764.
174. Verma J., Swain B.K., Johri T.S. Effect of various levels of aflatoxin and ochratoxin A and combinations thereof on protein and energy utilisation in broilers. J. Sci. Food Agric. 2002;82:1412–1417.
175. Wannop, C.C. 1961. Turkey "X" disease. Veterinary Record 73: 310-311.
176. Warren M.F., Hamilton P.B. Intestinal fragility during ochratoxicosis and aflatoxicosis in broiler chickens.Appl. Environ. Microbiol. 1980;40:641–645.
177. Wilkinson, D. Rood, D. Minior, K. Guillard, M. Darre, and L. K. Silbart. IMMUNOLOGY AND MOLECULAR BIOLOGY Immune Response to a Mucosally Administered Aflatoxin B1 Vaccine. 2003 Poultry Science 82:1565–1572
178. Wilson, H.R.; Douglas, C.R.; Harms, R.H. and Edds, G.T. (1975): "Reduction of aflatoxin effects on quail." Poult. Sci., 54: 923-925.

179. Wolzak A., Pearson A.M., Coleman T.H. Aflatoxin carryover and clearance from tissues of laying hens.Food Chem. Toxicol. 1986;24:37–41
180. Wyatt, R.D. and Hamilton, P.B. (1975): "Interaction between aflatoxicosis and a natural infection of chickens with salmonella" Appl. Microbiol., 30: 870- 872.
181. Zhao J, Shirley RB, Dibner JD, Uraizee F, Officer M, Kitchell M, Vazquez-Anon M, Knight CD. Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. Poult Sci. 2010 Oct;89(10):2147-56.

4.2. Avian ochratoxicosis

Ochratoxicosis is one of the most common mycotoxicoses in poultry, caused by the most dangerous mycotoxins called ochratoxins. It is characterized by nephrotoxicity, hepatotoxicity and carcinogenicity. It occurs less frequently in poultry than aflatoxicosis but is more lethal because of its acute toxicity. **It is one of the causes of economic losses in poultry industry due to increased mortality, reduced body weight gain, reduction of carcass quality, greater feed conversion rate and immunosuppression.**

Ochratoxins are a family of toxic compounds consisting of three members, A, B and C, which are structurally related and are produced as secondary metabolites of several species of fungus. The name “ochratoxin” derives from ***Aspergillus ochraceus***, the first fungus discovered to produce this toxin

- Ochratoxin A (OTA) is the most commonly detected and most toxic member of the family.
- OTA is a common contaminant of cereals (corn, wheat, barley, oats, rye, sorghum) and peanuts, as well as soya, coffee and cocoa beans.
- Environmental conditions for ochratoxin production are similar to those for aflatoxin and simultaneous contamination with both is common (Pattison et al., 2008).

.

Ochratoxin A (OTA) producing fungi are members of the genera *Aspergillus* and *Penicillium.*

Ochratoxins-producing Aspergillus species

Nowadays, there are about 20 species accepted as OTA producers, which are distributed in three phylogenetically related but distinct groups of aspergilli of the subgenus *Circumdati*

- **Eight species consistently produce large amounts of ochratoxin A**:
    - *Aspergillus cretensis,*
    - *Aspergillus flocculosus,*
    - *Aspergillus pseudoelegans,*
    - *Aspergillus roseoglobulosus,*
    - *Aspergillus westerdijkiae,*
    - *Aspergillus sulphurous,*
    - *Neopetromyces muricatus*.

- **Two species produce large or small amounts of ochratoxin A, but less consistently:**
    - *Aspergillus ochraceus*

- *Aspergillus sclerotiorum.*

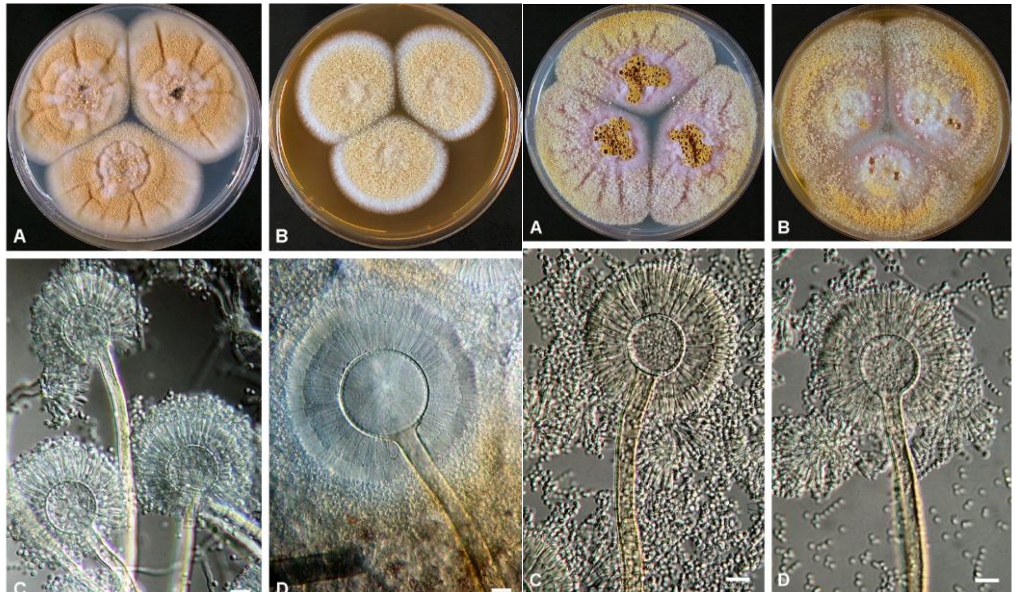
- **Further species that produce ochratoxin A inconsistently and in trace amounts according to the literature:**
  - *Aspergillus melleus,*
  - *Aspergillus ostianus,*
  - *Aspergillus petrakii,*
  - *Aspergillus persii.*

- **Ochratoxin A producing species of section Nigri**
  - *Aspergillus* carbonarius,
  - *Aspergillus* niger,
  - *Aspergillus* lacticoffeatus
  - *Aspergillus* sclerotioniger

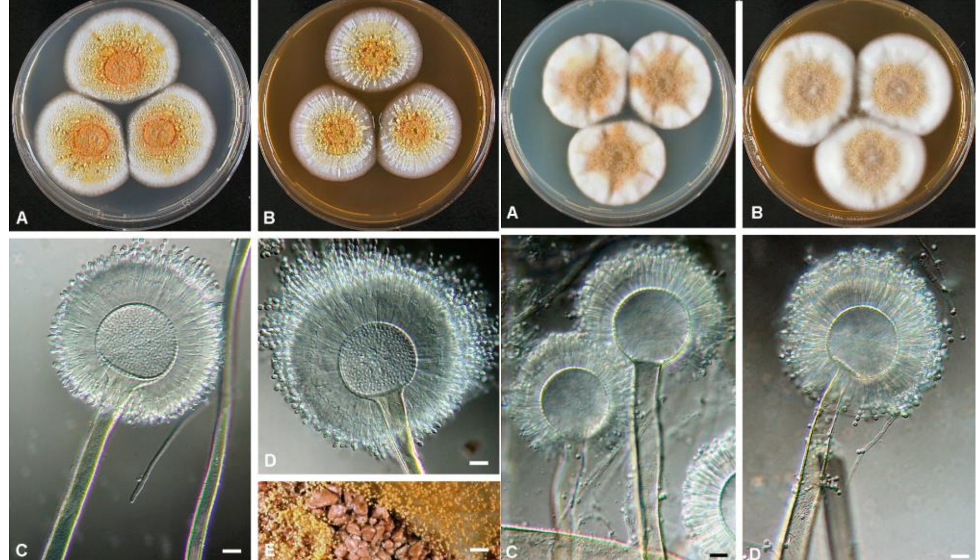
Species in Neopetromyces and Aspergillus subgenus Circumdati section Circumdati and their mycotoxin and mellein production. Species names in bold are newly described, **Visagie *et al.* 2014**

Species	Sclerotia	Ochratoxins	Penicillic acid	Xanthomegnins	Mellein
(*A. robustus*)	+	–	–	–	–
A. auricomus	+	–[1]	+	+	+
A. bridgeri	+	–	+	+	–
A. cretensis	+	+	+	–	+
A. elegans	+	–	–	+	–
A. flocculosus	+	+	+	+	+
A. insulicola	–	–	+	+	+
A. melleus	+	–[2]	+	+	+
A. neobridgeri	–	–	+	+	–
A. ochraceus	+/–	+/–	+	+	+
A. ostianus	+/–	–[2]	+	+	+
A. persii	+	–[2]	+	+	–
A. petrakii	–	–[2]	+	+	+
A. pseudoelegans	+	+	+	–	+
A. roseoglobulosus	+	+	+	+	–
A. sclerotiorum	+	+/–	+	+	–
A. steynii	+	+	–	+	+
A. sulphureus	+	+	+	+	–
A. westerdijkiae	+	+	+	+	+
N. muricatus	+	+	+	+	–

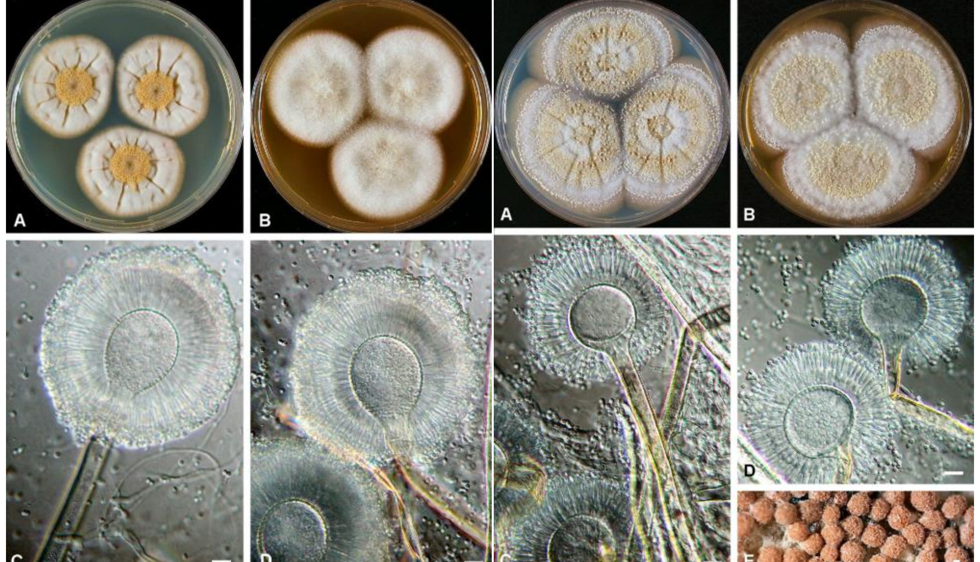
1 The strain A. auricomus FRR 3819, reported to produce ochratoxin A **by Varga et al. (1996)** was re-identified as Neopetromyces muricatus. 2 Type or authentic strains have been reported to produce trace amounts of ochratoxin A (Ciegler 1972, Hesseltine et al. 1972), but we have not been able to repeat the detection of OTA in those strains yet.



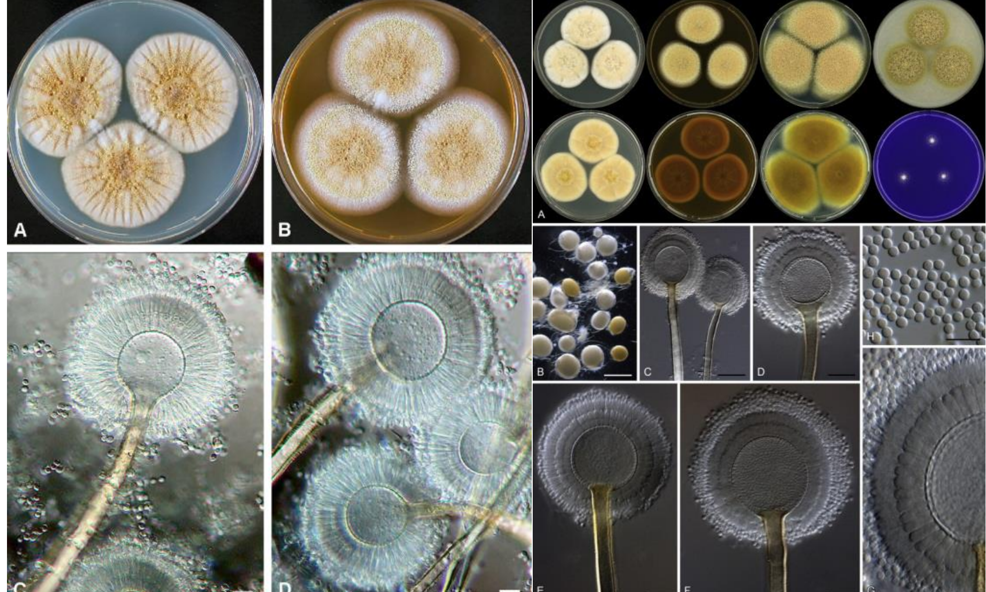
Aspergillus westerdijkiae. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. Aspergillus roseoglobulosus. Fourteen-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores **Visagie *et al*. 2014**



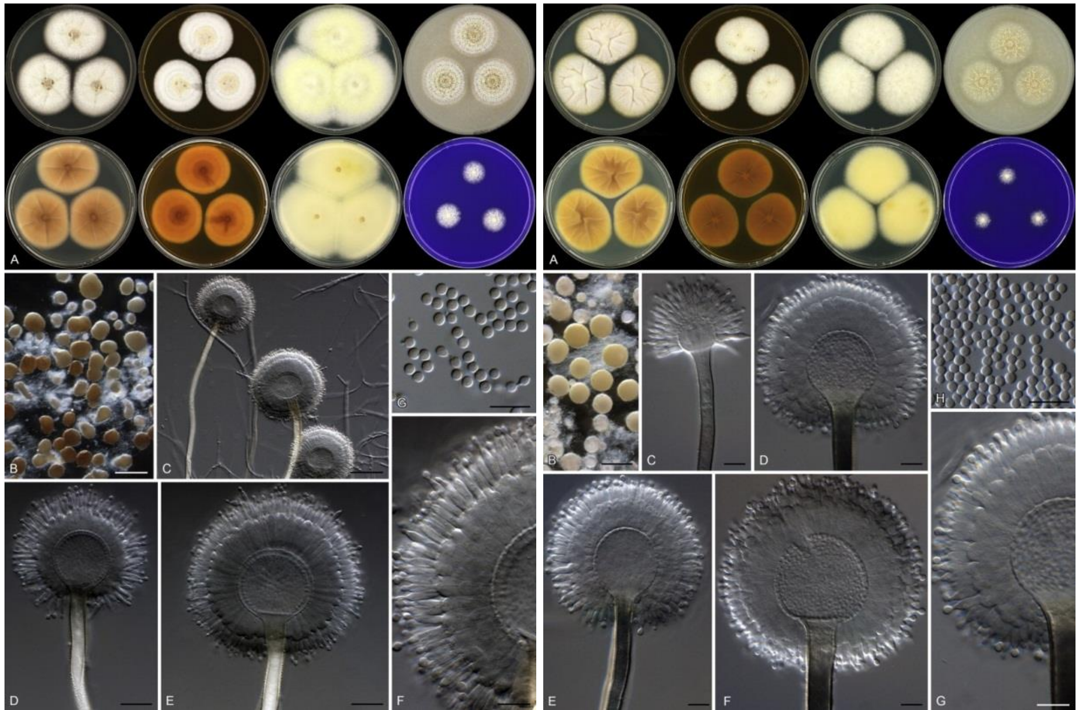
Aspergillus cretensis. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores Aspergillus flocculosus. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. **Visagie *et al*. 2014**



Aspergillus neobridgeri. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores.
Aspergillus pseudoelegans. Fourteen-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Detail of a 28- d-old colony showing sclerotia. **Visagie *et al*. 2014**

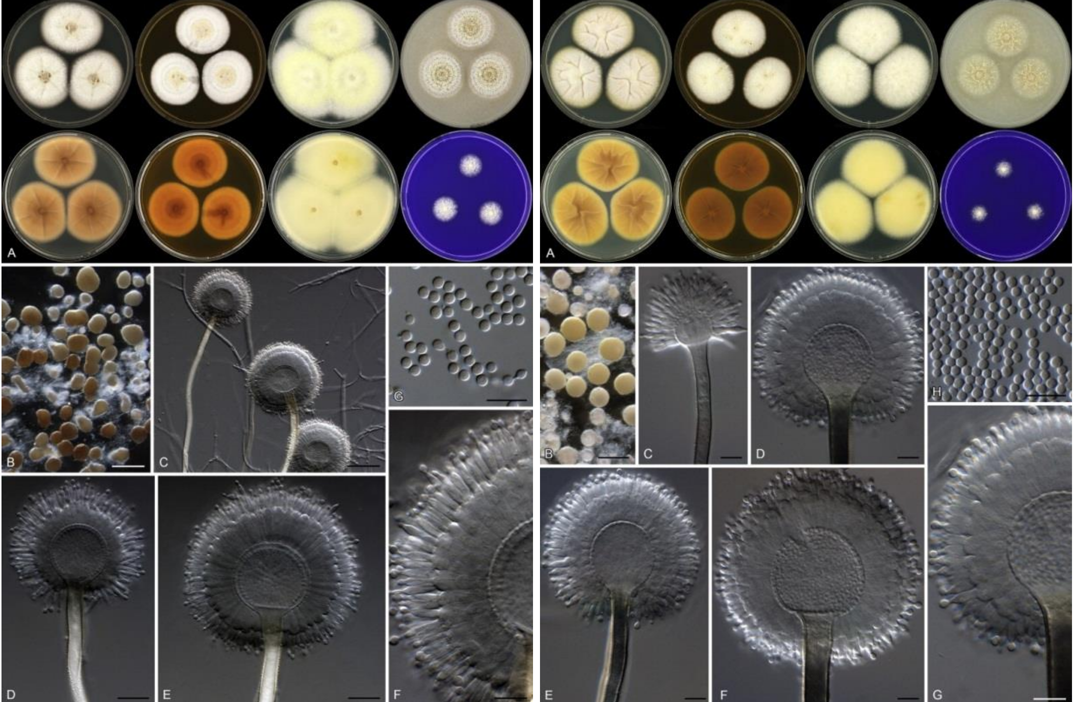


Aspergillus steynii. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores.
Aspergillus ochraceus. A. Colonies: top row left to right, obverse CYA, MEA, DG18 and OA; bottom row left to right, reverse CYA, MEA, DG18 and obverse CREA. B. Sclerotia. C–G. Conidiophores. H. Conidia. Scale bars: B = 1 mm; C = 50 μm; D–F = 20 μm; G, H = 10 μm.



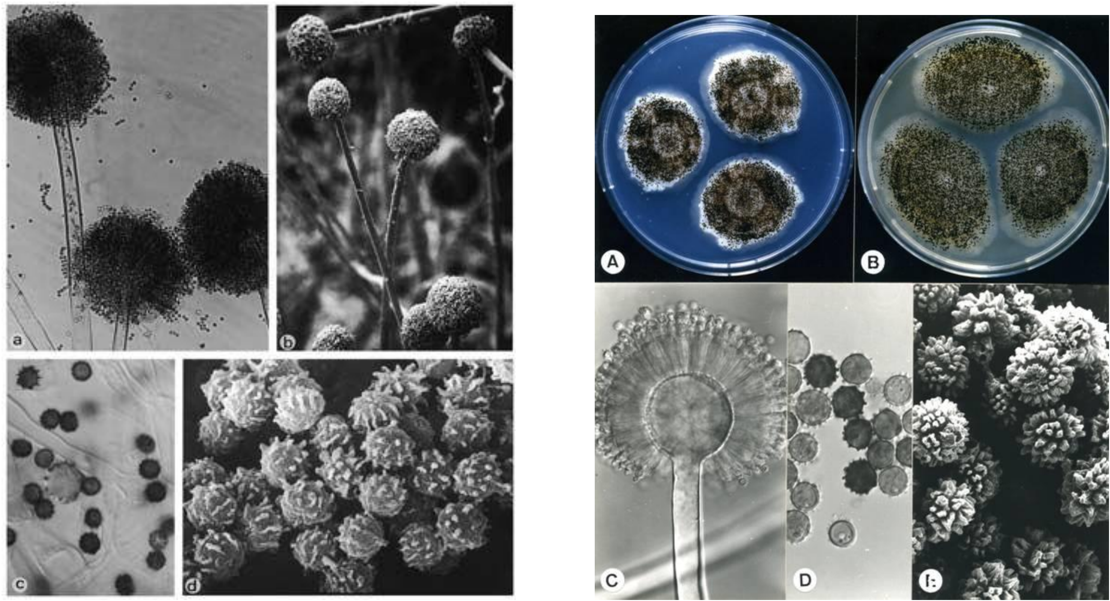
Aspergillus pseudoelegans. A. Colonies: top row left to right, obverse CYA, MEA, DG18 and OA; bottom row left to right, reverse CYA, MEA, DG18 and obverse CREA. B. Sclerotia. C–F. Conidiophores. G. Conidia. Scale bars: B = 1 mm; C = 20 µm; D–G = 10 µm.

Aspergillus muricatus. A. Colonies: top row left to right, obverse CYA, MEA, DG18 and OA; bottom row left to right, reverse CYA, MEA, DG18 and obverse CREA. B. Sclerotia. C–G. Conidiophores. H. Conidia. Scale bars: B = 500 µm; C–H = 10 µm. **Visagie *et al*. 2014**



Aspergillus pseudoelegans. A. Colonies: top row left to right, obverse CYA, MEA, DG18 and OA; bottom row left to right, reverse CYA, MEA, DG18 and obverse CREA. B. Sclerotia. C–F. Conidiophores. G. Conidia. Scale bars: B = 1 mm; C = 20 µm; D–G = 10 µm.

Aspergillus muricatus. A. Colonies: top row left to right, obverse CYA, MEA, DG18 and OA; bottom row left to right, reverse CYA, MEA, DG18 and obverse CREA. B. Sclerotia. C–G. Conidiophores. H. Conidia. Scale bars: B = 500 µm; C–H = 10 µm. **Visagie *et al*. 2014**



Aspergillus niger Mycoba *Aspergillus carbonarius* S. S. Tzean and J. L. Chen

Ochratoxins-producing Penicillium species :

- At present, *P. verrucosum* and *P. nordicum* are the only OTA producers known and accepted in this genus, despite some reports on OTA production by other species
    - *Penicillium casei* and *P. mediolanense* are synonyms for *P. verrucosum* and *P. nordicum*, respectively
    - Different examples of incorrect citations of some *Penicillium* spp. producing OTA (e.g., *P. cyclopium*, *P. viridicatum*, *P. chrysogenum*) have been recently listed
    - In the last century, OTA producers in this genus were classified as *P. viridicatum* for many years.
- Main species concepts for *P. viridicatum*, *P. verrucosum* and *P. nordicum* are summarized in Table

Main species concepts of OTA producing species in the genus *Penicillium*. Cabañes *et al.* 2010

References	Strains		
	OTA - and CIT-	**OTA + and CIT +**	**OTA + and CIT -**
Frisvad & Samson., 2004	*P. viridicatum*	*P. verrucosum*	*P. nordicum* *P. nordicum* II OTA?
Larsen *et al.*, 2001	*P. viridicatum*	*P. verrucosum*	*P. nordicum*
Frisvad & Filtenborg, 1989	*P. viridicatum*	*P. verrucosum* chemo.II	*P. verrucosum* chem. I

References	Strains		
	OTA - and CIT-	**OTA + and CIT +**	**OTA + and CIT -**
Pitt, 1987	*P. viridicatum*	*P. verrucosum* chemot.CIT	*P. verrucosum*
Pitt, 1979	*P. viridicatum*	*P. viridicatum*	*P. verrucosum*
Samson *et al.*, 1976	*P. verrucosum var. verrucosum*	*P. verrucosum var. verrucosum*	*P. verrucosum var. verrucosum*
Ciegler *et al.*, 1973	*P. viridicatum* I	*P. viridicatum* II	*P. viridicatum* III
Raper & Thom, 1949	*P. viridicatum*	*P. viridicatum*	*P. viridicatum*

OTA, ochratoxin A; CIT, citrinin; +, producing strains; - non producing strains.

www.mycobank.org

Clinical signs of avian ochratoxicosis

- unspecific clinical image of chronic ochratoxicosis
- decrease in egg production of laying hens,
- broilers growth is hindered and conversion of food is weakened.
- The egg shell often becomes thin and fragile, with different discoloration appearing on the surface.
- Growth inhibition is linked with malabsorption syndrome, as confirmed by the presence of hypocarotenoidemia.
- The minimum amount of ochratoxin also causes reduced bone firmness and poor pigmentation.

- Nephropathies are not clinically manifested, although polydipsia accompanied by a substantial amount of moist excrement appears.

- **Acutely intoxicated birds** are
    - depressed,
    - dehydrated and
    - often polyuric and
    - die in acute renal failure.
- **Survivors will be**
    - poorly feathered,
    - have delayed sexual maturity,
    - increased clotting times,
    - anaemia and immunosuppression.(Resanovic R, 2009)

Pathogenesis of avian ochratoxicosis

- After resorption, the highest quantity of ochratoxin can be found inside kidneys and liver and a considerably smaller extent in muscle.
- It is characteristic of poultry to have a more efficient and faster excretion of ochratoxins than other animals, approximately 48 hours.
- Ninety percent of the ingested OTA is excreted.

- OTA in poultry diets leads to
    - reduction in growth rate,
    - reduction in feed consumption and feed efficiency
    - increased mortality.
    - Alteration of the function of the immune system in avian species, causes:
        - severe leucocytopenia,
        - impaired complement activity,
        - reduction in immunoglobulin and
        - several functional properties of macrophages and heterophils and finally it causes
        - atrophy of the lymphoid organs along with
        - depletion of lymphocytes.
- OTA causes enlargement of the kidney and subsequently impairing its function, therefore, considered as a nephrotoxic mycotoxin in birds (http://ntp.niehs.nih.gov).
- OTA proximal tubular epithelial necrosis in the kidneys and
- OTA inhibits normal renal uric acid secretion.
- OTA inhibits respiration in mitochondria, where it acts as a competitive inhibitor of the carrier's proteins, localized on the inner membrane of mitochondria.
- OTA represents a teratogenic agent for chickens, but not for other domestic animals **(Bennett and Klich, 2003).**

Lesions of avian ochratoxicosis

- enlargement of the liver and kidney
- Affected kidneys are white to tan, swollen, hard and may have white pinpoint urate crystals.
- damage may be extensive enough to cause
  - renal failure,
  - dehydration,
  - hyperuricaemia
  - visceral urate deposition appears at kidney level.
  - Pasty white urates are deposited on pericardial, perihepatic, peritoneal and articular surfaces.
- More commonly, birds survive in compensated renal failure and kidneys appear enlarged, fibrotic and pale (**Biró *et al.*, 2002).**

- **Other lesions include**:
  - thickened basement membrane in the glomeruli
  - lymphoid depletion from the lymphoid organs.
  - mild to moderate glycogen deposition in hepatocytes, mainly at the periphery of the liver lobes at higher levels of dietary OTA (4 and 8 ppm), resulting in yellow enlarged livers.
  - mild decrease in bursal and thymic size consistent with immunosuppression.(**Herenda and Franco, 1996**).

Pathohistological changes associated with ochratoxicosis

- OTA caused marked degree of lymphocytic depletion and obscure distinction between red and white pulp were detected in some areas of the spleen. The lack of visible damage in heart and muscles indicates a low sensitivity of these tissues to OTA toxicity (**Dwivedi and Burns, 1984**)..
- OTA caused tubular dilatation and hypertrophy, swelling of tubular epithelial cells, localized necrosis, and desquamation of the tubular basement membrane as signs of tubulonephrosis (**Dwivedi and Burns, 1984**).
- OTA and penicillic acid (PA) intoxication in one hundred broiler chickens fed a diet containing 130, 300 and 800 ppb OTA and 1000-2000 ppb PA induced changes in the epithelium of the proximal tubules in the kidneys (slight edema and degenerative changes in capillary endothelium), slight changes in hepatocytes, and pronounced mitochondrial damage and loss of the membrane integrity of cell organelles leading to death (**Stoev, 2000a**).
- OTA induced an increase in the weight of the kidneys, liver, heart and ventriculum, a depletion of lymphoid tissue and a decrease in the lymphoid organs' weight and the body weight. The intensity of clinical signs, impairment of kidney functions, histopathological changes and deviations in growth depression were greater in chicks infected with E. tenella and OTA **(Stoev *et al.*, 2002)**
- Broiler chickens fed a mouldy diet containing 130, 300 or 800 ppb OTA and 1000-2000 ppb PA showed pathomorphological changes in the form of cloudy swelling and granular degeneration in the epithelium, mononuclear cell proliferation and the activation of capillary endothelium in the kidney and liver **(Stoev *et al.*, 2004)**.

- Birds exposed to OTA showed that the proximal tubules of the kidneys were prominently affected with microgranulation of cytoplasmic tubulocytes and masked nuclei. Morphological alterations in kidney samples of groups offered OTA in combination with modified clinoptilolite were expressed in intracellular edema (**Nedeljkovic-Trailovic *et al.*,2001**).
- The exposure of birds to 2 ppm OTA in the presence or absence of aluminosilicate reduced their humoral immune response and number of mitotic cells in the bursa. In the liver, microscopically, there was hepatocytes vacoulation and megalocytosis with accompanying hyperplasia of the biliary epithelium. Kidneys showed hypertrophy of the renal proximal tubular epithelium with thickening of the glomerular basement membrane (**Santin *et al.* 2002**).
- Glomerulonephrosis, tubulonephrosis, focal tubular epithelial cell proliferation and the multiplex adenoma-like proliferation of renal parenchyma are considered to be primarily related to the toxin, while focal intertubular infiltration of lymphocytes and histiocytes can also occur either primarily or secondly as reparation of tubulonephrosis or as a consequence of immune stimulation **(Elaroussi *et al.,* 2008).**

Host sensibility of avian ochratoxicosis

- Young poultry are more sensitive to ochratoxin ingestion than adults and
- Ducks are seven times more sensitive than chickens.
- Quail and turkeys are also more sensitive to ochratoxicosis than chickens.
- Variations in sensitivity towards OTA exists among avian species, as
    - LD 50 ranges from 0.5 to 16.5 ppm body weight for ducks and Japanese quail, respectively,
    - chickens 2-4 ppm **(Pattison et al., 2008).**

Effect of OTA on performance of broiler chicken

- Graded doses of OTA (0 to 4.0 ppm) given to broiler chicks for 6 weeks from hatching, resulted in depressed growth, poor feed conversion ratio, enlargement of the kidneys, liver, proventiculus, regressed spleen and bursa, and mortality. The minimum growth inhibiting level was 2 ppm (**Gibson *et al.,* 1990**).
- A group of 20 broiler chickens were fed a diet containing OTA alone at 0 or 2.5 ppm, or in combination with CPA for 3 weeks. A significant reduction in body weight gain was observed by the second week of feeding and continued at the third week (19 percent). The relative weights of the kidneys were increased in groups only fed OTA, and a significant increase in serum uric acid and triglycerides, but decreased total proteins, albumin and cholesterol were also seen (**Gentles *et al.*, 1999**).
- Significant growth depression, reduced feed consumption and poor feed conversion efficiency were recorded in broilers fed a diet containing the two

higher concentrations of AFB1 (1 and 2 ppm) and OTA (2 and 4 ppm) (**Verma *et al.*, 2004**)..

- Feeding OTA, even at low levels, as compared to previous studies, (at levels of 400 and 800 ppb) for 1-5 weeks of age resulted in a significantly decreased body weight, thymus weight, feed consumption, feed conversion ratio (FCR) and thyroxine concentration (**Elaroussi et al., 2006**).

Interaction of OTA with infectious diseases

- **Elissalde *et al.* (1994)** studied the effect of OTA (3 ppm) on ***Salmonella* Typhimurium** (1 x 106 cfu) challenged broiler chicks. S. Typhimurium alone had no effect on the variables measured except for the decrease in the body weight. With the exception of an increase in mortality and a decrease in body weight, Salmonella in combination with OTA did not alter the values of the remaining variables measured from those measured in the OTA diet alone.
- **Fukata *et al.* (1996)** revealed that OTA at the level of 3 ppm was observed to be one of numerous factors that affect the susceptibility of chicken to **Salmonella Typhimurium** colonization. The number of S. typhimurium in both duodenal and cecal contents of chickens administered high doses of OTA increased significantly when compared with control birds.
- **Stoev *et al.* (2002**) reported that deviations in the weights of some organs and the general depression in growth were greater when chicks infected with E. tenella were also given OTA.
- **Gupta *et al.* (2005)** mentioned that the mortality and severity of S. Gallinarum infection in broiler chicks was increased significantly by the presence of OTA in the diet.

Immune response to ochratoxicosis

- The immunosuppressive activity of OTA is characterized by the reduction in size of vital immune organs, depression of antibody responses, alterations in the numbers and functions of antibody responses, alterations in number and function of immune cells, and the modulation of cytokines production **(Al-Anati and Petzinger, 2006).**
- Immunotoxic effects of OTA after feeding a diet at 5 ppm for 56 days to broiler chicks revealed reduced contents of alpha1-, alpha2-, beta-, and gamma globulins in plasma (**Rupic *et al.,* 1978**).
- Ochratoxin A induced significant leukocytopenia, i.e. the reduction in white blood cell count, which they considered primarily a lymphocytopenia, and to a lesser extent, a monocytopenia (**Chang *et al*., 1979).**
- Suppression of bone marrow activity and lymphoid depletion from the spleen and bursa of Fabricius in young chicks, and the regression of the thymus in turkey poults were reported after an OTA treatment (**Chang *et al*.,1981**).
- Significant decrease in lymphoid cell population of immune organ was observed in broiler chicks fed a diet containing OTA at a concentration of 2-4 ppm for 20 days (**Dwivedi and Burns,1984a**).

- Depressed IgG, IgA and IgM levels were observed in the lymphoid tissues and serum of chicken fed diets containing OTA at a concentration of 2-4 ppm for 20 days(**Dwivedi and Burns,1984b**).
- The complement activity was slightly affected in birds fed diets containing 2 ppm of OTA for 5-6 weeks (**Campbell *et al.,* 1983).**
- Total lymphocyte counts, total serum proteins, serum albumin and serum globulin were significantly depressed on the twenty-first day of intoxication by dietary OTA (0.5-2 ppm), **Singh et al. (1990).**
- OTA at 0.5 and 1 ppm in the presence and/ or absence of a toxin deactivator on the histology of the bursa of Fabricius, liver and kidney reduced their humoral immune response to various vaccines (**Hanif and Muhammad, 2015**).

Vaccines

- **Stoev *et al.* (2000)** investigation revealed lower haemagglutination inhibiting antibody titers in chicks of groups (receiving 305 and 790 ppb OTA) immunized with the vaccine against Newcastle disease, than in the control group. A significant protective effect of artichoke extract on the humoral immune response and other clinical changes induced by OTA was established.
- **Raju and Devegowda (2002)** proved that esterified glucomannan significantly improved antibody titers, indicating its counteracting efficacy against immunosuppression in mycotoxicosis of multiple origins.
- **Santin *et al.* (2002)** revealed that exposure of birds to OTA, in the presence or absence of aluminosilicate, reduced their humoral immune response and the number of mitotic cells in the bursa.
- **Gounalan *et al.* (2006)** observed that the OTA fed birds were immunocompromised, even if adequately and predisposed to ND.
- **Koynarsky *et al.* (2007)** reported a rapid progress of coccidiosis occurred in OTA-treated turkey pouts than in those fed an OTA free diet. Coccidiosis in the presence of OTA further induced growth depression, impaired kidney functions, caecal hemorrhages and histopathological changes in certain body organs, as well as causing a depletion of lymphoid tissues.
- **Hanif and Muhammad (2015)** noticed that intoxication of OTA in broilers, significantly reduced HI titers of ND, IBD and HPS vaccine with declined weights of the bursa of Fabricius

Carry-over effect of OTA Ochratoxin

- Several studies determined and estimated OTA levels in the internal organs, blood, muscles, eggs of poultry, kidneys of cows and kidneys, liver, muscle, fat, blood of pigs (Table 2). As can be seen in this table, OTA tends to concentrate in tissues and organs.

- Residues of toxins in the tissue are influenced by various factors including the form of the toxin ingestion, health status of the animals, age and even the sex of animal.

- About 90% ingested OTA binds to plasma proteins that are why the elimination half life is more in the blood than any other organ (**Marquardt et al., 1997).**
- The tissue distribution in chicken follows the order kidney > liver > muscle > fat (**Harwing et al., 1983**).

Detection and estimation of ochratoxins

The ochratoxin content in food can be determined by analytical techniques such as:

- Thin layer, gas
- Liquid chromatography,
- Spectrofluorometry
- Spectrophotometry (**Talebi , 2011).**
- HPLC (high-performance liquid chromatography) still remains the technique of choice for aflatoxin and ochratoxin analysis. HPLC methods include HPLC with fluorescence detection and HPLC with near-ultraviolet, laser- induced fluorescence detection (near- UV LIF) (**Abbas, 2005).**
- ELISA test for poultry are available for identification of total aflatoxin and ochratoxin A.
- Detection of aflatoxin and ochratoxin residues in tissues requires 100 g of fresh or frozen liver or kidney.
- Samples for analysis should be placed in sealable plastic bags. Although not ideal, tissues from several dead birds can be pooled for analysis if necessary.(Ritchie, 1994);

Prevention and control of ochratoxin formation

- The best way to control ochratoxin formation is to prevent the growth of fungi on harvested and stored grains and other susceptible commodities.
    - Crops should be harvested at maturity
    - Pre- or post-harvest mechanical damage should be avoided.
    - Moisture contents of harvested crops should be reduced to a safe level.
    - Moisture build-up in the stored grain should be prevented by measures such as regular aeration.
    - Ochratoxin production can be decreased by storing food in a low oxygen, high-CO2 environment
- **Chemical detoxification methods**
    - **Activated charcoal (AC)**
        - AC was used in vivo and in vitro as an antidote for lethal doses of OTA. The findings revealed AC as an impractical method for reducing OTA toxicity in poultry chronically exposed to OTA (**Rotter et al., 1989**).
    - **Vitamin C**

        - All the negative effects of OTA, apart from body weight changes, reductions in feed intake, and increases in egg shell elasticity at 33°C were either moderated or significantly reversed by dietary ascorbic acid supplementation **Haazele et al., (1993).**
        - Better results were obtained in groups receiving a combination of vitamin E and C. In vitro mycotoxin adsorption capacity of 8 agents (**Huwig et al., 2001**)
    - **Hydrated Sodium Calcium Aluminosilicate (HSCAS)** from natural zeolite is the most widely studied mycotoxin sequestering agent among the mineral clay.
        - Aluminosilicate did not ameliorate the deleterious effects of OTA (**Santin *et al.*, 2002)**
        - OTA and their combination in feed given to 240 day old broiler chicks. revealed no protective effect but reduction in serum enzymes GGT and ALP was noticed (**Bhanuprakash *et al.*, 2006).**
- **Biological detoxification methods**
    - A statistically significant protective effect of 5% total water extract of an artichoke on humoral immune response (increase of haemagglutination inhibiting antibody titer), relative organ weight, as well as pathomorphological, hematological and biochemical changes induced by OTA was established **(Stoev *et al.,* 2000).**
    - Significant ameliorating effect of Bio-Bantox® was revealed on FCR and serum values of protein, albumin, globulin, albumin/globulin ratio, cholesterol and uric acid (**Pathan *et al*., 2006).**
    - The body weight, hemoglobin and total leukocyte counts, percentage changes in organ mass and impaired immune response were protected by Toxiroak (**Sakhare *et al*., 2007**).
    - improved performance of poultry by using a yeast cell culture based on the Saccharomyces cerevisiae strain 1026 was observd (.**Stanley *et al*., 1993**)
    - Beneficial effects of **esterified glucomannan** (E-GM 0 and 1 g/kg) on mycotoxicosis in broiler chickens by increasing the body weight (2.26 percent) and food intake (1.6 percent), decreasing weights of the liver (32.50 percent), adrenal (18.9 percent) and activity of serum GGT (8.70 percent), and elevating serum protein (14.7 percent), cholesterol (21.9 percent), BUN (20.80 percent) and hemoglobin (3.10 percent) contents were reported
    - Esterified glucomannan showed significantly higher binding with AFB1 (81.6 percent), whereas those recorded with T-2 (27.80 percent) and OTA (25.6 percent) were moderate (**Raju and Devegowda,2000)**

Reports

Van der Merwe *et al.* (1965) reported that the **oral LD50 o**f OTA in one day-old **ducklings** was 25µg. Later work using a larger number of ducklings suggested a higher value of approximately 150µg.

Doupnik and Peckham, (1970) fed OA 12 producing strains of Aspergillus to young broiler chicks. Two isolates of A. ochraceus grown on corn (final OA concentrations of 7.925 and 1.05 mg/kg) were highly toxic to day-old Babcock B-300 cockerels causing high mortality, whereas two other isolates were moderately toxic and resulted in growth suppression and low mortality.

Chu and Chang (1971) reported acute toxicity of OTA in poultry by several researchers. The LD 50 (lethal dose) of OTA given orally to seven-day-old New Hampshire Leghorn cross chicks was 166 µg

Peckham *et al.* (1971) reported acute toxicity of OTA in in seven-day White Leghorn cockerels. They gave ochratoxins A and B to 1-day-old Babcock B-300 cockerels to evaluate acute toxic effects. Two trials with ochratoxin A gave 7-day oral median lethal dose estimates of 116 ,g (3.3 mg/kg) and 135 ,ug (3.9 mg/kg) per chick. Chicks given daily oral doses of 100 ,g of ochratoxin A died on the second day. Single subcutaneous doses of 400 Mig of ochratoxin A were also lethal. The 7-day oral median lethal dose of B was estimated at 1,890 Mug (54 mg/kg) per chick. Chicks given oral doses of 100 ,Mg of ochratoxin B daily for 10 days survived. Sublethal doses of both ochratoxins A and B resulted in growth suppression which was proportional to the amount of ochratoxin given. Visceral gout was the principal gross finding. Microscopic examinations revealed acute nephrosis, hepatic degeneration or focal necrosis, and enteritis. Suppression of hematopoiesis in the bone marrow and depletion of lymphoid elements from the spleen and bursa of Fabricius were frequently seen. Both ochratoxins appeared to have similar pathological effects. This is the first report on the toxicity of ochratoxin B.

Trenk *et al.* (1971) studied the effects of duration of incubation and incubation temperature on production of OTA. For the maximum production of OTA, 28 ºC was considered as optimum temperature. At low temperature as 4 ºC, the levels of OTA produced were very low. The optimum duration of incubation depends on the substrate used; normally it ranged from 7 to 14 days at the temperature of 28 ºC. OTB and one of the hydrolysis products of OTA i.e. dihydroisocoumaric acid was also produced but at considerably lower than OTA in rice. No detectable levels of OTC were produced in rice at 28 ºC. Addition of OTA in oatmeal, rice and cereal remained quite stable for long time of storage and even to autoclaving for 3 hr. OTA in poultry feed

Choudhury and Carlson (1973) injected OTA into fertile chicken eggs to determine minimum lethal dosage. The approximate LD 50 were: for day 0 embryos- 0.04-0.05 µg, for day 6 embryo-less than 0.01 µg, for day 12 embryo 0.02 µg and for day 18 embryo 0.05-0.08 µg. Ochratoxin in eggs and tissues of poultry

Huff *et al.* (1974) reported that graded doses of ochratoxin (0, 0.5, 1.0, 2.0, 4.0, and 8.0 µg./g.) incorporated into the feed of broiler chickens from hatching until three weeks of age resulted in a decreased growth rate, enlarged kidney, crop, proventriculus, gizzard, and liver, while the bursa of Fabricius was regressed and the sizes of the heart, spleen, and pancreas were unaffected. The most sensitive indicators

of ochratoxicosis were reduced growth rate and enlarged kidneys which occurred at doses as small as 1.0 µg./g. The determination of LD_{50} values indicated that three-week old chickens (3.60 ± 0:57 mg./kg.) were more resistant than day old chickens (2.14 ± 0.37 mg./kg.). Observation of birds dying from acute ochratoxicosis revealed a progression of symptoms from listlessness, huddling, diarrhea, tremors and other nerual abnormalities, prostration, to death which occurred 22 to 25 hours after a single oral dose of 16 mg./kg. was given. Necropsy of these birds revealed food in the crop, proventriculus and gizzard. The lower intestine was empty of its normal contents. Petechiae of the papillae of proventriculus, slight gizzard erosions, and paleness of the liver, pancreas, and especially the kidney were the only gross pathological lesions noted. On the basis of LD_{50} dose and minimal growth inhibitory concentration, ochratoxin appears to be the most potent mycotoxin studied in chickens.

Elling *et al.* (1975) was the first to report spontaneously occurring nephropathy associated with OTA in poultry in Denmark describing gross and microscopic lesions in kidneys of the birds. The breast muscles of birds with renal lesions also contained residues of OTA.

HUFF *et al.* (1975) incorporated graded doses of pure ochratoxin A (0, 0.5, 1.0, 2.0, 4.0, and 8.0 ,g of toxin per g of feed) into a commercial diet which was fed to **chicks** from 1 day to 3 weeks of age, at which time the experiments were terminated. Growth was inhibited at 2.0, 4.0, and 8.0 ug/g, whereas the kidneys were enlarged at doses of 1.0 Mg/g and above. Renal function as measured by clearance of phenol red was decreased 15 and 31% by doses of 4.0 and 8.0 ig/g, respectively. Uric acid was increased 38 and 48% over the control values by doses of 4.0 and 8.0 jg/g, respectively. The plasma electrolytes Na, Cl, Ca, and K were measured; however, only K was significantly ($P < 0.05$) altered, showing a decrease at doses of 4.0 and 8.0 ,g/g. The percentage dry weight of the kidneys decreased significantly at dose levels of 4.0 and 8.0 ,g/g, indicative of edema. Histological examination of kidney sections gave the impression of edema and some tubular necrosis. Pathological changes were observed at all dose levels. These data demonstrate that ochratoxin A is a severe nephrotoxin in young broiler chickens.

Galtier *et al.* (1976) observed prostration, cachexia and **retardation of growth** in ten-day-old WL chicks given graded concentrations of OTA either as a single dose or by daily administration for ten days.

Krogh *et al.* (1976) studied the effect in young fowls by feeding subclinical levels of OTA in the diet 0.3 and 1mg/kg for 341 days. They observed impairment of glomerular and tubular functions, indicated by decreases in inulin clearance, in tubular excretion rate of para-aminohippuric acid and in urine concentrating capacity.

Prior *et al.* (1976) reported LD50 values of 3.4, 5.9 and 16.5 mg/kg body weight for WL chicks, turkeys and Japanese quail respectively. All the birds dying of acute ochratoxicosis showed symptoms of listlessness, huddling, occasional diarrhoea, ataxia and prostration.

Frye and Chu (1977) studied the kinetics of distribution of OTA in chicken tissues. Day old chicks were fed a diet containing 1mg/kg OTA for the period of 5 weeks. 27 After feeding OTA contaminated diet the chicks were intubated with 50 µg H-OA/chick. After 8 hrs of intubation the highest level of radioactivity was found in the liver and kidneys. Maximum levels of the OTA in the liver, kidneys and breast

muscles were 4, 12 and 0.2 µg/kg, respectively. More than 90 % of the radioactivity was eliminated at 48 hrs after intubation. In a separate experiment the laying hens were fed the diet containing 0.5 or 5.0 mg/kg of OTA for 2 weeks. Levels of OTA in the liver and kidneys were 80 and 124 µg/kg respectively while in leg, breast and eggs were 7, 8 and 2.8 µg/kg, respectively. Serum biochemical parameters Feeding of OTA in the poultry affects the various organs systems. Being the target organs kidneys and liver enzymes are severely affected by feeding of OTA. Below is the tabulated summary of the work of some researchers on the effect of OTA in the serum biochemical parameters.

Gilani *et al.* (1978) conducted experiments to study the teratogenic effects of OTA in the chick embryos. OTA at the dose of 0.005 to 0.007 mg/egg was injected in the embryonated hens egg through air cells at 48, 72 and 96 hrs of incubation while the control group was injected with propylene glycol. Teratogenic effects studied at the 8th day of incubation included the skeletal and visceral defects. The most common defects included anopthalmia, exencephaly, twisted limbs and ventricular septal defects.

Prior and Sisodia (1978) reported a significant reduction in egg production in the second to the sixth weeks as well as in egg weights in WL hens fed a diet containing 4 mg/kg OTA.

Page *et al.* (1980) conducted a trial in WL laying hens by feeding 0.5 and 1 mg/kg OTA in the diet for 3 weeks and found decreased egg production and increased serum uric acid levels, together with an increase in egg-shell stains.

Prior *et al.* (1980) reported lower feed consumption for the first five weeks of life and depressed growth was more marked in male than female broilers fed dietary OTA at the dose 2 mg/kg till 8 weeks of age from hatch.

Chang *et al.* (1981) found the oral LD50 for day-old and three week-old turkey poults to be 4.63 and 7.84 mg/kg, respectively while intraperitoneally, the values were 0.16 and 0.34 mg/kg, respectively.

Huff and Doerr (1981) noticed an enhancement of the toxic effects in the form of a more marked depression of growth, greater mortality and more damage to the kidney, although OTA inhibited the accumulation of lipids in the liver normally induced by AF.

Prior *et al.* (1981) in a study in WL hens on 4 mg/kg dietary OTA, did not find a decrease in egg production, though egg weights did decrease significantly in restricted feed intake and organoleptic trials, despite the severe growth depression caused by the reduced feed consumption.

Doerr and Campbell (1982) fed dietary OTA at 2 mg/kg and citrinin 400 mg/kg to broilers up to 3 weeks of age and observed enhanced toxicity which particularly affected serum protein, liver weight and growth. Feeding dietary OTA 3 mg/kg and tannic acid (1.5 per cent) to day-old broiler chicks for up to 26 days caused synergistic toxicity in terms of poor weight gain, decreased carcase pigmentation and poor efficiency of feed utilization (Kubena et al., 1983).

Hamilton *et al.* (1982) investigated 5 independent episodes of ochratoxicosis in about 970,000 **turkeys**, two episodes in about 70,000 **laying hens**, and two episodes in about 12,000,000 **broiler chickens**. Ochratoxin A concentrations in suspect feed and ingredients ranged from less than .2 to 16 ppm. Feed samples tested for T-2 toxin, F-2

toxin, heavy metals, and polychlorinated biphenyls were negative. Minor amounts of aflatoxin (less than 60 ppb) were found in suspect feed from two episodes. The main symptoms in turkeys were mortality (up to 59%), nephrotoxicity (pale, swollen kidneys that became tan colored in the sequel to acute toxicity), decreased feed consumption (as little as 20% of the normal feed intake) prior to death, and secondary air sacculitis. Histopathology revealed edema and necrosis of the proximal tubules of the kidneys and no changes in the liver or other organs. Suspect feed containing 2 ppm ochratoxin A increased uric acid levels in serum when fed to poults in the laboratory. The episodes in laying hens were characterized by reduced egg production, poor egg shell quality, and nephropathy. The episodes in broiler chickens were characterized by poor growth rate, poor feed conversion efficiency, poor pigmentation, nephropathy, and increased incidence of air sacculitis. Obtaining feed and ingredients free of ochratoxin, cleaning the feed and ingredient handling equipment, and adding antifungal agents to the feed proved beneficial. Eight of the 9 episodes were traced to the corn supply and the ninth episode was traced to corn gluten meal that became contaminated during storage after manufacture. Evidence was obtained that the ochratoxin was unstable and declined in concentration during storage. Aqueous acetone was a better solvent for extracting ochratoxin than was the recommended phosphoric acid: chloroform. The ochratoxin extracted from high potency samples consisted of ochratoxins A, B, and C in ratios of about 90:8:2.

Nelson *et al.* (1982) found that A. ochraceus contaminated corn containing 0.8 mg/kg OTA, when fed to 4 week-old chicks, reduced amino acid and dry matter digestibility, and energy utilization by chicks. When OTA in dietary concentrations of 0 to 4mg/kg, was fed to White Leghorn pullets from the age of 14 weeks up to 1 year, delayed sexual maturity, decreased egg production, depressed weight gain, increased feed consumption with decreased feed efficiency and severe mortality at the higher OTA levels, due to kidney and liver damage, were observed

Reichmann *et al.* (1982) did not find an adverse effect on growth, feed intake and serum enzyme levels or on the liver and kidney in broiler chicks fed 1mg/kg OTA and 1.5 mg/kg AF, alone or in combination. In an attempt to study the synergistic effects of two nephrotoxic mycotoxins, OTA and citrinin,

Campbell *et al.* (1983) studied the immune responses of 21 the broiler chicks fed OTA (0 and 2.0 µg/g) and AFB1 (0 and 2.5 µg/g) for 3 weeks of age from the day of hatching. Antibody titers against Brucella abortus and SRBC, and phagocytic potential of heterophils were not significantly affected in the any treatment group. The number follicle in the in the folds of bursa were significantly decreased along with the depression in the relative weight of bursa of Fabricius. Combination of toxins also leads to depression in the complement activity.

Tohala (1983), in a study of OTA toxicity in laying hens, given 0.25 to 2 mg/kg OTA for 12 weeks, observed reduced egg weight, increased egg spots and reduced specific gravity of eggs in a dose-dependent fashion. Water consumption, mortality rate and liver weights were increased at the highest level of OTA, and enlargement of the kidneys was seen at both 1 and 2 mg/kg OTA levels. The effects of graded dietary OTA levels of 0.3 and 1 mg/kg on renal functions were studied (Svendsen and Skadhauge, 1976). Glomerular filtration rates, renal concentrating abilities and plasma protein concentrations, were reduced in OTA fed birds.

Vesela *et al.* (1983) studied the toxic effects of OTA and citrinin by administering at different days of incubation. The dose at which embryotoxicity starts for OTA ranges

from 0.01 to 0.05 µg for OTA while for citrinin it ranges from 1 to 10 µg per egg. The maximum toxic response occurs in the embryos when the toxin was injected at day 3 of 25 incubation. Mycotoxins resulted various teratogenic effects including growth retardation, deformities of limbs, microphthalmia, cleft beak, and ventral septal defects.

Bodnarchuk and Kaspruk (1984) reported an outbreak of ochratoxicosis in ducks in Ukraine (with 42 per cent mortality after 2-6 days of illness. The feed was severely contaminated with A. ochraceus as well as OTA.

Dwivedi and Burns (1984) determined the immunoglobulin bearing cells in the sections of spleen, liver, kidneys and bursa of the OTA treated broiler chicks. Number of Igs containing cells were significantly reduced in the immune organs of the chicks fed OTA up to 4 mg/kg for 20 days.

Kubena *et al.* (1984) showed that the simultaneous administration of OA and penicillic acid to broilers resulted in an enhanced toxicity for a short period followed by recovery in the later stages. The main effect appeared to be retarded growth as they could not detect any gross lesions.

Manning and Wyatt (1984) compared the toxicity of wheat contaminated with A.ochraceus and of three different chemical forms of OTA (potassium salt, sodium salt or organic acid) in broiler chicks fed from hatching to 4 weeks at the rate of 3 mg/kg in the diet. All OTA contaminated diets caused the usual symptoms of depressed growth, dehydration, mortality varying from ten to seventeen per cent, enlargement of the liver and kidneys, decreased serum levels of total proteins, albumin, globulin, cholesterol and phosphorus and increased serum uric acid concentration. In a study of the combined toxicity of dietary OTA at 2 mg/kg and AF 2.5 mg/kg in birds up to 3 weeks of age,

Piskorska-Pliszezynska (1984) described a method for determination of OTA in the feeding stuff, animal tissues and eggs, based upon the thin layer chromatography with instrumental and visual detection. The method allows for accurate assay of 10µg of OTA per kilogram of the feed with 80% recovery. In the animal tissues and eggs 0.5 µg of ochratoxin per kilogram was determined and depending upon the detection method applied, recovery was 80-100 % and 57-60%, respectively

Dwivedi and Burns, (1985) studied the effects of OT on the immune system of turkey poults. Feeding OT at 4 mg/kg feed for 10 weeks caused regression in thymus, spleen and bursa of Fabricius. Cell-mediated immune responses, as measured by delayed hypersensitivity (DH) reactions to avian tuberculin and to BSA in pre-sensitised birds, were significantly depressed in OA-treated birds.

Harvey et al. (1987) administered OTA to in the chorioallantoic membrane of the chick embryos at the 13 days of incubation. The value LD 50 for OTA at the 20th day of incubation i.e. 7 days after inoculation was 7.9 µg per egg. The embryo inoculated with 2.5 µg had decreased but non significant, the number of immunoglobulin containing cells in the bursa of Fabricius. The hatched chicks were inoculated with 9 × 104 colony forming units Escherichia coli at the age of 7 days. Pathological effects of the inoculation of E. Coli were less severe or almost equal in the OTA treated embryos than control group, showing the no effect of in ovo OTA inoculation. Lesion

scores of OTA treated chicks were equal to or less severe than those of controls. To study the preventive effect of phenylalanine in the teratogenisity of OTA,

Rotter *et al.* (1989) studied the ability of activated charcoal to adsorb ochratoxin A (OA) in vitro and to reduce the toxic effects of OA in vivo when added to the diet of growing Leghorn chicks. Activated charcoal (50 mg) was able to adsorb 90% of the OA (150 micrograms) contained in 10 mL of citrate-phosphate buffer (pH 7.0). When 2 g of a complete chick diet were mixed with OA in buffer, it adsorbed 66% of the OA, while addition of 50 mg of charcoal to this mixture further reduced the concentration of OA to 11.8% of the control, an additional 65% compared to the diet alone. In the first of two feeding studies, charcoal addition of up to 10,000 parts per million (ppm) to diets (6.7% tallow) containing 9.93 mumol (4 ppm) OA kg-1 diet had no effect on OA toxicity. Feed consumption and weight gain, however, were reduced 10 and 20%, respectively, in chicks fed diets which contained 10,000 ppm of charcoal compared to those fed no charcoal. In the second study, reducing dietary tallow to 2% did not alter the effects of OA or charcoal on weight gain and feed to gain ratio, but birds fed OA with 10,000 ppm charcoal had an 8.5% increase in feed consumption. An additional management problem was associated with the propensity of charcoal to blacken the feed, the birds and their environment. Addition of charcoal to OA contaminated diets appeared to be an ineffective method for reducing the toxic effects of OA in growing chicks

Sreemannarayana *et al.* (1989) fed chicks diets containing 0, 1, 5 and 10 mg/kg ochratoxin A (OA) over a four-week period to study the effects of OA on growth, weight of internal organs, liver RNA, DNA, protein, and glycogen and serum enzymes. Ochratoxin A depressed the rate of growth and relative weight of the bursa of Fabricius, and increased the relative weights of the liver, kidney, pancreas, and various sections of the gastrointestinal tract, but had no effect on the heart and spleen. Concentrations of liver RNA, DNA and protein were decreased while glycogen was increased. Serum alkaline phosphatase and gamma-glutamyl transferase activities, and uric acid and creatinine concentrations were elevated while serum proteins, albumin, phosphorus, potassium, and cholesterol were depressed. The effects of OA were time- and dose-dependent.

Piskorska and Juszkiewicz (1990) administered laying Japanese quails (Coturnix coturnix japonica) single oral doses of 0, 1, 5 and 20 mg of OTA per kg of body weight. After 6, 12 and 24 hours and 2, 3, 4, 5, 6, and 7 days an appropriate number of birds were sacrificed and OTA in blood, muscles, liver, kidney, abdominal yolks and eggs was measured. The highest concentration of OTA was found in kidneys and the lowest in muscles. Four days following the application, OTA was still detected in kidney, liver, muscles, yolk, and eggs, although even after six days traces of OTA were found in the muscles of birds given 20 mg/kg. Fuchs et al. (1988) studied the tissue distribution of the OTA in laying Japanese quail by whole body autoradiography and scintillation counting using 14C-labelled toxin. Periodically for 8 days after one intravenous injection of OTA at the level of 70 ng/g body weight, birds were killed, frozen and sagittal sections of the whole body were placed on X-ray film. Specific retention of radioactivity was seen as a ring-like distribution in yolks and growing follicles. After sectioning, organs and intestinal contents were removed from

carcasses in a frozen condition, homogenized, extracted, chromatographed and the radioactivity in fractions was measured by scintillation spectroscopy. High concentrations of OTA were found in gastric intestinal contents, probably originating from toxin excreted in the bile.

Ayed *et al.* (1991) fed ochratoxin A at 0.5 ppm to Lohmann-type chicks from 7 d of age for 4 w. Body weights and efficiency of feed utilization were depressed and the activity of serum SDH and GDH and the concentration of uric acid were significantly increased. The concentration of serum total protein and potassium and Hb, PCV, RBC and WBC were significantly decreased in the test group. Lesions were seen in vital organs, with hemorrhage in the thigh. A slow recovery in the kidney was observed 3 w after removal from the experimental diet.

Abarca *et al.* (1994) reported for the first time the production of OTA by ***A. niger var. niger***. In a survey of the occurrence of OTA positive strains isolated from feedstuffs, two of the 19 isolates of A. niger var. niger that were studied produced OA in 2% yeast extract-15% sucrose broth and in corn cultures. Varga et al. (1996) tested one hundred and seventy two different species of the genus Aspergillus for the production of OTA. For the detection of OTA immunochemical method was in which monoclonal antibodies against OTA were used. OTA was detected in A. alliaceus, A. ochraceus, A. sclerotiorum, A. albertensis A. sulphureus, A. wentii and A. auricomus strains. This was the first report of production of ochratoxins in A. albertensis, A. wentii and A. auricomus species. For the confirmation of OTA high performance thin-layer chromatography (HPTLC) and by high-performance liquid chromatography (HPLC) was employed.

Niemiec et al. (1994) conducted a study on ochratoxicosis in laying hens by feeding 0, 2.1 and 4.1 mg OTA per kg feed. The daily feed intake decreased as the OTA contents increased and was 156, 135 and 105 g/day respectively. The toxin negatively affected the quality of eggs, especially thickness and crushing strength of the shell and 26 hatchability. The mass of embryo at 6 and 8 day of incubation and the hatched chicks was lowered in the experimental than in the control group. OTA was found in the eggs and also in the blood serum, liver and kidneys of hens, rooster and one day old chicks.

Leszkowicz & Castegnaro (1995) described the LD50 of ochratoxin as a function of the animal sensitivity and administration route, and determined 3.3mg/kg body weight as the 50% lethal dose for broilers when administered orally.

Wei and Sulik (1996) studied the effect of OTA injection on the air cells of fertile hens eggs 48 hrs after incubation. For this purpose they injected 1 µg OTA per egg and after six to twelve hrs cell deaths was observed in the selected cells population as determined by vital staining and histological procedure. No significant effects were found on the development of notochord. But degenerative changes were found on the presomatic mesoderm. After six days of treatment caudal dysgensis was observed in the 30% of the surviving embryos.

Shlosberg *et al.* (1997) reported that feeding of a shipment of imported corn was associated with a severe reduction in growth and increased mortality in geese, and increased mortality in broilers. Pathological examinations revealed hepatopathy, visceral gout and mild nephropathy in geese, and in broilers an hepatopathy, which

was often severe, and ascites. Samples of feed from affected geese farms were examined for up to 24 mycotoxins, and ochratoxin was found in 6 of 15 samples at levels up to 930 ng/g. The syndrome was experimentally reproduced by feeding geese and broilers suspect feeds with the natural ochratoxin contamination. It is believed that another, unidentified, mycotoxin was the major cause of the hepatotoxicity, and that ochratoxin served in this case as an indicator of a multi-mycotoxin involvement.

Gentles *et al.* (1999) evaluated the individual and combined effects of ochratoxin A (OA) and cyclopiazonic acid (CPA) in Petersen x Hubbard broiler chickens from 1 d to 3 wk of age. The experimental design was a 2 x 2 factorial with treatments of 0 and 2.5 mg OA/kg feed and 0 and 34 mg CPA/kg feed. Production performance, serum biochemistry, and gross pathological observations were evaluated. Body weight gain was reduced ($P < 0.05$) by OA, CPA, and OA-CPA in combination at the end of 3 wk. Ochratoxin A significantly increased the relative weight of the kidney and serum concentrations of uric acid and triglycerides and decreased total protein, albumin, and cholesterol. The toxicity of CPA was expressed primarily through increased relative weights of the proventriculus and increased activity of creatine kinase. Exposure to OA-CPA was characterized by increased relative weights of the liver, kidney, pancreas, and proventriculus; decreased concentrations of serum albumin, total protein, and cholesterol; increased activity of creatine kinase; and increased concentrations of triglycerides and uric acid. Postmortem examination revealed that the chickens fed CPA or OA-CPA had thickened mucosa and dilated proventricular lumen. Data from this study demonstrate that OA, CPA, and the OA-CPA combination can limit broiler performance and adversely affect broiler health. The interaction of the compounds was primarily additive or less than additive in the parameter in which the interaction occurred.

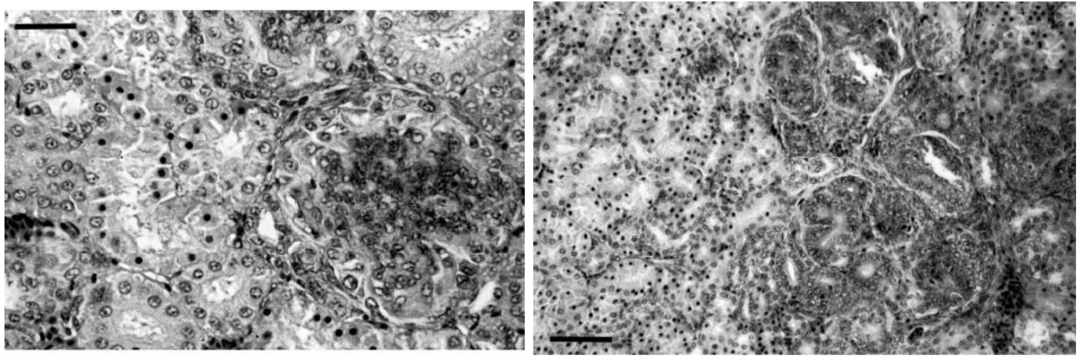
Raju *et al.* (2000) conducted a study to evaluate the individual and combined effects of aflatoxin B1 (AF), ochratoxin A (OA) and T-2 toxin (T-2) on performance, organ morphology serum biochemistry and haematology of broiler chickens and the efficacy of esterified-glucomannan (E-GM), a cell wall derivative of Saccharomyces cerevisiae1026 in their counteraction. 2. Two dietary inclusion rates of AF (0 and 0.3 mg/kg), OA (0 and 2 mg/kg), T-2 (0 and 3 mg/kg) and E-GM (0 and 1 g/kg) were tested in a 2 x 2 x 2 x 2 factorial manner on a total of 960 broiler chickens from 1 to 35 d of age in an open sided deep litter pen house. 3. Body weight and food intake were depressed by all the mycotoxins, OA being the most toxic during early life. 4. Weights of kidney and adrenals were increased by AF and OA. Liver weight was increased by AF (17.8%), while OA increased gizzard weight (14.6%) and reduced bone ash content (8.1%). T-2 toxin showed no effect on these variables. 5. Serum cholesterol content was decreased and activity of serum gamma glutamyl transferase (GGT) was increased by AF and OA while serum protein content was decreased by AF. These effects were more pronounced at 21 d than at 35 d of age. Inconsistent responses were seen in the other variables: blood urea nitrogen (BUN) content, activities of serum alanine amino transferase and aspertate amino transferase. Blood haemoglobin content was depressed by AF and T-2, whereas blood coagulation time was prolonged by OA. 6. Significant interactions were observed between any 2 toxins for their additive effects on body weight, food intake, bone ash content and serum GGT activity at 21 d. Conversely, antagonistic interactions were observed among any 2 of the toxins for their effects on variables such as serum protein and serum cholesterol content. Simultaneous feeding of all 3 mycotoxins did not show increased toxicity above that seen with any 2. 7. Esterified-

glucomannan increased body weight (2.26%) and food intake (1.6%), decreased weights of liver (32.5%) and adrenals (18.9%) and activity of serum GGT (8.7%), and increased serum protein (14.7%), cholesterol (21.9%), BUN (20.8%) and blood haemoglobin (3.1%) content, indicating its possible beneficial effect on mycotoxicosis in broiler chickens.

Stander *et al.* (2000) studied the effects of halogens salts on the production of OTA by A. ochraceus. Potassium fluoride and potassium iodide inhibited the growth of the fungus, whereas potassium chloride stimulated the production of OTA in shaken solid substrate fermentation on whole wheat or shredded wheat, generally giving a high yield of ochratoxins. Increasing levels of potassium bromide led to a decline in OTA production and an increase in bromo-ochratoxin B, ochratoxin B and 4-hydroxy ochratoxin B. Nevertheless, Asp. ochraceus was much less versatile in the bromo analogues than other fungi, which produce metabolites containing chlorine.

Bayman *et al.* (2002) collected 72 fungal isolates in A. alliaceus and A. ochraceus group, from nuts, figs and nut orchards. These fungi were grown in yeast extract sucrose broth 6 and potato dextrose broth at 30 °C for 10 days. A. melleus and A. ochraceus were the most common species among the isolates from tree nuts, orchards and figs. The field isolates of A. melleus and A. ochraceus did not produced OTA above the detection limit of 0.01 µg/ml.

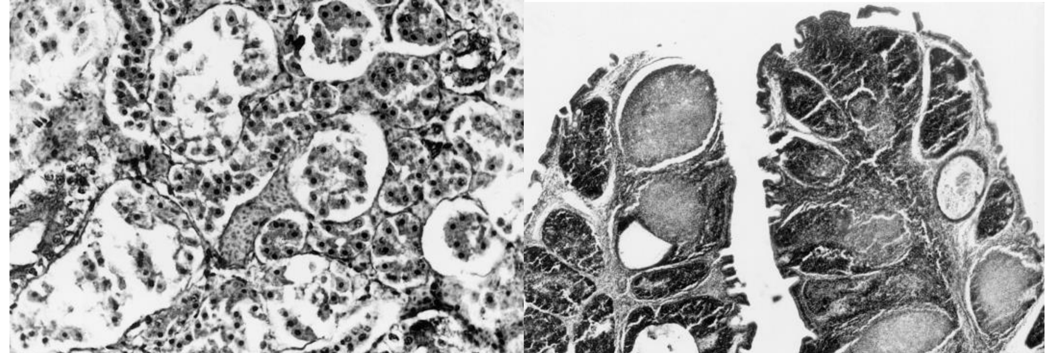
Biro *et al.* (2002) exposed broiler chicks to a total of 0.5 mg ochratoxin A per week for each of 4 weeks. Plasma toxin levels and tissue residues were measured by high-performance liquid chromatography (HPLC) and microplate enzyme-linked immunosorbent assay (ELISA). Results indicate an accumulation in plasma and wide distribution into all organs, with high levels in the liver and the kidney. Microscopical changes that could primarily be associated with toxin exposure were glomerulonephrosis, tubulonephrosis, focal tubular epithelial cell proliferation and multiple, adenoma-like structures in the renal parenchyma. The HPLC and ELISA methods gave similar results for both tissue distribution and depletion. Differences in absolute tissue toxin concentrations obtained by the two methods might be attributed to the different extraction and clean-up procedures, along with antibody specificity. The findings indicate that the dose applied causes subclinical tissue lesions and measurable tissue residues.



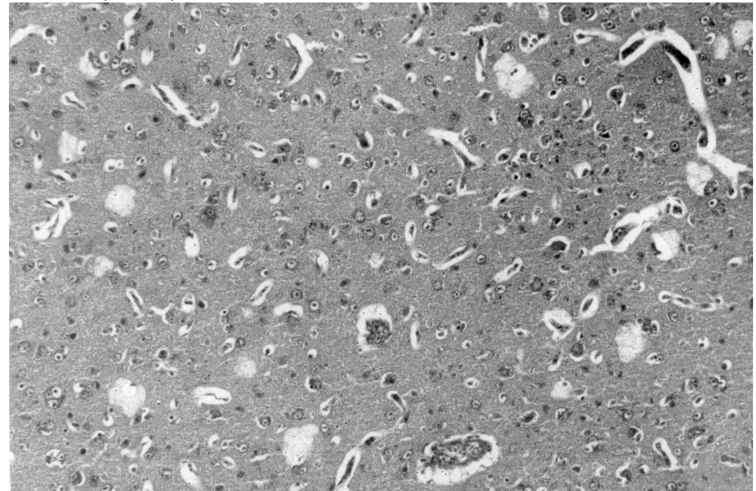
Histopathological changes in the kidney of toxin-treated animals after 4 weeks of ochratoxin A intake. Note the enlarged glomerulus, swollen capillary endothelial and mesangial cells (glomerulonephrosis), and degeneration of epithelial cells in the whole cross-section of proximal tubules (tubulonephrosis). HE, ´200 = 2 m., Kidney of experimental animals exposed to ochratoxin A for 28 days showing focal proliferation of tubular epithelial cellsand multiple adenoma-like structure. HE, ´100 = 4 m. **Biro *et al.* (2002)**

Santin et al. (2002) reported a depression of humoral immune response of broiler chicks by feed OTA at 2 mg/kg feed with or without aluminosilicate against NDV. 22 OTA and embryotoxicity

Stoev *et al.* (2002) carried out histopathological, biochemical and toxicological investigations of tissues and blood of normally slaughtered chickens exhibiting different frequencies (1–2%, 40–50% and above 80%) of nephropathy changes (congested or pale and enlarged kidneys) at the slaughtering meat inspection to elucidate the aetiology of nephropathies of chickens encountered in Bulgaria. A close relationship was observed between the frequency of this nephropathy and the rate of nephrotoxic mycotoxin ochratoxin A in muscles, kidneys and livers of chickens, but the levels of ochratoxin A in corresponding feed samples (0.1–0.3 ppm) were significantly lower than the levels (2–4 ppm) required to reproduce such nephropathy. Clinicomorphological changes such as nervous symptoms, vascular and oedematous changes in various internal organs and the brain, and subcutaneous or liver and kidney haemorrhages in addition to known degenerative changes in the kidneys, liver and lymphoid organs differed from the classical description of the nephropathy made in Scandinavia. The conclusion is that the Bulgarian chicken nephropathy may have a multitoxic aetiology because it cannot be explained by the concentration of ochratoxin A alone.



Photomicrograph of kidney in chicken with spontaneous mycotoxic nephropathy. Strong degenerative changes in epithelial cells of the proximal convoluted tubules. H/E. 260× .Photomicrograph of bursa Fabricii in chicken with spontaneous mycotoxic nephropathy. Degenerative changes, necroses and cyst formations in the lymph follicles. H/E.100× . **Stoev *et al.* (2002)**



Photomicrograph of brain in chicken with spontaneous mycotoxic nephropathy. A pericellular or pericapillary oedema and lytic changes in ganglionic and glia cells. H/E. 200× . **Stoev *et al.* (2002)**

Thirumala-Devi *et al.* (2002) assayed 216 ingredients intended for incorporation into chicken feed, which included groundnut cake, maize, millets, rice bran, sorghum, soybean, sunflower, and mixed feeds, for aflatoxins and ochratoxin A contamination using an indirect competitive enzyme-linked immunosorbent assay. Thirty-eight percent of the samples were contaminated with aflatoxins and 6% with ochratoxin A. The incidence scores of aflatoxin contamination in excess of 10 microg/kg were 41 of 95 for maize, 18 of 30 for mixed feeds, 10 of 37 for groundnut, 6 of 29 for sorghum, 5 of 10 for sunflower, 3 of 14 for rice bran, and 1 of 8 for millet. Ochratoxin A contamination, in excess of 10 microg/kg, was found in 9 of 29 sorghum samples, 1 of 27 groundnut samples, 1 of 14 rice bran samples, 1 of 10 sunflower samples, and 2 of 8 millet samples. Ochratoxin A was not found in maize and mixed feeds. None of the three soybean samples contained ochratoxin A. This is the first report, to our knowledge, of co-occurrence of aflatoxins and ochratoxin A in Indian poultry feeds. The results confirm the importance of analysis of ingredients before incorporating them into mixed feeds.

Garcia *et al.* (2003) conducted both in vitro and in vivo trials with broilers in order to assess ochratoxin A (OA) and T-2 toxin (T-2) binding ability of two commercial sorbents,. Crude OA and T-2 extracts from contaminated grain were used to assess in vitro binding ability of two sorbents (Zeotek [Zk] and Mycofix [Mx]), by quantifying free mycotoxin through an enzyme-linked immunosorbent assay (ELISA) test. For in vivo trial, a 3 x 2 x 2 factorial arrangement was used for this experiment, being the factors: adsorbents (none, Zk, and Mx), OA (0 and 567 parts per billion [ppb]) and T-2 (0 and 927 ppb). OA and T-2 contaminated wheat and corn, respectively, were added to sorghum-soybean meal diets to meet 567 ppb of OA and 927 ppb of T-2. Mycotoxins were fed alone or combined in treatments. After 21 days, blood chemistry, gross, and histological evaluations were performed. Relative weights of liver, kidney, and bursa of Fabricius were obtained. Zk had the highest OA and T-2 in vitro binding ability (100% and 8.67%, respectively). Chickens fed OA with or without sorbents had a lower body weight and feed intake reduction. However, those birds fedT-2 were partly protected by a sorbent. Birds fed both toxins showed toxic additive effects, and no protection of any adsorbent was observed. A significant reduction in plasma proteins, albumin, and globulins was a characteristic observed in all birds fed diets with OA both with or without adsorbents. Uric acid level in blood was increased in all chickens fed OA-contaminated diets. Histological findings observed in birds fed OA-contaminated diets were necrosis of kidney tubular cells, swollen and necrotic hepatocytes, bile ducts hyperplasia, and increased diameter of proventriculus glands. In birds that received T-2 alone, only the liver, with the same kind of lesions, was affected. According to these results, it can be concluded that there is not a relation between in vitro and in vivo trials. OA toxic effects could not be counteracted by any sorbent. T-2toxicity could be partially counteracted by an adsorbent used in this research.

Sur and Celik, (2003) administered AFB1 in fertile hens eggs and studied its effects on the bursa of Fabricius. Embryonic mortalities were significantly higher in the groups administered AFB1 compared with control. Highest mortalities were found in the group injected with 10 and 20 ng AFB1 per egg at HH scale 25 while in the group injected with 40 ng AFB1/egg most of the embryonic mortalities occurred at early stages i.e. HH scale 20. At the 7th day of incubation no developmental effect were found on the bursa while at 10th day of incubation impairment of bursal development was found in the group injected with 10, 20 or 40 ng of AFB1 per egg. All the chicks

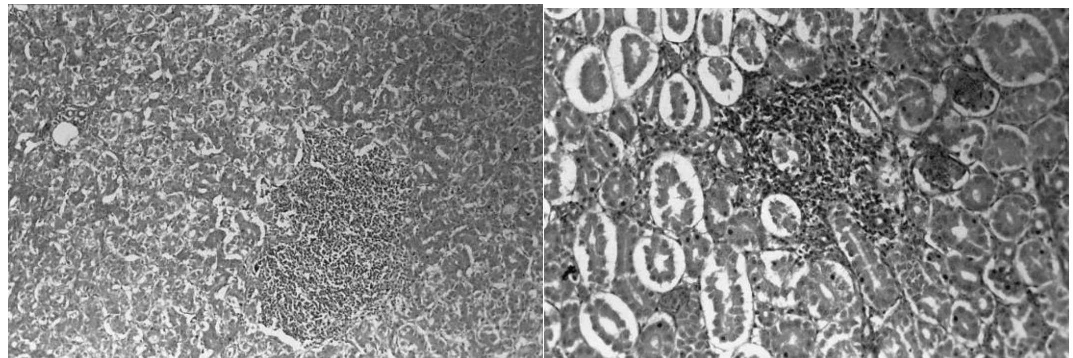
hatched from the intoxicated groups showed poor development of bursae compared with control.

Frisvad *et al.* (2004) reported that Aspergillus section Circumdati contains species with yellow to ochre conidia and non-black sclerotia that produce at least one of the following extrolites: ochratoxins, penicillic acids, xanthomegnins or melleins. The exception to this is A. robustus, which produces black sclerotia, phototropic conidiophores and none of the extrolites listed above. Based on a polyphasic approach using morphological characters, extrolites and partial β-tubulin sequences 20 species can be distinguished, that, except for A. robustus, are phylogenetically and phenotypically strongly related. Seven new species are described here, A. cretensis, A. flocculosus, A. neobridgeri, A. pseudoelegans, A. roseoglobulosus, A. steynii, and A. westerdijkiae. Twelve species of section Circumdati produce mellein, 17 produce penicillic acid and 17 produce xanthomegnins. Eight species consistently produce large amounts of ochratoxin A: Aspergillus cretensis, A. flocculosus, A. pseudoelegans, A. roseoglobulosus, A. westerdijkiae, A. sulphurous, and Neopetromyces muricatus. Two species produce large or small amounts of ochratoxin A, but less consistently: A. ochraceus and A. sclerotiorum. Ochratoxin production in these species has been confirmed using HPLC with diode array detection and comparison to authentic standards. Four further species produce ochratoxin A inconsistently and in trace amounts according to the literature: A. melleus, A. ostianus, A. petrakii, and A. persii. The most important species regarding potential ochratoxin A production in coffee, rice, beverages and other foodstuffs are A. ochraceus, A. westerdijkiae and A. steynii.

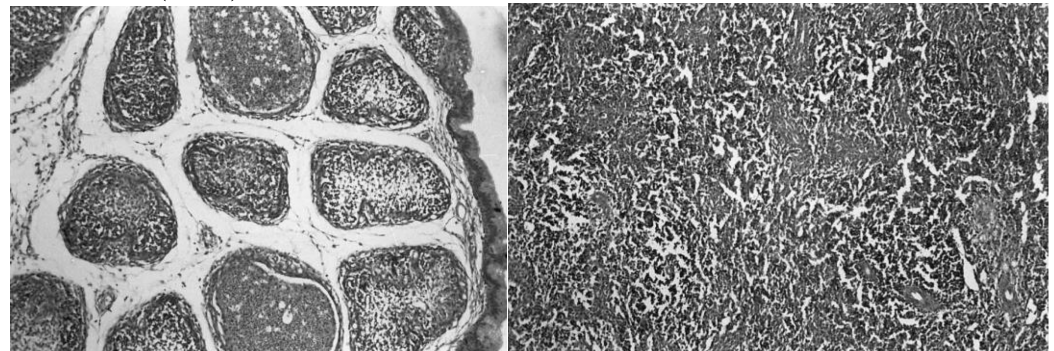
Joo *et al.* (2004) investigated the effects of dietary contamination with various levels of ochratoxin A (OTA) and potential preventive action of mycotoxin-deactivation product on two hundred one-day-old male broiler chicken. The birds were divided into 20 groups (5 treatment x 4 replication x 10 bird each) and fed 5 different diets for 5 weeks. Group 1: control (OTA free); group 2: OTA (1 mg/kg) without mycotoxin deactivator; group 3: OTA (1 mg/kg) with addition of mycotoxin deactivator at 0.2 % of the diet; group 4: OTA (2 mg/kg) without mycotoxin deactivator; group 5: OTA (2 mg/kg) with mycotoxin deactivator at 0.2 % of the diet. As dietary OTA increased, feed intake and weight gain were gradually and significantly decreased. These negative effects were partially counteracted by feeding the mycotoxin deactivator. The relative weights of liver and kidney, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the groups fed diets containing OTA alone were significantly higher compared to the control group. The level of serum total-cholesterol was significantly reduced by feeding OTA contaminated diets. As dietary OTA increased, the levels of OTA in liver and kidney tissue were significantly higher. Presence of mycotoxin deactivator in contaminated diets significantly decreased the OTA accumulation in organs. Moreover the fecal excretion of OTA and its metabolite OTα were significantly increased by feeding the mycotoxin deactivator. These results demonstrated that feeding the mycotoxin deactivator reduced the organ accumulation of OTA and OTA - induced performance reduction. In conclusion the contents of OTA in liver and kidney tissue were found to be a suitable indicator of OTA presence in broiler feed.

Kumar *et al.* (2004) conducted a study to evaluate the effects of ochratoxin A (OA) on Escherichia coli-challenged broiler chickens. One hundred and eighty-four one-

day-old broiler chicks were divided into two groups of 92 chicks each, with one group fed a control mash diet and the other fed a mash diet containing 2 parts/106 OA. On day 14, each group was further subdivided into two groups with one group inoculated with E. coli O78 (1/107 colony-forming units/0.5 ml) by the intraperitoneal route, whereas the other group was not inoculated with E. coli. Four birds from each group were sacrificed at 1, 2, 3, 5, 7, 10, 14 and 21 days post-inoculation to record pathological changes in the liver, kidneys, heart, lungs, bursa, spleen and thymus. E. coli infection induced perihepatitis and pericarditis in the liver and heart, respectively, in chickens infected with E. coli alone or in OA fed birds from 1 day post-infection (DPI) onwards. At 1 DPI, a thin fibrin layer covered the liver and heart; however, at subsequent days, the layer became thicker. E. coli infection did not produce appreciable changes in the kidneys, bursa or thymus. However, there was congestion of the lungs along with mononuclear cell infiltration. Ochratoxin feeding induced changes from 10 DPI onwards in chicks fed OA alone and those infected with E. coli. The changes in kidneys included swollen proximal convoluted tubules, degeneration of tubular epithelium and interstitial nephritis. Degenerative changes and mononuclear cell infiltration were recorded in the liver. There was atrophy of the lymphoid organs along with depletion of lymphocytes. Gross and histopathological changes were more severe in chickens fed OA and inoculated with E. coli than the chickens fed OA alone or those infected with E. coli, indicating combined action of these two.



Photomicrograph of the liver of a bird fed OA (group OX) showing mononuclear cell infiltration at 14 DPI (28 days of age). Haemotoxlyin and eosin _ 100. Photomicrograph of the kidney of a bird fed OA(group OX) showing swollen tubules and focal interstitial nephritis characterized by lymphocytic infiltration around atrophied tubules at 21 DPI (35 days of age). Haemotoxlyin and eosin _ 200. **Kumar *et al.* (2004)**



Photomicrograph of the bursa of Fabricius of a bird fed OA (group OX) showing atrophy of the bursal follicles, increased interfollicular space and lymphoid depletion at 21 DPI (35 days of age). H&E _ 100 Photomicrograph of the spleen of a bird given OA and E. coli infection (group OE) showing RE cell hyperplasia and mild lymphoid depletion at 7 DPI. H&E _ 100. **Kumar *et al.* (2004)**

Moura ***et al.*** **(2004)** evaluated alterations in the qualitative cellular profile of leukocytes caused by the administration of low doses of ochratoxin-A (OTA) in poultry. Sixty chicks were separated in three experimental groups: control, PBS-treated and OTA-treated. Blood smears from all birds were analyzed three and six hours post-treatment. Differential leukocyte counting demonstrated that OTA reduced the percentage of lymphocytes and eosinophils and significantly increased the number of heterophils and monocytes.

Nedeljković-Trailović ***et al.*** **(2004)** designed a study to examine the harmful effects of low level ochratoxin A (OTA) for different period and different dietary OTA levels for same period of ingestion as well as to assess the resting period necessary to revoke any adverse effects of OTA. The trial was performed on 72 day-old chickens with 12 broilers in each group. The experimental groups were offered feed contaminated with 0.5 ppm OA during 7, 14 or 21 days in the first evaluation, and feed contaminated with 0.5, 1.0 or 1.5 ppm OTA for 7 days in the second evaluation. The control group (K) received feed free of toxin. Broilers in the control groups had an average daily gain of 43.5 g and a feed: gain ratio of 1.97 kg. OTA expressed a negative effect on performance proportion to the time of exposure and to the amount of dietary toxin showing prolong and cumulative effects. Early detection of OTA and exclusion of contaminated feed could partially prevent its adverse effects, but not less than three weeks of recovery is needed to nullify the damage.

Samson ***et al.*** **(2004)** mentioned that, Aspergillus section Nigri includes some of the most important species for biotechnology and its species are of widespread occurrence. During our surveys of various food products and tropical soil they isolated several aspergilli belonging to section Nigri. In this paper, four new sclerotium and/or ochratoxin A producing species belonging to this section are proposed. In addition, based on a polyphasic approach using traditional characters, extrolites and β-tubulin sequencing, a provisional revision and synoptic key of section Nigri is proposed. Aspergillus costaricaensis was isolated from soil in Costa Rica and produces large pink to greyish brown sclerotia. Aspergillus lacticoffeatus was found on coffee beans in Venezuela and Indonesia, and is an effective producer of ochratoxin A. Aspergillus piperis was isolated from black ground pepper and produces large yellow to pink brown sclerotia. Aspergillus sclerotioniger was isolated from a green coffee bean and produces large yellow to red brown sclerotia and abundant ochratoxin A. The species A. homomorphus is validated. The ochratoxin A producing black aspergilli are revised. Fifteen species are provisionally accepted in Aspergillus section Nigri, four of these produce ochratoxin A. Ochratoxin A producing species of section Nigri occurring on grapes, raisins and in wine include A. carbonarius and to a lesser extent A. niger. Four species recovered from coffee, viz. A. carbonarius, A. niger, A. lacticoffeatus and A. sclerotioniger, all produce ochratoxin A, but other species of Nigri also occur on this substrate, including A. japonicus and A. tubingensis. The 10 species not producing ochratoxin A are especially interesting for biotechnological exploration, as many other extrolites are produced by these species.

Verma ***et al.*** **(2004)** studied the effects of dietary ochratoxin, aflatoxin and their combination on the humoral and cell mediated immune response in broilers. Cell mediated immunity was significantly reduced in the chicks fed AFB1 at the dose of 2

mg/kg feed and OTA at the dose of 4 mg/kg feed in combination. Antibody titer against SRBCs was also significantly lower in the group fed higher doses of OTA alone or in combination with AFB1.

Alvarez et al. (2005) studied the toxic effect of OTA (0.5, 2, 20 ppm) in lymphoproliferative response, natural killer (NK) cell activity, 20 cytotoxic T lymphocytes (CTL) activity and macrophages bacteriolytic capability in vitro after 1 hour of treatment. The proliferative response of lymphocytes to concanavalin A and lipopolysaccharide was not affected by OTA; the cytotoxic activity of NK cells was dose-dependent decreased; the CTL activity was significantly decreased at the lowest concentration; the bacteriolytic activity of macrophages varied only slightly.

Gupta *et al.* (2005) conducted a study to evaluate the effects of ochratoxin A (OA) on broiler chicks challenged with Salmonella gallinarum. One hundred and seventy-six 1-d-old broiler chicks were divided into two groups of 88 chicks each, with one group fed on a control mash diet and the other given a mash diet containing 2 ppm OA. On d 14, each group was further subdivided into two groups with one group infected with S. gallinarum and the other uninfected. Following S. gallinarum inoculation on d 14, 4 birds from each group were killed at 1, 2, 3, 5, 7, 10, 14 and 21 d post inoculation. S. gallinarum infection caused dullness, depression, weakness, increased thirst, droopy wings, ruffled feathers and greenish-yellow diarrhoea. S. gallinarum infection in the absence of OA caused 11.5% mortality which increased to 28.8% in the presence of OA. 5. Decreased body weight and reduced feed intake were observed in chicks fed on the diet containing OA. S. gallinarum infection also reduced the body weights of chicks, with the effects being more marked in chicks receiving OA. The OA diet led to increased serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, uric acid and creatinine, and decreased levels of total proteins, albumin, globulins, calcium and phosphorus. S. gallinarum infection did not cause significant alteration in any of the serum biochemical parameters. 6. Mortality and the severity of S. gallinarum infection in broiler chicks were increased by the presence of OA in the diet.

Kalorey *et al.* (2005) conducted an experiment to study the protective role of polyherbal feed supplement (Growell) during induced mycotoxicosis in broilers. A total of 240 Vencobb broilers were divided at day old stage into eight equal groups. Group A served as control and was given plain feed, group B, D, F and H were given Growell at 0.35 g/kg of feed. Group C, D, G and H were given dietary aflatoxin B1 at 0.2 ppm and groups E, F, G and H were given ochratoxin A at 0.2 ppm in feed to study effect of Growell on individual aflatoxicosis, ochratoxicosis and combined mycotoxicosis of broilers. The chicks were given their respective feeds from 1st day to 6th week of age and were vaccinated at 7th and 28th day of age with Lasota strain of Newcastle disease virus. There was no statistically significant effect of mycotoxins individually or in combination on body weight of broilers. However, body weights were highest in group B and lowest in co-mycotoxicated group G. Feed conversion ratio was best in group B followed by A, D, F, E, H and G. Significant improvement in haemoglobin values was observed in broilers due to feeding of Growell in ochratoxin and co-mycotoxicated groups. There was no significant effect of mycotoxin treatment on PCV, TEC and TLC of broilers. Due to single and combined mycotoxicosis, there was reduction in serum total protein, albumin, cholesterol and triglyceride and rise in alkaline phosphatase, creatinine and uric acid levels. Supplementation of diets with Growell reduced the alterations induced due to mycotoxins. There was a significant rise in per cent organ weight of liver and

reduction of that of spleen, bursa of Fabricius and thymus of broilers fed mycotoxins. Protection from alteration in per cent organ weight of these organ by supplementation of Growell was recorded. The observed impaired immune response and histopathological changes in liver, kidney, spleen, bursa of Fabricius and thymus of broilers given mycotoxins were protected by supplementation of Growell.

Beg *et al.* (2006) conducted a survey in Kuwait for mycotoxins contamination in the poultry feed ingredients. Results showed average AF concentration in soybean meal at 0.20 µg/kg (range 0 to 1.27 µg/kg), maize at 0.27 µg/kg (range 0 to 1.69 µg/kg), wheat bran at 0.15 µg/kg (range 0 to 1.07 µg/kg), broiler 7 finisher at 0.39 µg/kg (range 0 to 1.05 µg/kg), prepared poultry feed for broiler starter at 0.48 µg/kg (range 0 to 3.26 µg/kg) and layer mash at 0.21 µg/kg (range 0 to 1.30 µg/kg). The average levels of **fumonisin** from 1.4 to 3.2 mg/kg, **OTA** ranged from 4.6 to 9.6 µg/kg, **DON** from 0.17 to 0.29 mg/kg **zearalenone** from 46.4 to 67.6 µg/kg in various feed ingredients and prepared poultry feed.

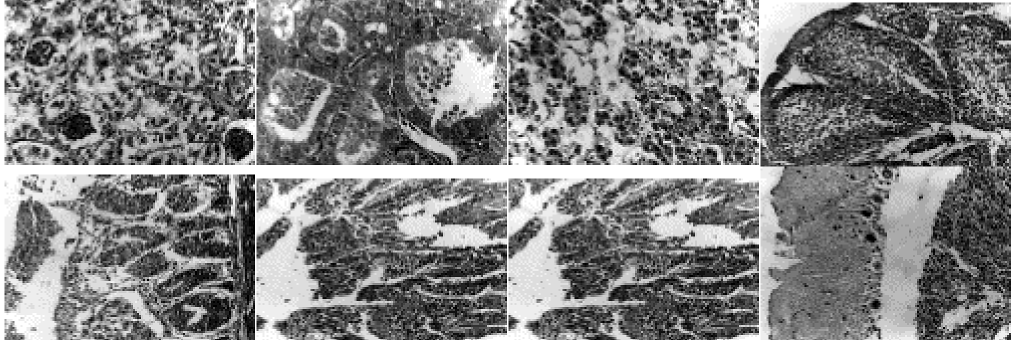
Elaroussi ***et al.* (2006)** described the toxicity signs that developed when the diet of male broiler chickens was artificially contaminated with different levels of the mycotoxin ochratoxin A (OTA). Chicks were assigned randomly to three groups of 80 chicks that were fed a diet containing 0 parts per billion (ppb) (control, group 1), 400 ppb (group 2) or 800 ppb (group 3) OTA from day 1 to 5 weeks of age. Signs of ochratoxicosis were assessed on the basis of changes in the following criteria: body weight, relative weights of two representative internal organs (gizzard and thymus), feed consumption, feed conversion ratio, mortality, thyroid activity, blood profile, humoral and cell mediated immunity. Feeding OTA at levels of 400 and 800 ppb (groups 2 and 3) significantly decreased the body weight, thymus weight, feed consumption, feed conversion ratio and thyroxine concentration ($P < 0.05$). The OTA groups developed anaemia manifested by a significant decrease in the red blood cell count, packed cell volume percentage and haemoglobin concentration ($P < 0.05$). By the end of the experiment both groups that received OTA showed a 37% reduction in red blood cell count compared with the control group. Furthermore, a significant decrease in the white blood cell count, humoral immune response and cell-mediated immunity was found in both groups fed ochratoxin compared with the control group ($P < 0.05$). The reduction in the above parameters was more noticeable with time and was proportional to the level of OTA exposure. A significant increase in relative gizzard weight, cumulative mortality and triiodothyronine concentration was found in OTA-fed chicks ($P < 0.05$). These data provide a description of ochratoxicosis in broilers that should be useful in diagnosis and in improved understanding of the practical implications on broiler performance and health, a problem that can threaten the poultry industry.

Hanif *et al.* (2006) analyzed 865 samples of poultry broiler and layer rations for mycotoxins contamination using TLC and HPLC. The mycotoxin AFB1 was noted to be the major contaminant in the feed samples analyzed (84.70% in 182 feeds), followed by OTA (51% in 41 feeds), Zon (49.33% in 150 feeds), Don (38% in 150 feeds), T-2 (34.65% in 101 feeds), 3ac-Don (19.41% in 67 feeds), and 15ac-Don (11.94% in 67 feeds). Mean values with standard deviation for AFB1, OTA, Zon, Don, T-2 toxin, 3ac-Don and 15ac-Don were 13±16.80, 10±19.63, 213.58±440, 456±1122, 442.56±1191, 41±102, and 38.92±149.58 µg/kg, respectively. All samples

were observed to be negative for HT-2 toxin, Das, neosolaniol, nivalenol, and fusarenon-x.

Rosa *et al.* (2006) in Brazil determined the incidence of mycoflora in poultry feeds and evaluated their OTA production potential. Sample of poultry feed were collected from different factories and studies for total moulds, Penicillium and Aspergillus spp. The most prevalent species were A. flavus and Penicillium citrinum, also high percentage of OTA produced fungi were present.

Koynarski *et al.* (2007) fed chicks on OTA-contaminated as well as on OTA-free diets. More heavy progress of duodenal coccidiosis, including mortality, occurred in OTA-treated chicks as can be seen from the higher value of lesion (3.50) and oocyst (31.65) indices. A stronger decrease of serum total protein was found in OTA-treated chicks (22.80 g/l) than in chicks infected with E. acervulina(24.20 g/l), but that decrease was strongest in chicks treated with OTA and simultaneously infected with E. acervulina (19.71 g/l). The serum concentration of uric acid was significantly increased in all chicks exposed to OTA, most notably in those additionally infected with E. acervulina (1020.6 (micro mol/L), whereas the serum enzyme activity of AST was increased only in chicks infected with E. acervulina and highest in those fed OTA contaminated diet (122.2 U/L). OTA induced degenerative changes in kidneys, liver and heart as well as a depletion of lymphoid tissue in the lymphoid organs and a decrease of body weight. Coccidiosis induced only a slight growth depression and duodenal hemorrhages in addition to characteristic duodenal damages. The impairment of kidney function, histopathological changes and general growth depression were stronger when chicks infected with E. acervulina were also given OTA.

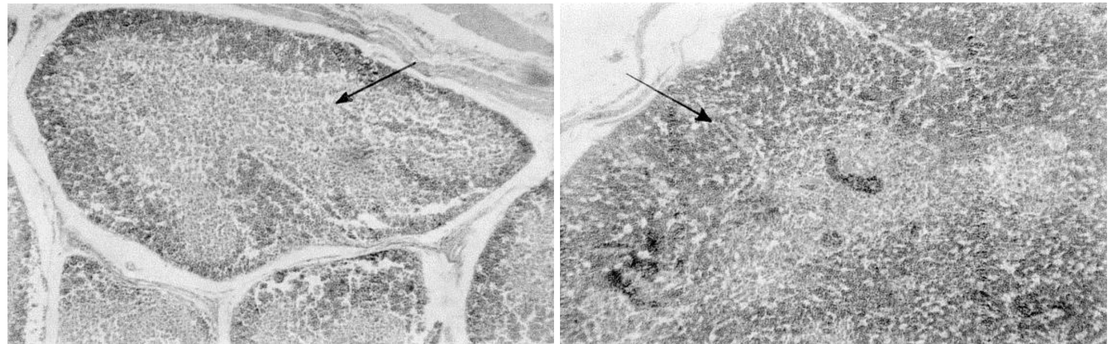


Koynarski *et al.* (2007)

Sakhare *et al.* (2007) conducted an experiment to study the protective role of polyherbal feed supplement (Toxiroak®) during induced mycotoxicosis in broilers. A total of 240 Vencobb one-day-old broilers were divided into eight equal groups. Group A served as control and was given plain feed. Groups B, D, F and H were given Toxiroak® at 0.75 g/kg of feed. Groups C, D, G and H were given dietary aflatoxin B1 at 0.2 ppm, and Groups E, F, G and H were given ochratoxin A at 0.2 ppm in feed to study the effect of Toxiroak® on individual aflatoxicosis, ochratoxicosis and combined mycotoxicosis of broilers. Chicks were given their respective feeds from the 1st day to 6th week of age and were vaccinated at 7th and 28th days of age with a Lasota strain of Newcastle disease virus. There was a significant effect of mycotoxins, individually and in combination, on body mass of broilers. Toxiroak® protected the effect of individual mycotoxins on body mass. Feed

conversion ration was highest in Group B birds, followed by Groups A, F, D, H, C, E and G. Significant restoration of haemoglobin and total leukocyte count values in broilers due to feeding of Toxiroak® in co-mycotoxicated and aflatoxins-fed groups, respectively, was observed. There was no significant effect of mycotoxin treatment on packed cell volume and total erythrocyte count in broilers. Due to single and combined mycotoxicosis there was a reduction in serum total protein, cholesterol and triglyceride and a rise in creatinine and uric acid levels. Supplementation of diets with Toxiroak® reduced the changes induced due to mycotoxins. There was a significant increase in the percentage organ weight of liver, and a reduction in that of spleen, bursa of Fabricius and thymus of broilers fed mycotoxins. Protection of changes in the percentage of organ mass of these organs by supplementation of Toxiroak® was recorded only in respect of bursa of Fabricius. The observed impaired immune response and histopathological changes in liver, kidney, spleen, bursa of Fabricius and thymus of broilers given mycotoxins was protected by Toxiroak® supplementation

Szeleszczuk ***et al.*** **(2007)** evaluated the effect of different doses of ochratoxin A (2 and 4 ppm) administrated to feed for 30-weeks old RIR hens for the period of 5 weeks on selected parameters of cellular (PHA – skin test) and humoral (immunization with SRBC and *Brucella abortus* antigens) immunity in the hens and humoral immunity (immunization with SRBC, *Brucella abortus* and NDV) in their progeny. In the hens which received ochratoxin A in the diet an impairment of cellular immunity was found. The wing web index in the control group was 1.82 whereas in the birds receiving 4 ppm OA – only 0.68 ($P<0.05$). After immunization with SRBC and *Brucella abortus* antigen, the antibodies titre was considerably lower in the birds receiving OA in their diet ($P<0.05$). This difference was especially clear in the group III receiving the dose of 4 ppm OA in the diet. In chickens at the 2 nd week of life, the histopathological examination of liver, kidneys, heart and of organs of immunological system, i.e. thymus, bursa of Fabricius and spleen, were also performed. The most strongly expressed changes appeared in bursa of Fabricius of chickens derived from the hens fed the diet containing 4 ppm of ochratoxin A. A considerable loss of lymphocytes in this organ was manifested by narrowing of border zone of lymphonoduli, especially in part nearby the epithelium lining a lumen of bursa of Fabricius. In some cases the lymphoid tissue in lymphonoduli was completely depleted. In the group of chickens, the mothers of which received 2 ppm OA in the diet, narrowing of border zone of lymphonoduli in bursa of Fabricius was observed but this lesion was not so intense as in the chickens coming from laying hens fed with 4 ppm OA. A small degree of lymphocytes' loss was revealed in thymus and in spleen of the chickens from both experimental groups. In kidneys and liver, there were no lesions apart from congestion.



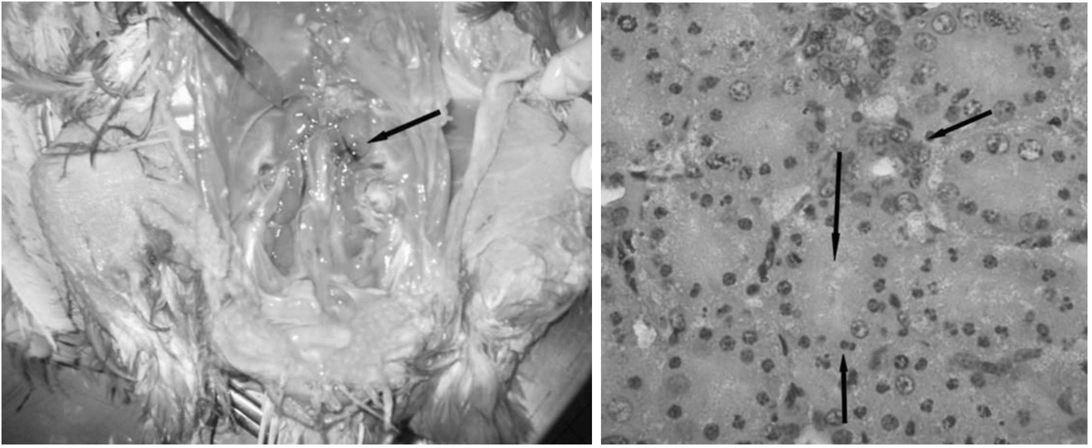
Bursa of Fabricius (200 x magnification). Depletion of cortex of lymphatic nodes. Thymus (100 x magnification). Focal depletion of cortex lymphocytes Szeleszczuk ***et al.*** **(2007)**

Trailović ***et al.*** **(2007)** investigated in vitro and in vivo (in broiler chickens) ochratoxin A (OTA) adsorption efficiency of three different adsorbents: inorganic (modified zeolite); organic (esterified glucomannans) and mixed (inorganic and organic components plus enzymes). 2. The aim of the study was to investigate which of these adsorbents provided the best protection against the presence of residues of OTA in the pectoral muscle and liver of broilers given an OTA-contaminated diet. In addition, it was important to test and compare the results of adsorbent efficiency using two different in vitro methods. 3. The results from classical in vitro investigations carried out in the artificial intestinal fluid, showed that the inorganic adsorbent (Mz), exhibited the highest adsorption, having adsorbed 80.86 ± 1.85% of OTA, whereas average in vitro adsorption abilities of organic (30.52 ± 3.50%) and mixed (32.00 ± 2.60%) adsorbents were significantly lower. 4. In the investigation of absorption in everted sacs of broiler duodenal segments (Everted Duodenal Sacs Procedure), higher OTA adsorption in gut was exhibited by organic adsorbent, 74.26 ± 4.48%. Furthermore, the mean adsorption efficiency of mixed and inorganic adsorbent was 65.26 ± 4.76% and 45.75 ± 7.14%, respectively. 5. In the in vivo investigation, broilers were fed for 21 d on diets containing 2 mg/kg of OTA and supplemented with inorganic (Mz), organic (Ms) or mixed adsorbent (Mf) at the recommended concentration of 2 g/kg of feed. All three adsorbents significantly decreased OTA residue concentrations in the pectoral muscle and livers, but the order of effectiveness was mixed > organic > inorganic. The most efficient was the mixed adsorbent which decreased residue concentration by 72.50% in pectoral muscle and 94.47% in livers. 6. The Everted Duodenal Sac in vitro method provided results similar to those obtained in the in vivo study. However, further studies are required to investigate the efficiencies of adsorbents against various mycotoxins using this method.

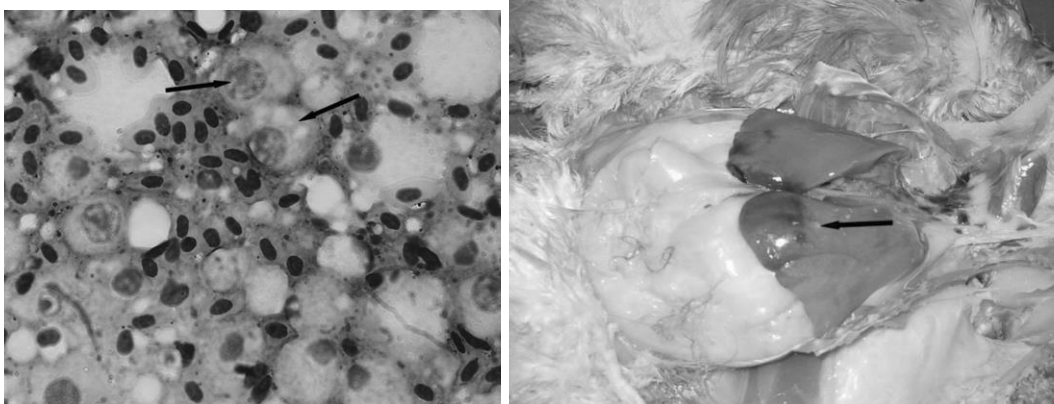
Wangikar ***et al.*** **(2007)** performed an experiment on the rat embryos at 10th day of gestation, to study the effects of mycotoxins on the development of organs. For this purpose they explanted rat embryos and cultured them in the medium containing rat serum, OTA alone (0.004, 0.008 µg/ml culture), AFB1alone (0.5, 1.0 µg/ml culture) and combination of OTA+AFB1 (0.004+1.0 µg/ml culture). Both the toxin affected the development neural tube when given alone, but when these were given in combination no effects were found on the development of neural tube instead the defects on heart were found. The defective hearts when subjectd to histological studies showed the degenerative change and vaculation

Zaghini ***et al.*** **(2007)** checked the in vivo capability of Saccharomyces cerevisiae (SC), and of an esterified glucomannan (EGM) to reduce the oral bioavailability of ochratoxin A (OTA) added to a basal diet for laying hens over a 12 week period. The residues of OTA in kidney, muscle and blood were studied. Eighty-four Isa Brown laying hens were divided into 6 experimental groups, fed 6 different diets: 0-0: basal diet; EGM-0: diet supplemented with 0.2% EGM; SC-0: diet supplemented with 0.2% SC; 0-OTA: diet supplemented with 0.2 ppm OTA; EGM-OTA: diet supplemented with 0.2% EGM and 0.2 ppm OTA; SC-OTA: diet supplemented with 0.2% SC and 0.2 ppm OTA. During the trial feed and water were provided ad libitum and all the animals were clinically observed. At the end of the experimental period and immediately before the hens were euthanized, blood samples were collected; kidneys, and muscle were sampled. The ochratoxin A was checked using a HPLC flourometric method. During the trial all the hens were healthy. All the biological matrices of the OTA administered hens were positive to the mycotoxin; the recorded levels were very low and decreased in the order: kidneys > blood > muscle

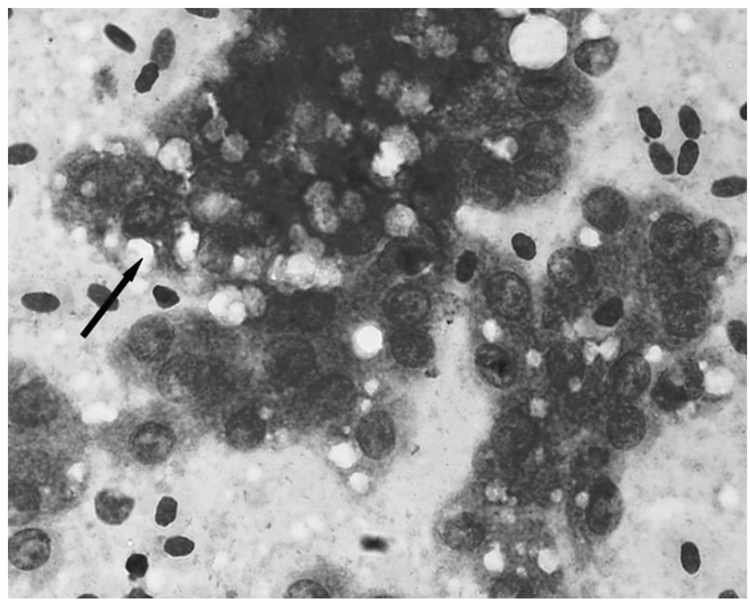
Bozo ***et al.*** **(2008)** administered OA-contaminated feed to laying hens for at least 2 months. Analysis by HPLC with fluorometric detection of the tissues of 4 layer hens that displayed gross and microscopical lesions identified OA in the kidney (8.7 to 16.9 g/ kg, average 13.65 ± 3.58 g/kg) and liver (3.7 to 5.1 g/kg, average 4.43 ± 0.64 g/kg) but not in the other tissues.



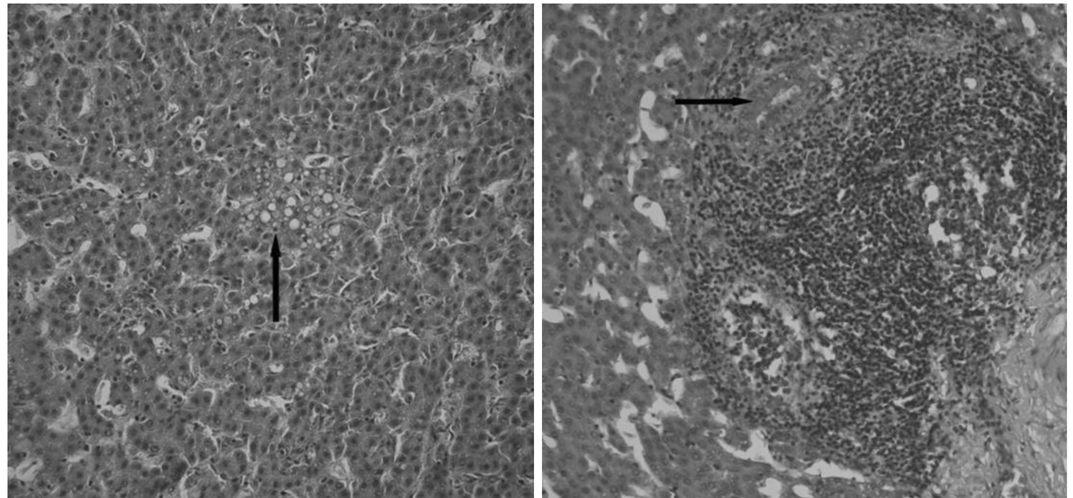
Kidney. Nephritis: congestion, hemorrhage, and cortical hyperemiaKidney. Distension, enlargement, and degeneration of epithelial cells of the proximal tubules. Presence of pyknotic nuclei. Hema **Bozo** ***et al.*** **(008)**



Kidney. Cells with abundant cytoplasm and a round-oval, slightly eccentric nucleus. May-Grunwald- Giemsa · 100. Liver. Enlargement, diffuse yellow color of the surface, and necrotic foci of the surface. **Bozo** ***et al.*** **(008)**



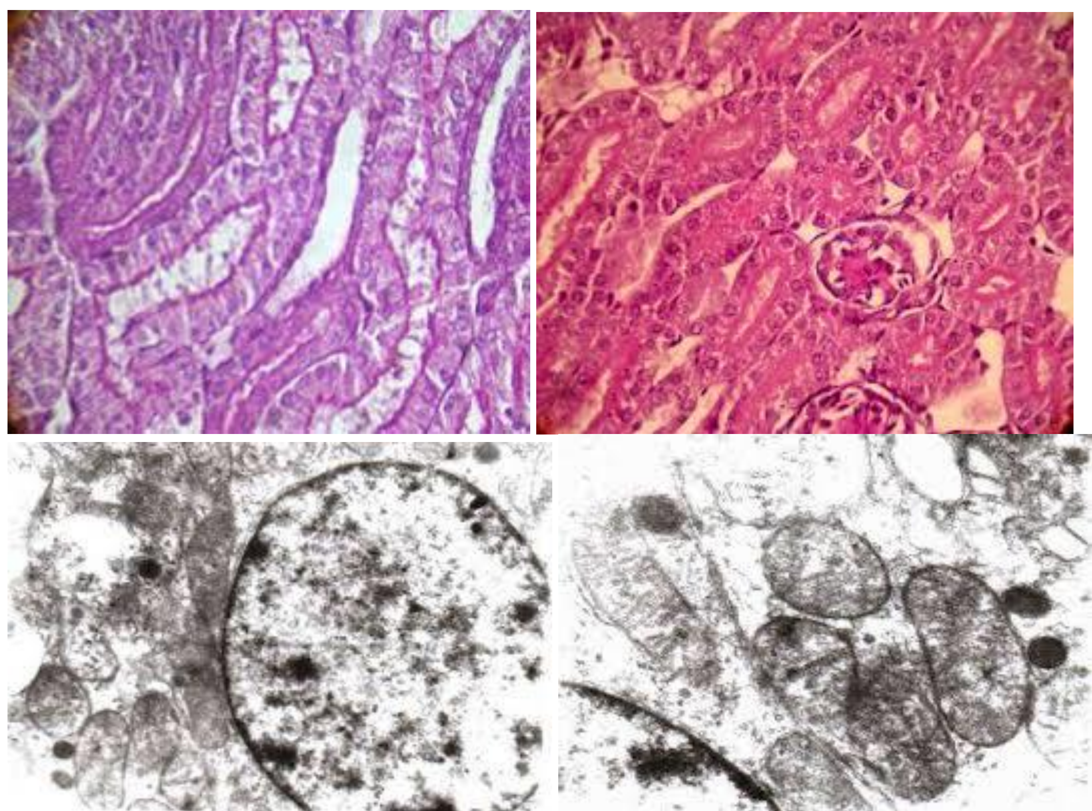
Liver. Numerous hepatocytes with fatty infiltration and vacuolated hepatocytes. May-Grunwald-Giemsa · 100 **Bozo *et al.* (008)**



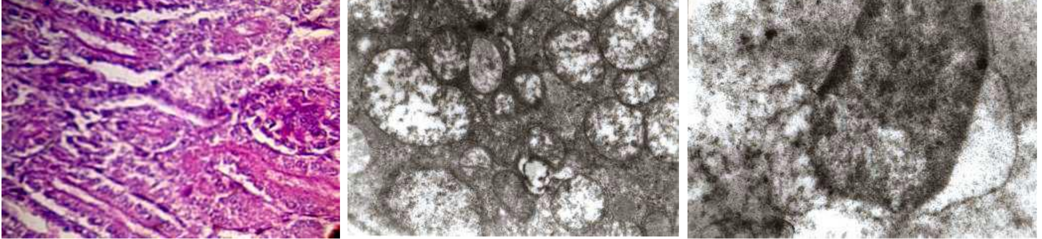
Liver. Group of vacuolated and heavily stained hepatocytes. Hematoxylin-eosin · 20. Liver. Nodular inflammatory cells around the bile ducts and focal fibrosis in the parenchyma. Hematoxylin- eosin · 40. **Bozo *et al.* (008)**

Carmen *et al.* (2008) described the **histological and ultrastructural** lesions of the **kidney** in an experimental ochratoxicosis pattern of broiler chickens from 1st to 21st day of life. Administration of OA in dose of 1mg/kg determined small degenerative lesions of epithelial cells of convoluted tubules; alternating with unchanged zones after 7th and 14th day of the experiment. After the 21st day mitochondria reduction in size; loose of mitochondrial membrane integrity and nuclear lesions were clearly observed. In dose of 9 mg/kg/day OA induced more significant lesions characterized by hypertrophia of epithelial cells of convoluted tubules and degenerative changes of them and tickening of basal membrane of Malpighi corpuscles. Administration of OA in dose of 20mg/kg determined severe lesions of both convoluted tubules and glomeruli of the kidney. The collagen fibers increased into the interstium and the basal membrane of the tubes increase. Ultrastructurally; in 14th and 21st day of OA poisoning abnormal shape and dimensions of mitochondria and peroxysomes; lipidic

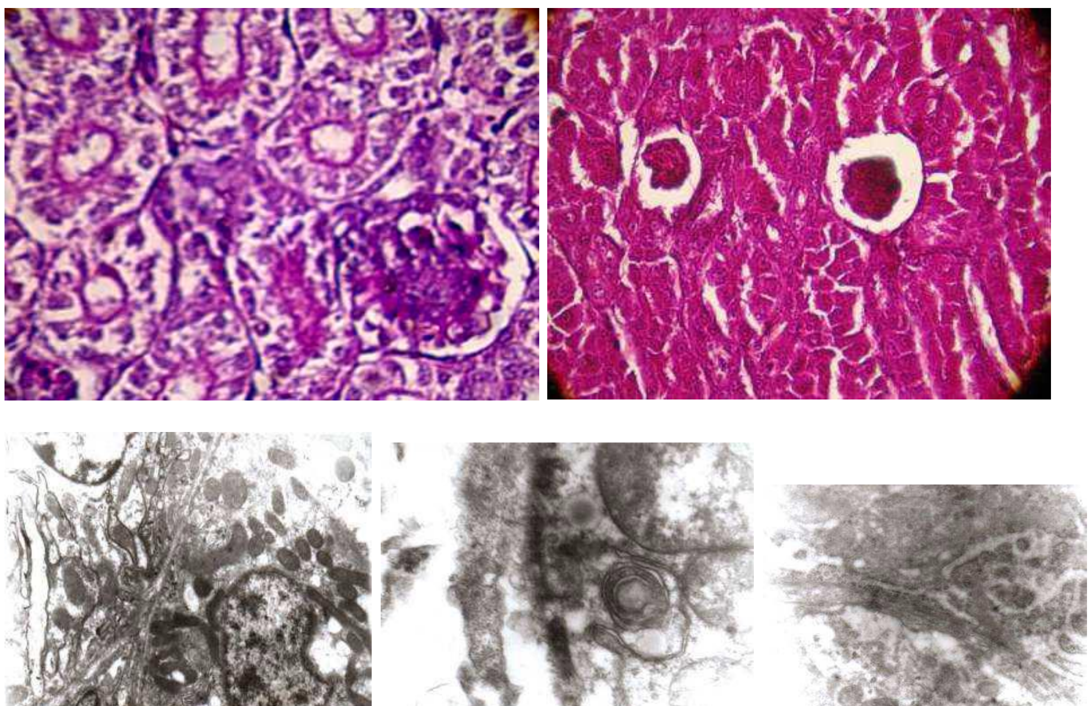
droplets into the citoplasma and nucleus; round; electronodense bodies; enlarged smooth endoplasmatic reticulus and intracitoplasmatic and intranuclear myelin-like; figures were observed into epithelial cells of convoluted tubules.



Kidney of chickens; E1 group 14th (a) and 21st day of the experiment (b). Degeneration of the epithelium of proximal convoluted tubules and Malpighi corpuscles. Col. Giemsa (a); PAS x200 (b); Shortened mitochondria; with few cristae (c); nucleus with a small quantity of condensed chromatine (d) Col. Uranyl acetate x10000 (c); x14000 (d) **Carmen *et al.* (2008)**



Kidney of chickens; E2 group 21st day of the experiment. Degeneration of the epithelium of proximal convoluted tubules and Malpighi corpuscles. Col. Giemsa x400 (a); Balonised and shortened mitochondria with degenerated cristae (b); Nucleus with corticale hyperchromatosis and lipidic inclusions betwen the two layers of nuclear membrane (c); Col. Uranyl acetate x10000 (b; c) **Carmen *et al.* (2008)**

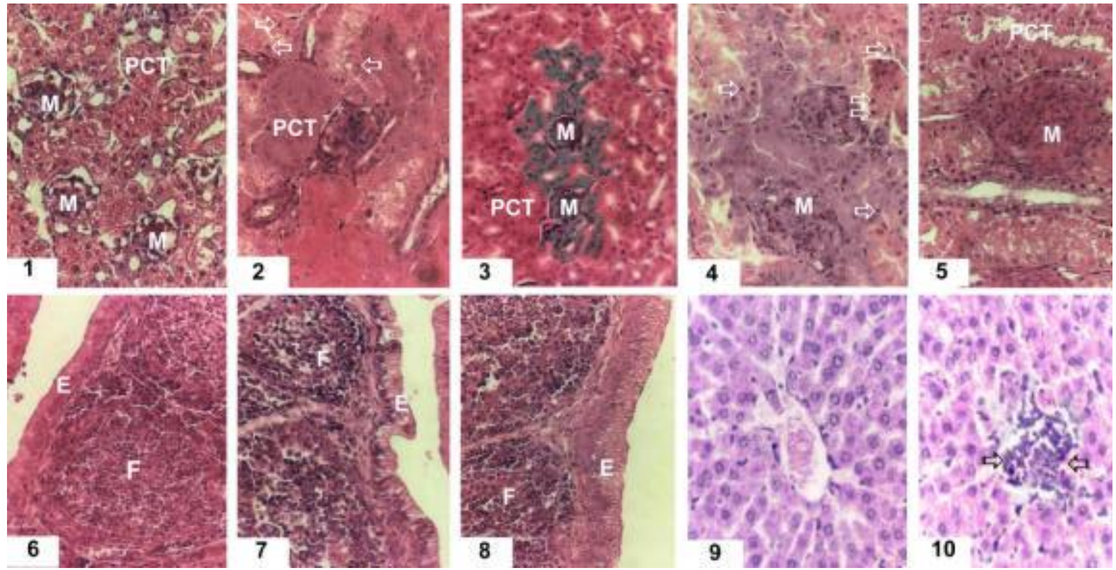


Kidney of chickens; E3 group; 14th and 21st day of the experiment. Degeneration of the epithelium of proximal convoluted tubules and Malpighi corpuscles. Col. PAS x400 (a; b); Lipidic droplets into the citoplasma and nucleus (c); myelin-like figures (d); collagen fibers into the basal membrane (e). Col. Uranyl acetate x4000 (c); x8000 (d; e). **Carmen *et al.* (2008)**

Denli et al. (2008) fed OTA at the level of 0 and 2 mg/kg feed to the laying hens for the period of 3 weeks. The OTA diet significantly increased the serum activity of alkaline phosphates, relative liver weight and the serum concentration of uric acid as compared with the control diet. Birds fed the OTA treatment diet showed a greater content of OTA in the liver compared with control group. Residues of OTA were not detected in any of the eggs samples analysed.

.

Elaroussi *et al.* (2008) conducted an experiment to evaluate the effects of ochratoxin A (OTA) on the function and histology of the liver, kidney and bursa of broiler chickens fed OTA contaminated rations. Two hundred forty one-day-old male Ross broiler chicks were used, they were randomly divided into 3 dietary experimental groups of 80 birds and given rations containing 0 (control), 400 or 800 µg OTA/Kg feed. The chicks were maintained on these treatments through 5 weeks of age with feed and water available for ad libitum intake throughout the experimental duration. Dietary OTA contamination at both levels resulted in significant increase (P 400 ppb could adversely affect kidney, liver and bursa function and histology and thus broiler performance and health.



1: kidney of control broiler chick showing the proximal convoluted tubules (PCT) and Malpigian corpuscles (M). (HE x 400). 2: kidney of male broiler chick receiving OTA in their diet for 2 weeks at a dose of 400µg/Kg feed, showing cells of PCT with cloudy swelling in the cytoplasm and few degenerating pyknotic nuclei (arrows) (HE x 400). 3: kidney of male broiler chick receiving OTA in their diet for 4 weeks at a dose of 400 µg/Kg diet showing cells of PCT with more prominent cloudy swelling in the cytoplasm and few degenerating cells with pyknotic nuclei and decreased capsular space in Bowman's capsule of Malpigian corpuscles (M) (HE x 400). 4: kidney of male broiler chick receiving OTA in their diet for 2 weeks at a dose of 800 µg/Kg feed showing more prominent changes in the form of more degenerating cells with pyknotic nuclei (arrows) in cells of (PCT) and obliteration of capsular space in Bowman's capsule of Malpigian corpuscles (M) (HE x 400). 5 kidney of male broiler chick receiving OTA in their diet for 4 weeks at a dose of 800 µg/Kg feed showing more prominent changes in the form of degenerating cells with pyknotic nuclei in cells of PCT and Malpigian corpuscles (M) showing marked degeneration, hyalinization and obliteration of the capsular spaces (HE x 400). 6: a section in the bursa of control male broiler chick showing lymphatic follicles (F) with the follicle associated simple columnar epithelium (E) (HE x 200). 7: a section in the bursa of a male broiler chick receiving OTA in their diet for 2 weeks in a dose of 800 µg/Kg feed showing the lymphatic follicles (F) and that follicle associated epithelium (E) became disorganized with apical accumulation of secretions, which appeared as vacuolated cytoplasm (HE x 200). 8 a section in the bursa of a male broiler chick receiving OTA in their diet for 4 weeks in a dose of 800 µg/Kg feed showing swollen follicle associated epithelium (E) and depletion of lymphocytes in lymphoid follicles (F) with larger paler nuclei in cells of the germinal center of the follicle (HE x 200). 9: a section in the liver of control male broiler chick showing the central vein with the surrounding hepatocytes arranged in plate (HE x 200). 10: a section in the liver of a male broiler chick receiving OTA in their diet for 4 weeks in a dose of 800 µg/Kg feed showing mild mononuclear cellular infiltration (arrows) (HE x 200). **Elaroussi *et al.* (2008)**

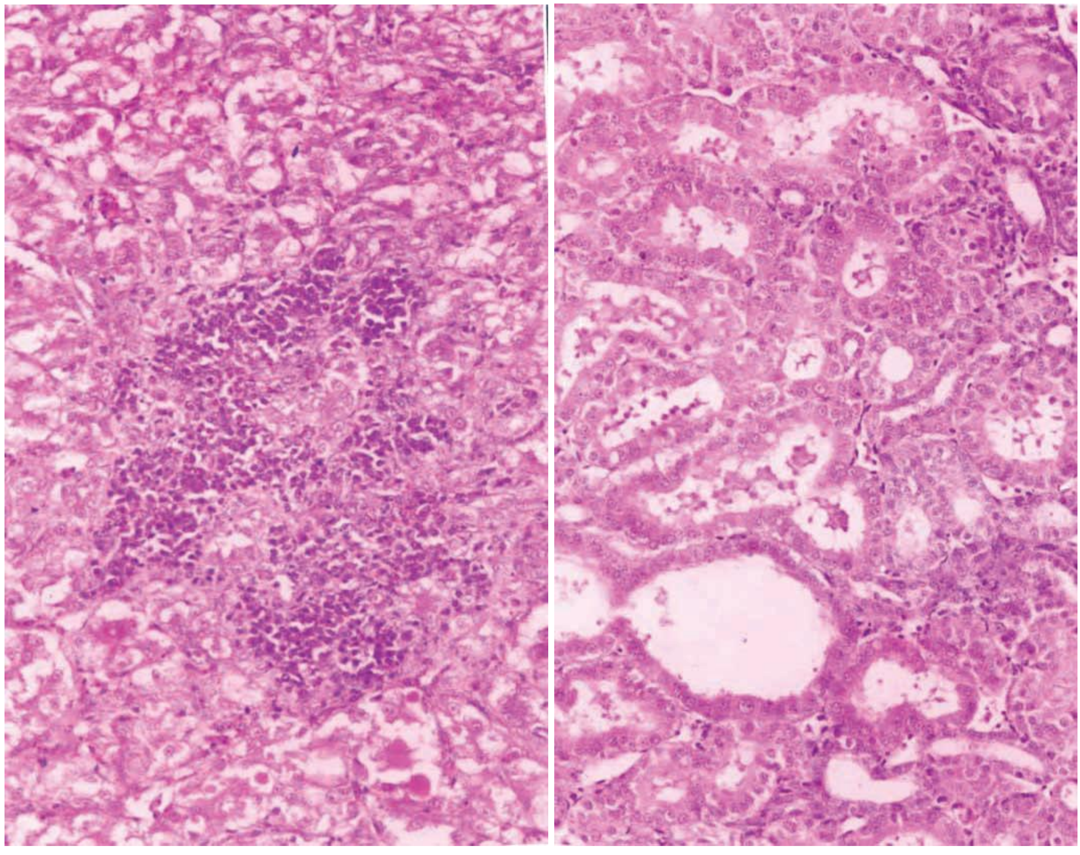
Ganif *et al.* (2008) studied the toxic effects of two concentrations (0·5 and 1 mg/kg) of ochratoxin A (OTA) and attenuating effects of a toxin deactivator (Mycofix® Plus$^{MTV\ INSIDE}$) containing the yeast *Trichosporon mycotoxinivorans* on the performance (feed conversion ratio; body weight gain), serum enzymes (lactate dehydrogenase, gamma-glutamyltranspeptidase and aspartate aminotransferase) and clinico-pathomorphology of internal organs were studied in 270 one-day-old broiler chicks divided into 9 groups over a 42-d period. Feed conversion ratios (FCR) in groups fed toxin deactivator were improved compared with groups receiving OTA only. An increase in the relative weight of kidney and liver was observed in groups fed 0·5 and 1 mg/kg OTA on day 42 of the experiment as compared with the control group. In contrast, relative weights of bursa of Fabricius and spleen were not significantly affected in experimental groups exposed to OTA as compared to control groups determined on days 28 and 42 of age. Serum enzymes (LDH, GGT and AST) values in OTA treated groups determined on days 28 and 42 were higher than those of the control group. Histopathological examination of kidney on day 42 revealed degenerative changes in the epithelial cells of the proximal convoluted tubules and massive necrosis of the proximal tubular epithelial cells. These changes were less marked in birds receiving 0·5 mg/kg OTA than in those receiving 1 mg/kg. In general, histological changes in kidneys, liver, bursa and spleen were less pronounced in birds receiving OTA and toxin deactivator concomitantly. Dietary OTA at 0·5 and 1 mg/kg adversely affects FCR, increases the serum liver enzymes and induces pronounced pathomorphological and histological changes in internal organs of broiler chicks. Co-administration of OTA with deactivator attenuated the harmful effects.

Schiavone *et al.* (2008) conducted a survey in Italy to study the correlation in the levels of OTA in the poultry feed and sera of the birds. Sample from 10 poultry farms (20 feed and 94 blood) were analyzed for the levels of OTA. All the feed samples were contaminated by OTA with values ranging from 0.04 to 6.50 μg kg-1. Fifty three percent of the sera samples were positive, with values ranging from 0.003- 0.165 ng/ ml. No statistically significant differences in OTA contamination of feed or sera were observed either between the organic vs conventional group or between the laying hens vs broiler group.

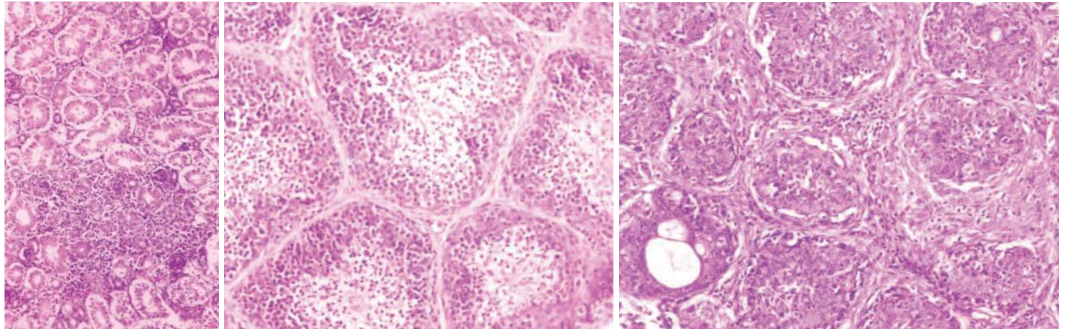
Shah *et al.* (2008) conducted a survey in the Swat Valley of Pakistan for the determination of toxigenic fungi and mycotoxins contamination in the maize kernels. Penicillium Aspergillus, Fusarium, and Rhizopus were the most predominant fungal genera identified and amongst the mycotoxigenic species, A. flavus had the highest incidence, followed by A. parasiticus, A. ochracious, A. carbonarous and P. verrocosum. AFB1 levels ranged from trace to 30.92 μg/ kg with the average value 15.58 μg/ kg. Contamination level ranged from trace to 7.32 μg/ kg with average value of 3.08 μg/kg.

Gupta *et al.* (2008) conducted to a study to evaluate the individual and combined effects of ochratoxin A (OA) and Salmonella enterica serovar Gallinarum (S. Gallinarum) on gross and histopathological changes in broiler chickens. One hundred and seventy-six 1-day-old broiler chicks were divided into two groups of 88 chicks each; one group was fed a control mash diet, and the other group was fed a mash diet containing 2 parts/10(6) OA. On day 14, each group was further subdivided into two

groups, with one group inoculated with S. Gallinarum intraperitoneally (1.25 x 10(10) colony-forming units/0.5 ml) whereas the other group was not inoculated with S. Gallinarum. Four birds from each group were sacrificed on 1, 2, 3, 5, 7, 10, 14 and 21 days post inoculation to record pathological changes in different organs. Gross and microscopic changes in OA-fed birds indicated the kidneys and bursa of Fabricius as the primary organs to be affected by this toxin. Gross and microscopic changes due to S. Gallinarum infection indicated the liver and spleen as the primary organs affected by this infection. The effects of OA on the kidney and bursa of Fabricius were enhanced following S. Gallinarum infection. Degenerative changes and interstitial nephritis in the kidneys, and lymphocyte depletion from bursal follicles were more pronounced and were observed earlier in the combination group. In conclusion, data indicate that birds fed OA and infected with S. Gallinarum will demonstrate increased pathology compared with birds fed OA alone or those infected with S. Gallinarum but not fed OA.



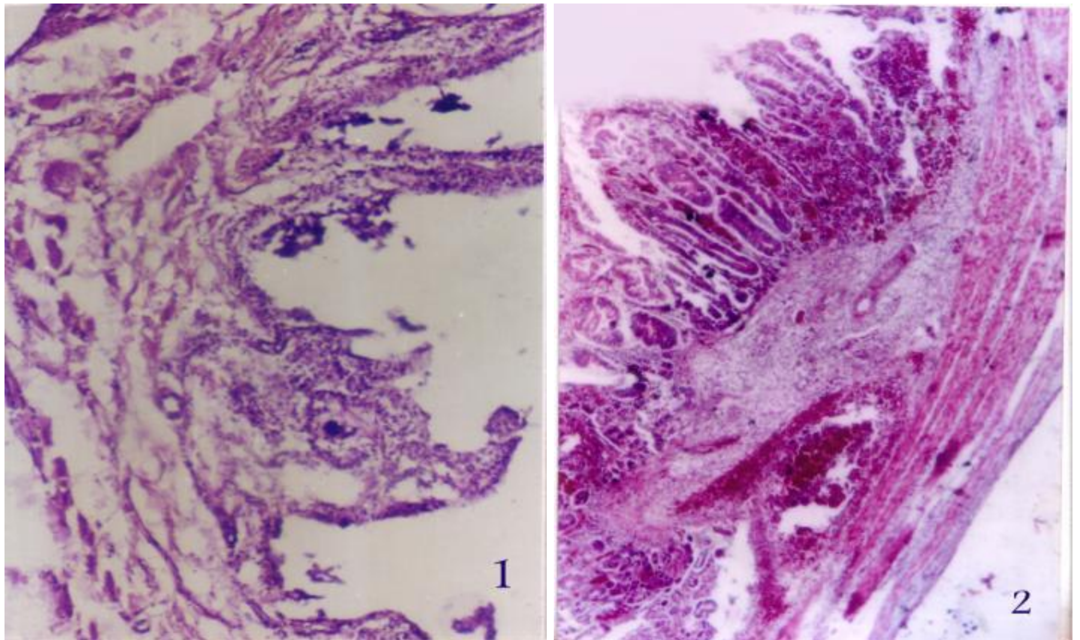
Typhoid granuloma along with an enhanced glandular appearance of hepatic cord structures at 21 d.p.i. in chicks fed OAand infected with S. Gallinarum. Haematoxylin and eosin,_66. Markedly swollen kidney tubules with necrotic and sloughed epithelial debris in the lumen at 21 d.p.i. in chicks fed OA. Haematoxylin and eosin,_66. **Gupta *et al.* (2008)**



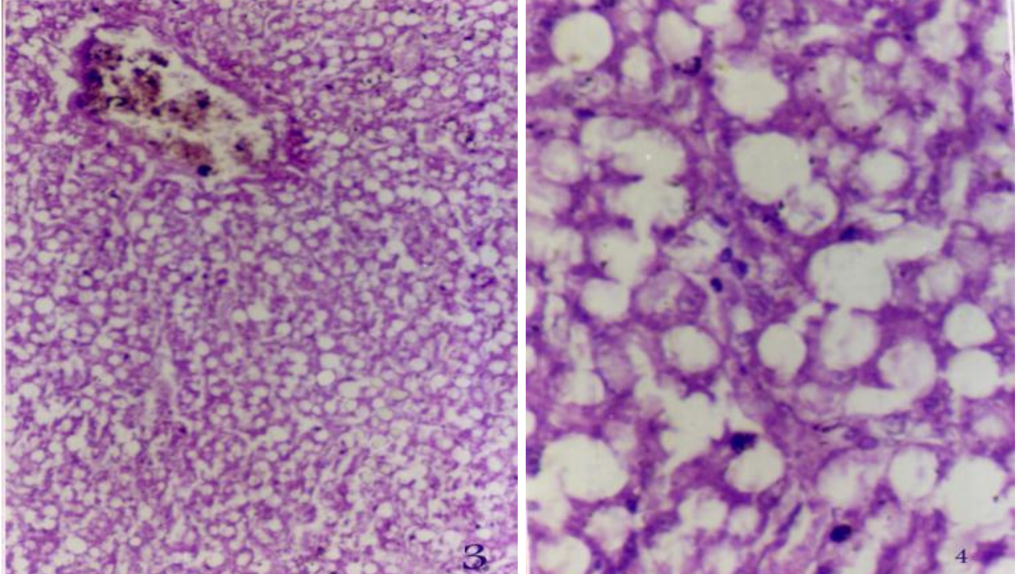
Focal area of mononuclear cell infiltration in the interstitial tissue of kidney at 5 d.p.i. in chicks fed OA and infected with S. Gallinarum. Haematoxylin and eosin,_66., Depletion of lymphocytes from the medullary region of bursal follicles at 2 d.p.i. in chicks fed OA and infected with S. Gallinarum. Haematoxylin and eosin,_66. **Gupta *et al.* (2008)**

Sawale *et al.* (2009) performed a study in 80 white leghorn 26 week old laying hen to evaluate toxic effects of ochratoxin A and preventive efficacy of herbomineral toxin binder product (Toxiroak®). Birds were randomly divided into four groups of 20 each. Group I served as control and given no treatment, Group II comprised healthy birds fed standard basal diet and administered Toxiroak@1.25 kg/tonne of feed for 60 days, birds of group III received ochratoxin A@1 ppm while those of group IV were given ochratoxin A@1 ppm and herbomineral toxin binder product Toxiroak@1.25 kg/tonne of feed for 60 days. Ochratoxin A adversely affects body weight gain, feed consumption, laying performance of hens besides haematobiochemical disturbances & severe immunosupression. However, supplementation of herbomineral toxin binder feed supplement has provided a moderate amelioration in mycotoxicosis

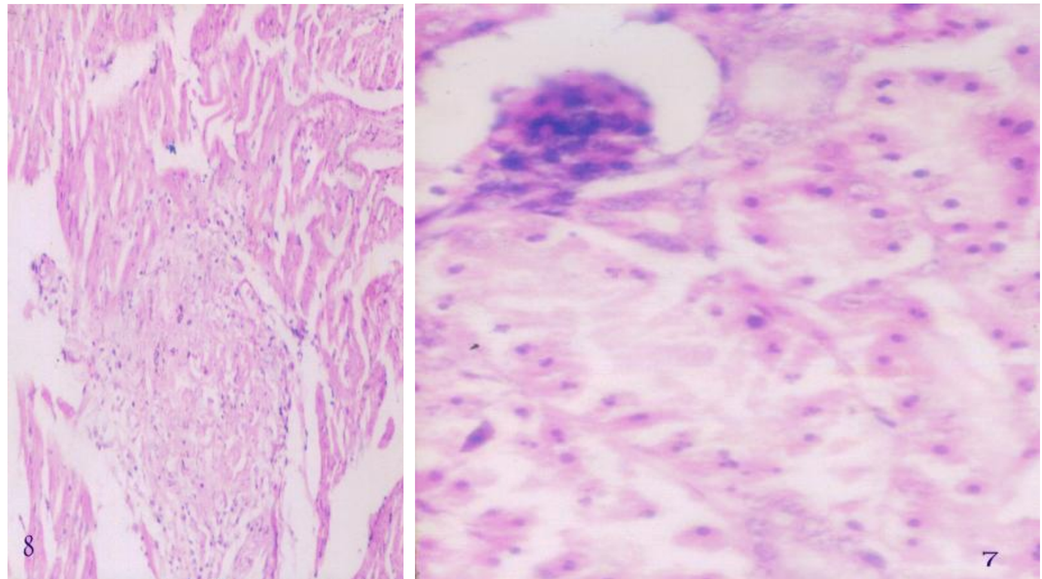
Elwan *et al.* (009) studied ochratoxicosis in ostrich. One hundred diseased Ostrich, one-month age, showed dullness, weakness, loss of body weight and high mortality. They were reared on mycotoxin contaminated ration (9ppm Ochratoxin, 12ppm aflatoxin and 25ppp fumonisin). Blood picture denoted, significant decrease in Total RBCs, Hb concentration, PCV%, MCV and MCHC. Significant decrease in WBSc was observed. Significant decrease were seen in total protein, albumin and gama globulin. Significant increase in uric acid and creatinine were also recorded. The post-mortem examination showed enlarged proventriculus, swollen pale liver and kidneys. Microscopically there were proventriculitis and hemorrhagic enteritis. Vaculation and focal necrosis of hepatocytes with biliary epithelial hyperplasia were noticed. Necrosis of the renal tubules and Zinker's necrosis of the cardiac muscles were seen. We recommend the periodical detection of mycotoxins in feed of ostrich to minimize the economic losses within ostrich farms



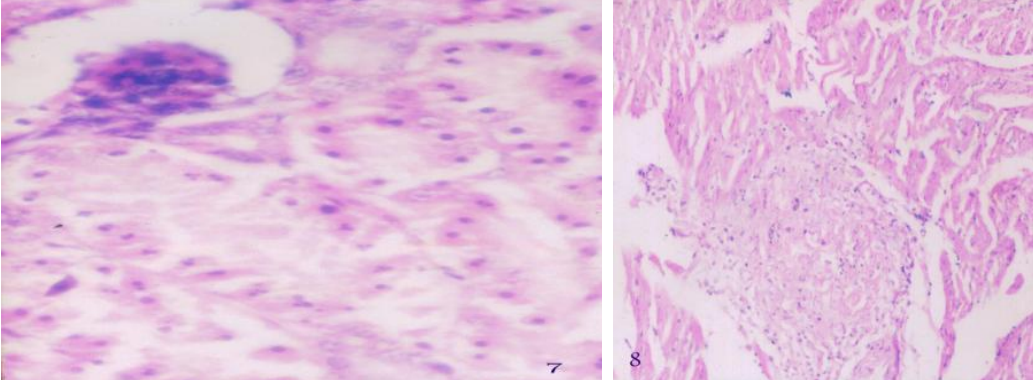
Proventiriculus showing edema and desquamation of epithelial lining (H&E x 300) ,Intestine showing hemorrhage and necrosis (H&E x 300) **Elwan *et al.* (009)**



Liver showing congestion, vaculation and focal areas of necrosis (H&E x 300) ,High magnification showing cytoplasmic vaculation of hepatocytes (H&E x 600). **Elwan *et al.* (009)**



Liver showing portal aggregation of lymphocytes and hyperplasia of bile ductules (H&E x 300). Kidney showing congestion, hemorrhage and necrosis of the renal tubules and glomeruli (H&E x 150) **Elwan *et al.* (009)**.



Kidney showing coagulative necrosis of renal tubules (H&E x 300). Heart showing coagulative necrosis of cardiac muscles with lymphocytic infiltration (H&E x 150). **Elwan *et al.* (009)**

Sawale *et al.* (2009) conducted a study in 80 white leghorn 26 week old laying hen to evaluate toxic effects of ochratoxin A and preventive efficacy of herbomineral toxin binder product (Toxiroak®). Birds were randomly divided into four groups of 20 each. Group I served as control and given no treatment, Group II comprised healthy birds fed standard basal diet and administered Toxiroak@1.25 kg/tonne of feed for 60 days, birds of group III received ochratoxin A@1 ppm while those of group IV were given ochratoxin A@1 ppm and herbomineral toxin binder product Toxiroak@1.25 kg/tonne of feed for 60 days. Ochratoxin A adversely affects body weight gain, feed consumption, laying performance of hens besides haematobiochemical disturbances & severe immunosupression. However, supplementation of herbomineral toxin binder feed supplement has provided a moderate amelioration in mycotoxicosis

Wang *et al.* (2009) reported the immunosuppresent and mitogenic effects of combinations of OTA and T-2 toxin in yellow-feathered broiler chickens at the age of 21days for 3 weeks. The 3 groups were fed the basal diet, L, basal diet 0.25 mg/kg of OTA, 0.5 mg/kg of T-2 toxin; and basal diet 0.5 mg/kg of OTA, 1 mg/kg of T-2 toxin. The feeding of OTA and T-2 toxin contaminated diets decreased not only the relative

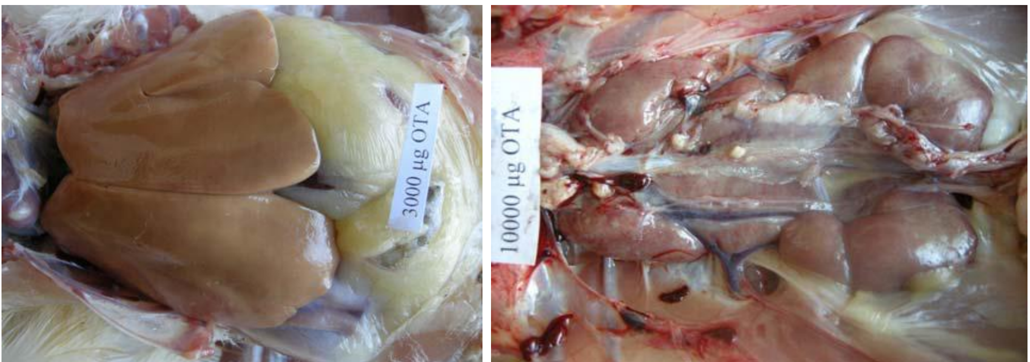
weight of spleen, thymus, and bursa of Fabricius, but also serum concentrations of total protein, albumin, and globulin. The mitogenic effects of both toxins were evident by the results of methyl thiazolyl tetrazolium reduction assay used on peripheral blood lymphocytes. Both toxin treatments significantly decreased the CD4+ /CD3+ and CD4+ /CD8+ ratios as determined by flow cytometery.

Sakthivelan *et al.* (2010) investigated the effect of ochratoxin A (OA) on the body weight, feed intake, and feed conversion was investigated in broiler chicken fed dietary levels of OA at 0, 1, and 2 ppm for 28 days from hatch. Feeding OA significantly reduced the growth rate of broiler chicken. The reduction was observed from the first week onwards in OA-treated groups. Feed consumption and feed conversion also showed a diminishing trend from the first week of feeding toxin. Its implication on the performance of broiler chicken is discussed.

Xue *et al* (2010) investigated the immunopathological effects of combinations of ochratoxin A (OTA) and T-2 toxin on broilers. Four hundred eighty 1-d-old broilers were randomly assigned to 4 groups, each group consisting of 4 duplicates each with 30 broilers. The 4 groups were fed the following diets for 4 wk: group 1 = basal diet (control, mycotoxinfree); group 2 = basal diet + 2,000 mg/kg of Mycofix Plus; group 3 = basal diet + 0.25 mg/kg of OTA and 0.5 mg/kg of T-2; and group 4 = basal diet + 0.25 mg/kg of OTA and 0.5 mg/kg of T-2 + 2,000 mg/kg of Mycofix Plus. The feeding of OTA-T-2 toxin diets reduced ($P < 0.05$) the level of anti-Newcastle disease virus antibody titers by 10.4%. When broilers were administered lipopolysaccharide, the results of real-time PCR showed that broilers fed OTA-T-2 toxin reduced the cytokine mRNA expression levels of interleukin-2 and interferon-γ to some extent but not significantly ($P > 0.05$). The concentrations of interleukin-2 and interferon-γ in serum were significantly decreased ($P < 0.05$) by OTA-T-2 toxin combination. Histopathological studies demonstrated that OTA-T-2 toxin combination caused abnormalities in the thymus, bursa of Fabricius, spleen, and liver. Ochratoxin A-T-2 toxicity could be counteracted by Mycofix Plus partially but not significantly ($P > 0.05$). The concentrations of OTA and T-2 toxin used in this study are under the maximum tolerated levels recommended by Canadian Food Inspection Agency. Our study clearly put the standard and detoxification method for these toxins into question. We suggest that it may be time to reduce the maximum allowable limits of OTA and T-2 mycotoxins in feeds to improve animal health and the safety of the food chain.

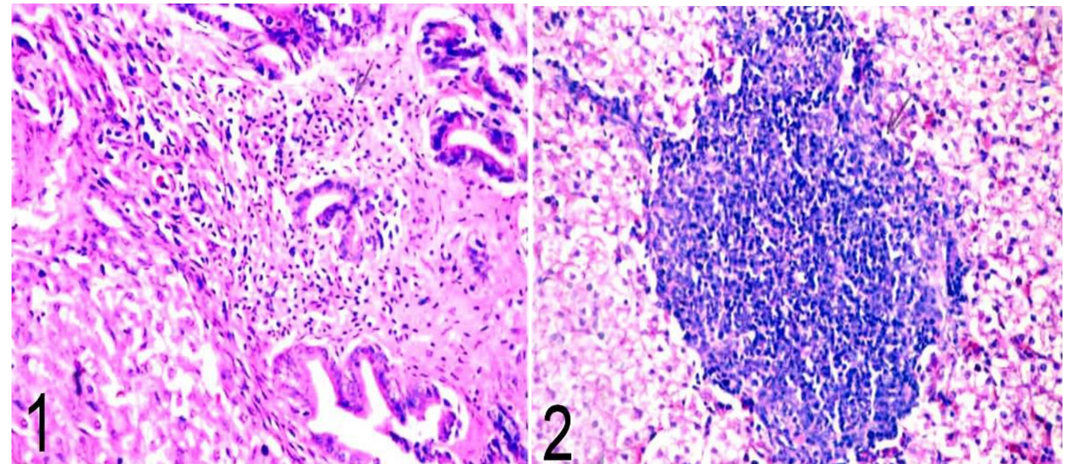
ZAHOOR-UL-HASSAN *et al.* (2010) produced ochratoxin A (OTA) by propagation of Aspergillus ochraceus and fed to breeder hens. For this purpose, 84 breeder hens were divided into seven groups (A-G). Group A served as control, while groups B, C, D, E, F and G were fed OTA at 0.1, 0.5, 1.0, 3.0, 5.0 and 10.0 mg/Kg feed, respectively for 3 weeks. Clinical signs, feed intake, feed conversion ratio and egg mass production were recorded on daily basis, while body weight was recorded on weekly basis. Lesions on visceral organs and serum biochemical parameters were determined. Significant decrease in feed intake, body weight and egg mass production was found in the OTA treated groups compared to control. Among different groups, diarrhea, unthriftiness, water intake and depression increased with increase in dietary OTA levels. Enlargement and hemorrhages on liver and kidney were more severe in birds fed higher dietary OTA levels. Serum ALT, urea, creatinine and total protein levels were significantly higher in OTA treated groups. It was concluded that

production performance, pathological alterations and serum biochemical changes determined became more severe with increase in dietary levels of OTA.

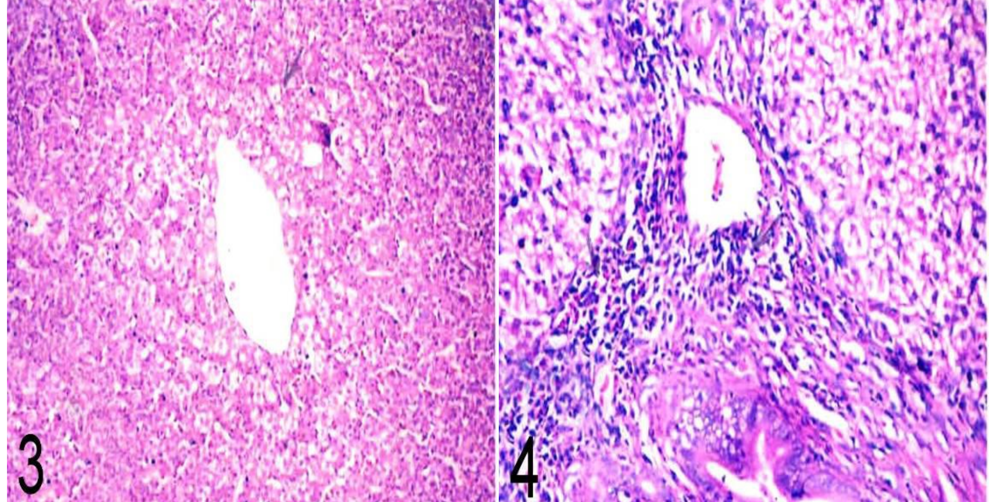


liver of a hen fed OTA contaminated feed at 3 mg/Kg. Liver is large and light in color, kidneys of a hen fed OTA @ 10 mg/Kg. Kidneys are swollen and bulging out of sockets.

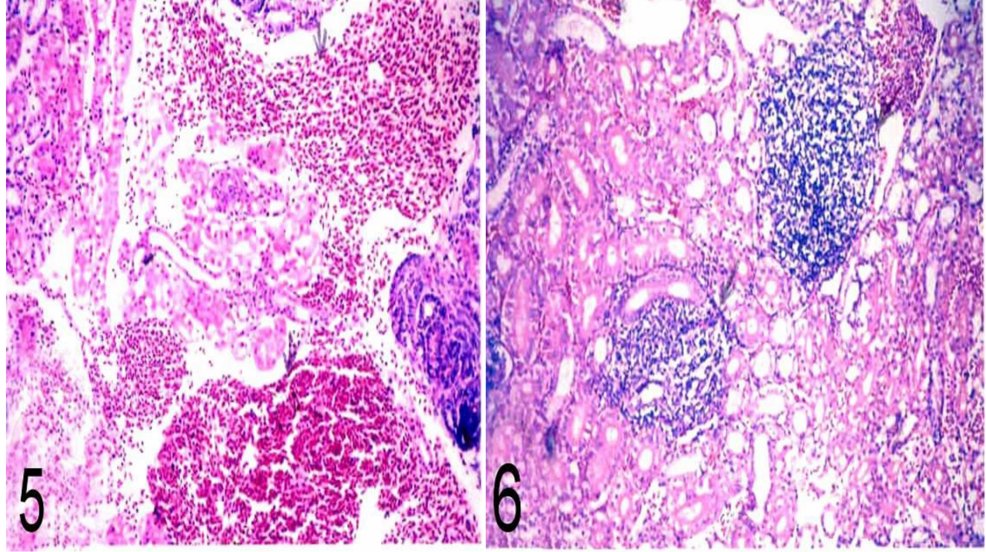
Awaad *et al.* (2011) investigated the effect of a specific combination of Mannan-oligosaccharides (MOS) and β-glucans extracted form the cell wall of a specific strain of Saccharomyces cerevisiae (AGRIMOS®) on zootechnical performance, ochratoxicosis and immune dysfunction caused by ochratoxin in broiler chickens. Three hundred and sixty, one day-old chickens were randomly allocated in a 2x2 factorial design for 5 weeks: supplementation of 2kg/ton of MOS (presence or absence) and feed contamination (presence or absence) with 50 µg/kg of ochratoxin A (OTA) for the first 3 weeks of life was done. Obtained results revealed that OTA did affect bird's growth one week after the contamination, although the final weight gain after 5 weeks was not different from the control. The use of AGRIMOS® stimulated the overall daily gain compared to the OTA group. Feed intake and feed conversion were not affected by the dietary treatments. Cumulative mortality was similar between treatments and performance indexes significantly improved with AGRIMOS® for the OTA challenged regimes. AGRIMOS® supplementation reduced macroscopic and microscopic lesion scores associated with ochratoxicosis. Also, it corrected the depression in phagocytosis induced by ochratoxin intoxication and it had strong immunomodulation as it stimulated the immune response to vaccination. It could be concluded that administration of a specific combination of Mannanoligosaccharides and β-glucans extracted form yeast cell wall (AGRIMOS®) to chickens improved zootechnical parameters had a potent immunomodulatory effect, evoked immune response and enhanced vaccination effectiveness. It helps not only in controlling chicken ochratoxicosis but also can play a positive role in treating chicken immune dysfunction



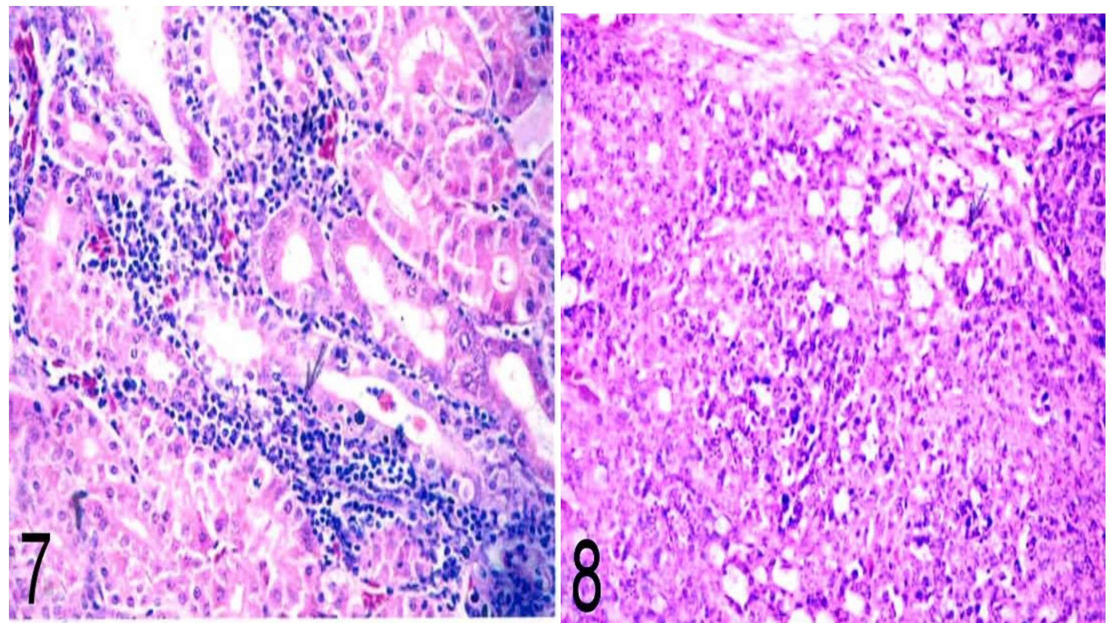
Liver (gr.I) showing chronic cholangitis. Notice the fibrous connective tissue proliferation and massive inflammatory cells infiltration in the wall of bile duct (arrow) (H&E x200) , Liver (gr.I) showing focal hepatic necrosis replaced by mononuclear leucocytes (arrow) (H&E x200) Awaad ***et al.*** **(2011)**



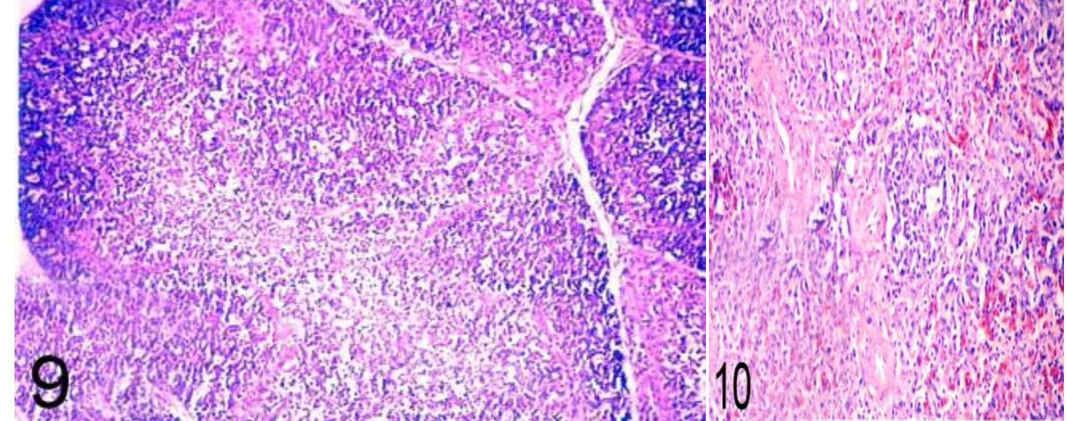
Liver (gr.IV) showing vacular degeneration of centrolobular hepatocytes (arrow) (H&E x200), Liver (gr.III) showing vacular degeneration of hepatocytes, slight thickening in the wall of bile ducts associated with leucocytic cells infiltration (arrow) (H&E x200)) **Awaad *et al.* (2011)**



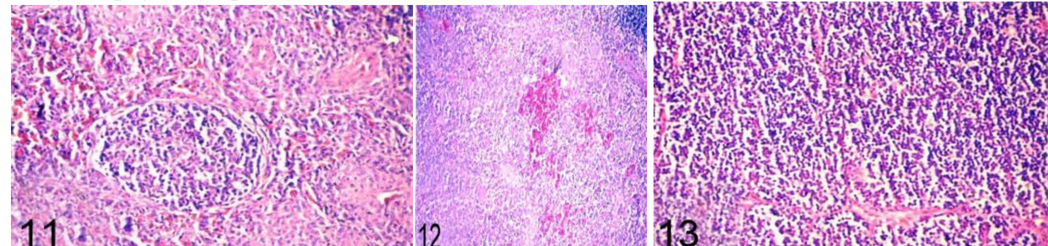
Kidney (gr.I) showing massive interstitial haemorrhage (arrow) (H&E x100) Kidney (gr.I) showing multiple focal areas of necrosis completely replaced by massive leucocytes (arrow) (H&E x100) **Awaad *et al.* (2011)**



Kidney (gr. III) showing peritubular leucocytic cells infiltration (arrow) (H & E x200) Bursa of Fabricius (gr. I) showing vaculations of lymphoid follicles (arrow) (H & E x200) **Awaad *et al.* (2011)**



Bursa of Fabricius (gr. III & IV) showing no histopathological changes (H & E x100) Spleen (gr. I) showing atrophy of lymphoid follicles (arrow) **(H & E x200) Awaad *et al.* (2011)**



Spleen (gr. III & IV) showing no histopathological changes (H & E x200) Thymus gland (gr. I) showing focal thymic haemorrhage (arrow) (H & E x100), Thymus gland (gr. III & IV) showing no histopathological alterations (H & E x00) **Awaad *et al.* (2011)**

Milicevic *et al.* (2011) carried out toxicological and histopathological investigations of tissues of commercially slaughtered chickens were carried out to provide a preliminary evaluation of the incidence of occurrence of ochratoxin A (OTA) in chicken sold in Serbian retail market. In addition, the etiology of nephropathies of these chickens was elucidated. The majority of these tissue samples were not found to contain measurable amounts of OTA. Moreover, the OTA levels found in analyzed

tissues were generally low and there was no positive correlation between the presence of OTA and the frequency of histopathological changes. Histopathological changes such as degenerative changes in the kidneys and liver differed from the classical description of the mycotoxic nephropathy, indicating that the chicken nephropathy observed in Serbia may have a multitoxic etiology with possible synergistic effect between microorganisms and natural toxins, usually present in low concentrations. The low OTA results also suggested that chicken meat available in the retail market in Serbia are unlikely to pose any significant adverse health risk to the consumers with respect to OTA toxicity.

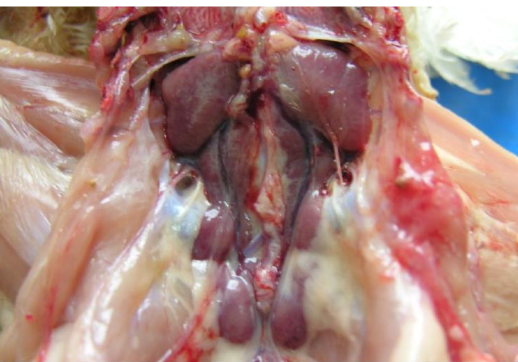
Sawarkar *et al.* (2011) conducted a study in 75 dayold Vencobb broiler chicks to evaluate toxic effects of aflatoxin B1 and ochratoxin A and efficacy of herbomineral toxin binder product (Toxiroak Gold) in preventing co-mycotoxicosis. Chicks were randomly divided into three groups of 25 each. Group I served as healthy control (C) and given standard basal ration and no treatment, Group T_0 and T_1 comprised healthy birds fed standard basal diet and mycotoxicated with 100 ppb each of aflatoxin B1 and ochratoxin A from 0-42 days. Group T_0 is not given any treatment and served as positive control; however, mycotoxicated group T_1 was administered herbomineral toxin binder product Toxiroak Gold@1kg/tonne of feed for 6 weeks. Mycotoxin adversely affected body weight gain, feed consumption, feed efficiency, haematobiochemical profile. However, supplementation of herbomineral toxin binder feed supplement has provided amelioration in mixed mycotoxicosis in broilers.

SOLCAN *et al.* (2011) evaluate the prophylactic of oil Hypophae rhamnoides, when included in a diet containing ochratoxins and fed to broiler chicks. The criteria of the evaluation included body weight gain, haematological profile and biochemistry, in addition to associated lesions in chicks. The biochemical analysis showed a considerable decrease in the serum alanine aminotransferase (ALT), increase of aspartate aminotransferase (AST), uric acid, cholesterol levels, a reduction in the serum total proteins, albumin and globulins. The addition of H. rhamnoides oil, diminished the adverse effects of ochratoxins. Chickens who received H. rhamnoides oil had a better body weight gain. Finally, it was concluded that effective in the amelioration of the toxic effects of aflatoxins that may be present in poultry rations. OTA residues from liver and kidney were significantly reduced in chickens treated with H. rhamnoides oil.

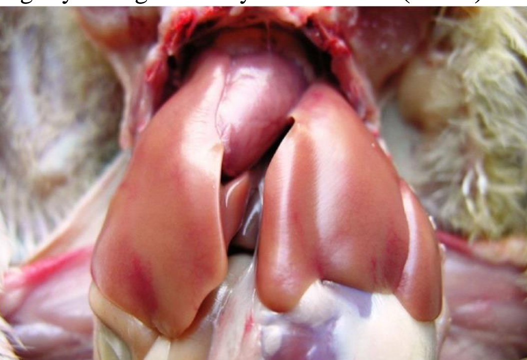
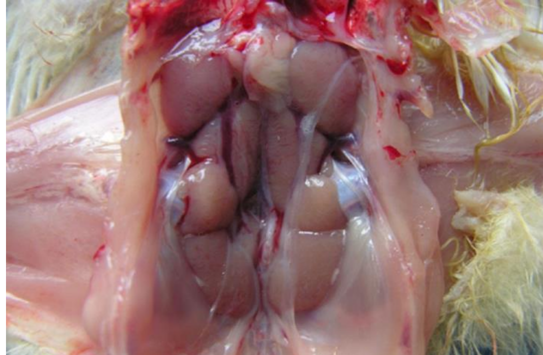
Zahoor-ul-Hassan *et al.* (2011) conducted a study to evaluate the effect of concurrent feeding of ochratoxin A (OTA) and aflatoxin B1 (AFB1) to breeder hens, upon their deposition in different tissues and eggs. Residues of OTA and AFB1 in (ng g−1) were significantly higher in liver followed by kidneys and breast muscles by 22.54±1.48, 4.22±0.93 and 0.56±0.06 for OTA (group fed OTA at 5 mg kg−1 diet) and 1.44±0.21, 0.25±0.01 and 0.03±0.01 for AFB1 (group fed AFB1 at 5 mg kg−1 diet), respectively. Residues of OTA and AFB1 in eggs appeared at days 3 and 5 of toxin feeding and disappeared at days 5 and 6 of withdrawal of mycotoxins contaminated feed, respectively. The residues of OTA and AFB1 were significantly lower in the tissues of hens fed these toxins concurrently compared with the groups fed OTA and AFB1 independently. It was concluded that residues of OTA and AFB1

appeared in the tissues and eggs of laying hens kept on OTA- and AFB1-contaminated diets. Concurrent feeding of OTA and AFB1 to hens significantly decreased the concentration of OTA and AFB1 residues in the tissues and eggs.

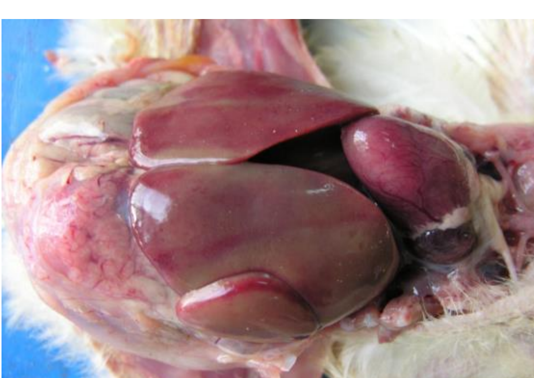
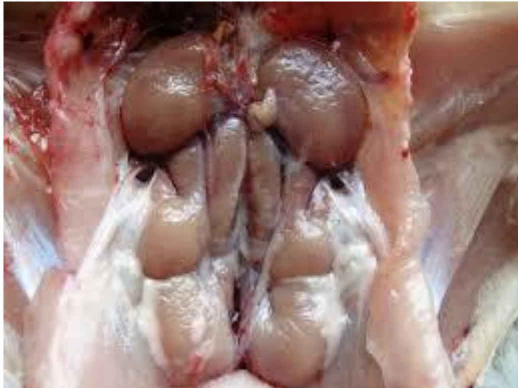
Hameed (2012) conducted experiments to induce acute ochratoxicosis in one day old (experiment 1) and 21 day old (experiment 2) broiler chicks by feeding rations containing 0, 1.6, 3.2, and 6.4 mg/kg OTA for 10 days. Chronic ochratoxicosis was induced in day old broiler chicks by feeding 0, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/kg OTA for duration of 21 (experiment 3) and 35 days (experiment 4). The severity of clinical signs was at their highest intensity at day 10-11 of the experiment. After withdrawal of OTA from the feeds the severity of signs gradually decreased and at day 22 of the experiment the birds of all treatment groups had similar behavior with minor differences from of control group. The mortality of the chicks of kept on different levels of OTA contaminated feeds have been recorded. In group A (control) no mortality was observed throughout the course of the experiment. In groups B-D mortality increased with increase in dietary levels of OTA. Maximum mortality in all OTA fed groups occurred in 2nd week of experiment during the feeding of OTA contaminated diets. In the subsequent weeks, after withdrawal of OTA contaminated feeds, mortality decreased and became 0 during weeks, 4, 5 and 6 in groups B, C and D, respectively. No gross lesions could be observed in different visceral organs of birds of all treatment groups till day 5 of the experiment. Gross lesions in visceral organs of birds of different groups were discernible on day 8 of the experiment. These changes increased in intensity on day 12 and then gradually subsided. Hemorrhages were present on the subcutaneous tissue and muscles of chicks. Liver of broiler chicks of different groups given OTA contaminated feed showed enlargement, pale discoloration, friable consistency and hemorrhages on the surface. All the changes increased in severity in dose related manner. Kidneys of treatment groups were hemorrhagic and bulged out of sockets. Lesions were more intense in chicks of 6.4 mg/kg OTA group than others. No significant gross changes could be observed in gross morphology of spleen, bursa of Fabricius and thymus of chicks of different groups. Microscopic alterations in liver were acute cellular swelling, vacular deneration and pyknosis of the hepatocytes. In kidneys, necrotic and degerative changes were recorded in the proximal convoulated tubules along with congestion of renal parenchyma. Histological alterations in bursa and thymus also showed degenerative changes and lymphoid cell depletion. The severity of microscopic changes in these organs increased in dose related manners.



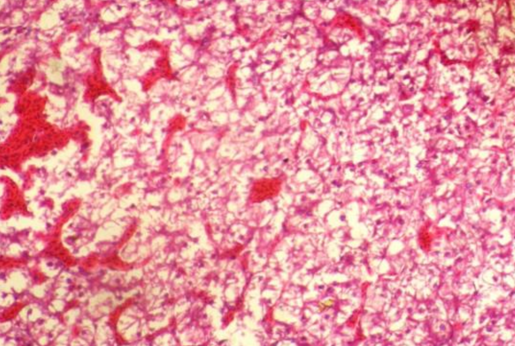
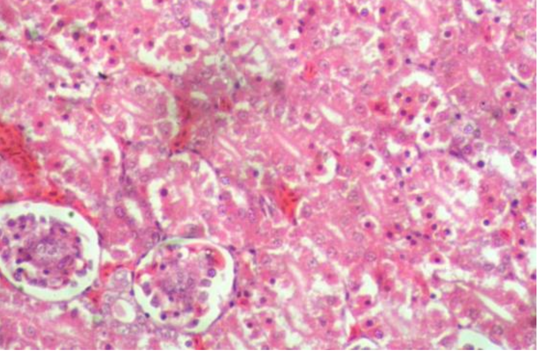
Liver from broilers fed 1.6 mg/kg OTA for 10 days from one day of age. Liver is enlarged. A photograph of kidneys from broilers fed 1.6 mg/kg OTA for 10 days from one day of age. Swollen and slightly enlarged kidneys. **Hameed (2012)**

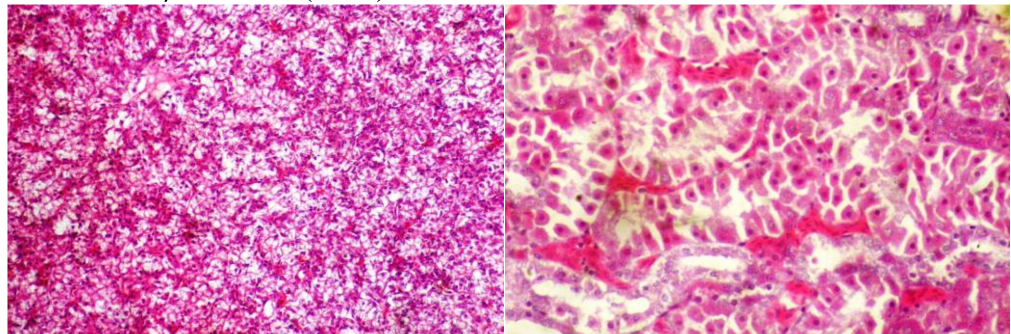
liver from broilers fed 3.2 mg/kg OTA for 10 days form one day of age. Swollen and pale liver with mild hemorrhages Liver is enlarged, kidneys from broilers fed 3.2 mg/kg OTA for 10 days from one day of age. Kidneys are enlarged and have pallor discoloration **Hameed (2012)**

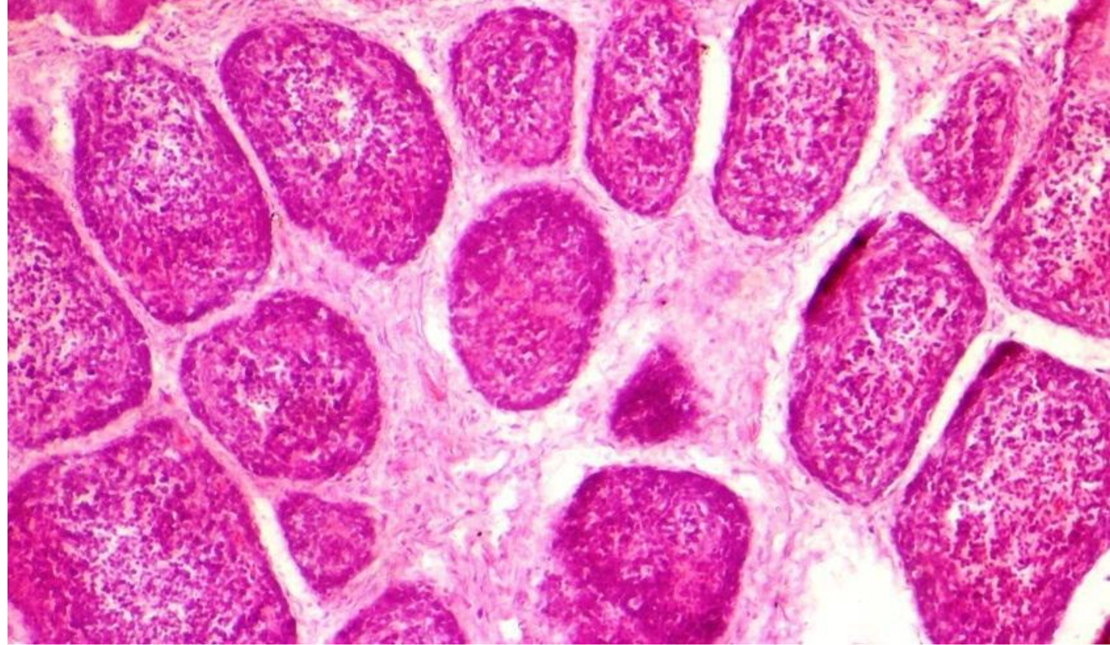
liver from broilers fed OTA 6.4 mg/kg OTA for 10 days from one day of age, liver is swollen and hemorrhagic, kidney from broilers fed OTA 6.4 mg/kg OTA for 10 days from one day of age, Kidneys are swollen and enlarged bulging out from sockets. **Hameed (2012)**

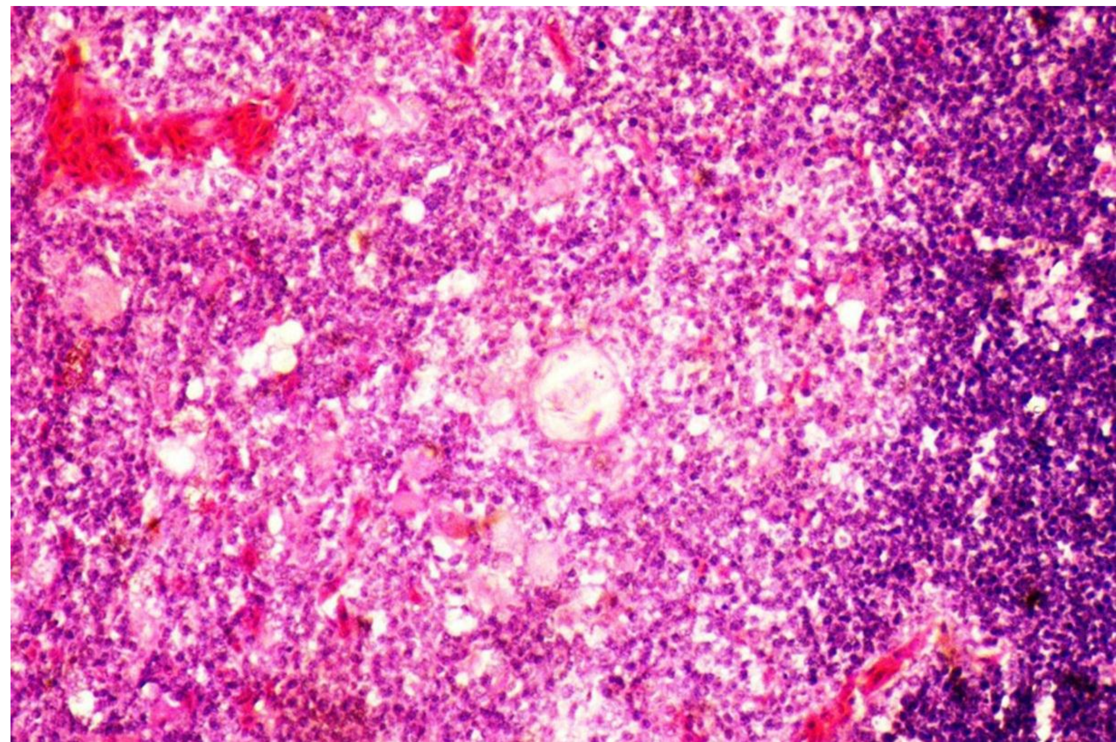
liver from broilers fed 3.2 mg/kg OTA contaminated feed for 10 days from one day of age. Vacuolar degeneration, congestion and individual cell necrosis of the hepatocytes. (H and E Satin 200X), kidney from broilers fed 3.2 mg/kg OTA contaminated feed for 10 days from one day of age. Tubular epithelial cell necrosis indicated by pyknotic nuclei and mild congestion. (H and E Satin 400X) **Hameed (2012)**



liver from broilers fed 6.4 mg/kg OTA contaminated for 10 days from one day of age. Vacuolar degeneration in hepatic parenchyma, along with pyknosis and congestion (H and E Satin 200X)., kidney from broilers fed 6.4 mg/kg OTA contaminated feed for 10 days from one day of age. Severe tubular epithelial cell necrosis as indicated by pyknosis. Congestion is also prominent. (H and E Satin 400X) **Hameed (2012)**

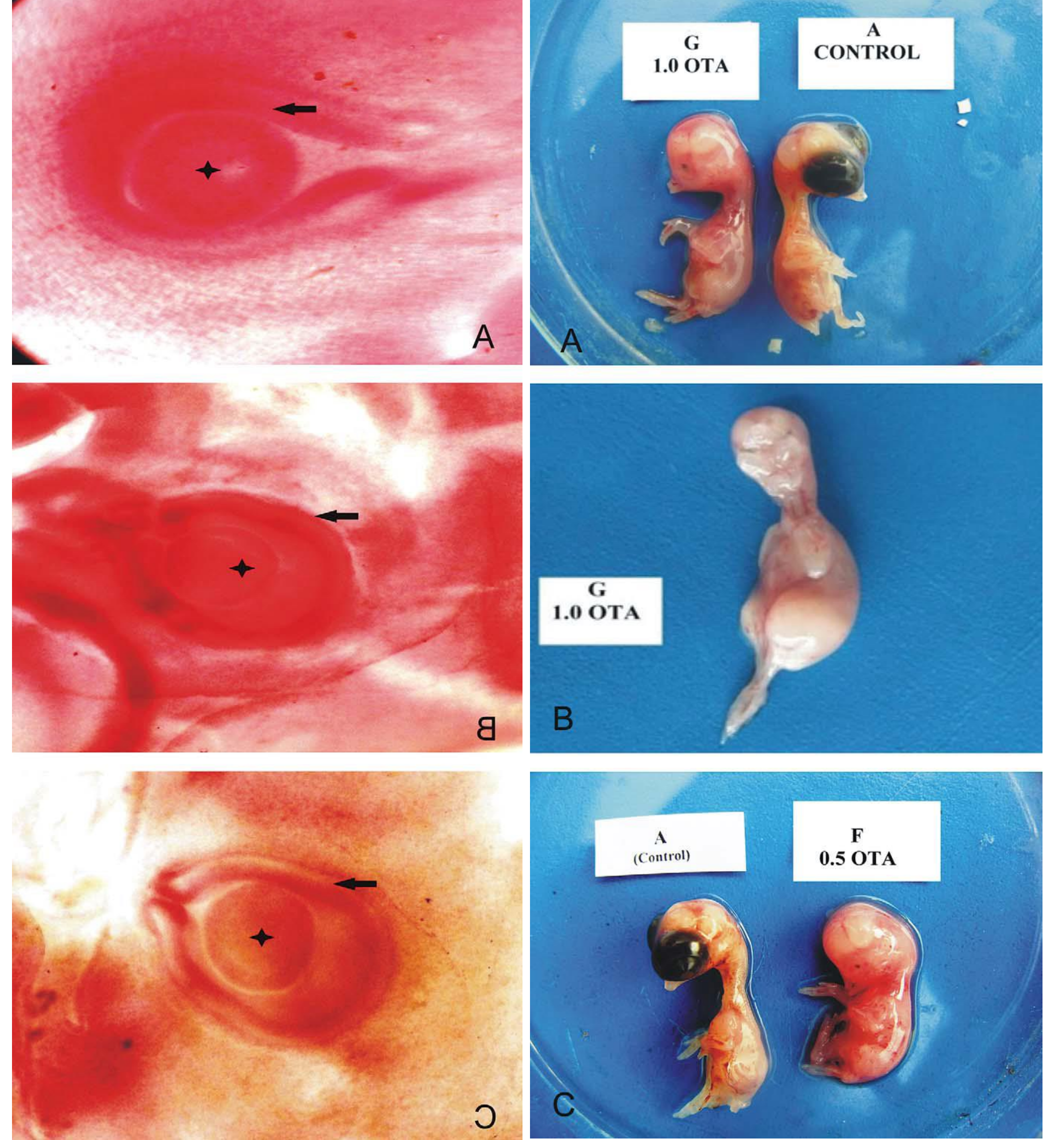


bursa of Fabricius from broilers fed 6.4 mg/kg OTA contaminated for 10 days from one day of age. Increased thickness of interfollicular connective tissue and lymphoid cell depletion in the follicles (H and E Satin 200X). **Hameed (2012)**

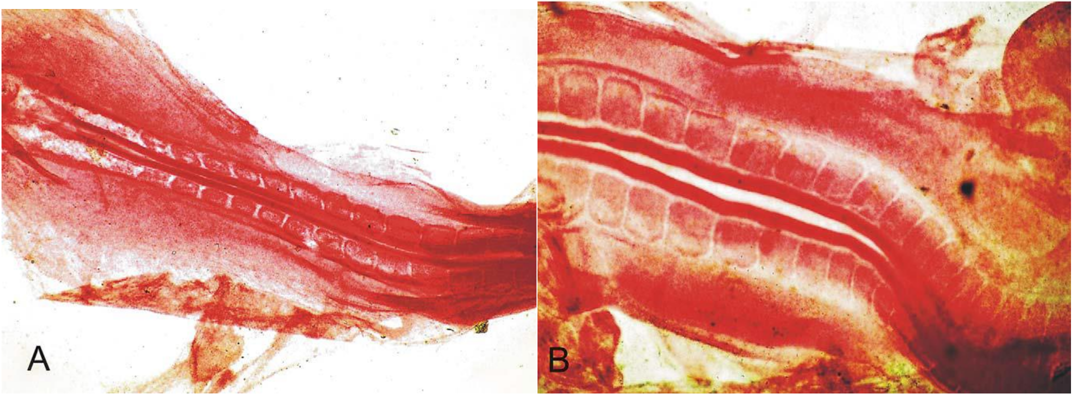


thymus from broilers fed 6.4 mg/kg OTA contaminated for 10 days from one day of age. Degenerative changes in the Hassel's corpuscles, pyknotic nuclei and vacuolation in medulla, mild hemorrhages (H and E Satin 200X) **Hameed (2012)**

ZAHOOR-UL-HASSAN *et al.* (2012) designed a study to investigate the toxico-pathological effects of in ovo inoculation of ochratoxin A (OTA) in chicken embryos and subsequently in the hatching chicks. Nine hundred fertile white leghorn (WL) layer breeder eggs were divided into eight groups (A–H). Group A was maintained as untreated control, whereas group B was kept as sham control (10 mL of 0.1 M NaHCO3 solution). Before incubation, groups C, D, E, F, G, and H were injected with 0.01, 0.03, 0.05, 0.10, 0.50, and 1.00 mg OTA/egg, respectively. At 53 hrs of incubation, crown to rump length, optic cups, and eye lens diameters were significantly (p .05) lower, whereas neural tube closure defects were higher in the OTA-treated embryos. Teratogenic defects (studied at day 9 of incubation) and embryonic mortalities were higher in the groups administered high doses of OTA. A significant increase was noted in the serum concentration of ALT, urea, and creatinine, along with higher weights of liver and kidney, in chicks hatched from OTA-contaminated eggs. These findings suggested that there are teratogenic and substantive toxicological risks in the developing chicken embryos and hatched chicks that could be exposed to OTA in ovo.

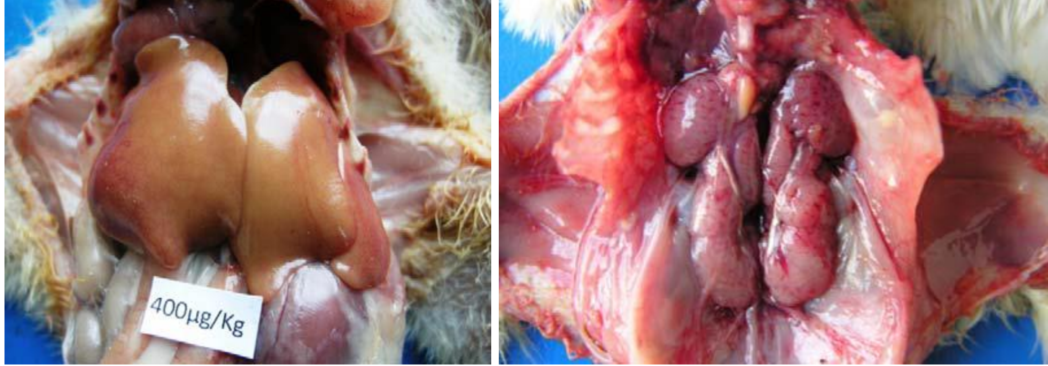


Photomicrograph of chick embryos at 53 hrs of incubation. A. Control group showing normal development of eye lens (star) and optic cup (arrow). B. 1.0 mg OTA-inoculated group. C. 0.5 mg OTA group. B & C are showing defective development of eye lens (Borax Carmine staining)., Photograph of chick embryos at day 9 of incubation. A. Embryo from group H, inoculated with OTA at 1.0 mg/egg, is showing anophthalmia. B. Embryo showing maxillary retrognathism, mandibular hypoplasia, and anophthalmia (bilateral). C. Embryo from group G, inoculated with OTA at 0.5 mg/egg, is showing anophthalmia. **ZAHOOR-UL-HASSAN et al. (2012)**

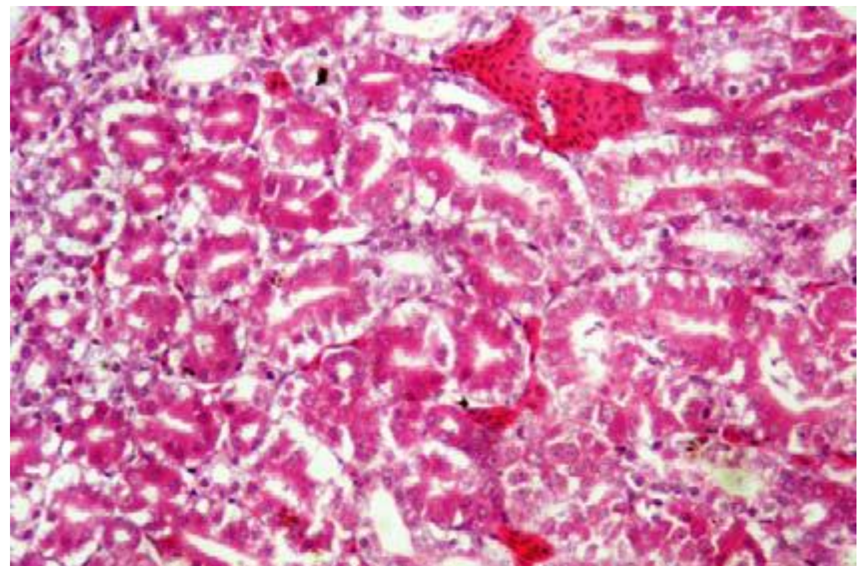


Photomicrograph of the chick embryo at 53 hrs of incubation. A. Embryo from control group showing normal closure of neural tube. B. Embryo from group H (1.0 mg OTA), showing defective closure of neural tube (Borax Carmine staining). **ZAHOOR-UL-HASSAN et al. (2012**)

Hameed *et al.* (2013) designed an experiment to evaluate the toxicopathological effects of feeding of ochratoxin contaminated feeds to broiler chicks for 21 and 35 days. Two experiments were conducted simultaneously. In these experiment six groups each having 75 chicks were maintained and offered feeds containing 0, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/kg OTA. Half of the birds from each group of both experiments were killed on days 21 and 35, respectively. Remaining birds of all the groups were switched to basal feed and killed on day 42 of the experiment. Birds killed in both experiments showed a significant decrease in the feed intake and body weight in OTA fed groups. OTA associated clinical signs and behavioral alterations included diarrhea, depression, increased water intake and ruffled feathers. The highest mortality was 12 and 20 percent observed in birds fed 0.4 and 0.8 mg/kg OTA, respectively. OTA fed birds showed a significant increase in the relative weights of liver and kidneys while decrease in weight of bursa of Fabricius and thymus. Gross lesions in liver and kidneys included enlargement, paler discoloration, friable consistency and hemorrhages. Microscopic changes in the kidneys included congestion and tubular epithelial cell necrosis. Liver showed vacuolar degeneration along with individual cell necrosis in birds fed 0.2-0.8 mg/kg OTA. Birds killed on day 35 of the intoxication showed changes similar to those observed in 21 days old birds with the exception of increased severity of these alterations in 0.4 and 0.8 mg/kg OTA groups. In conclusion, present study suggested that OTA induced pathological alterations were dependent upon dose and duration of exposure.



A liver from broilers fed 0.4 mg/kg OTA for 21 days from one day of age. Liver is pale, enlarged and hemorrhagic., kidneys from broilers fed 0.8 mg/kg OTA for 21 days from one day of age. Kidneys are swollen, enlarged and bulging out from sockets. **Hameed *et al.* (2013)**

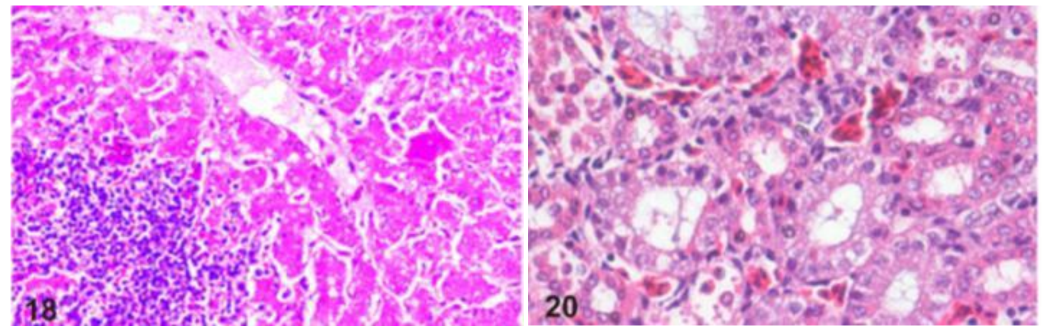


kidney from broilers fed OTA 0.4 mg/kg OTA contaminated for 21 days from one day of age. Degenerative changes along with mild congestion in the tubular epithelial cells (H and E Satin, 200X). **Hameed *et al.* (2013)**

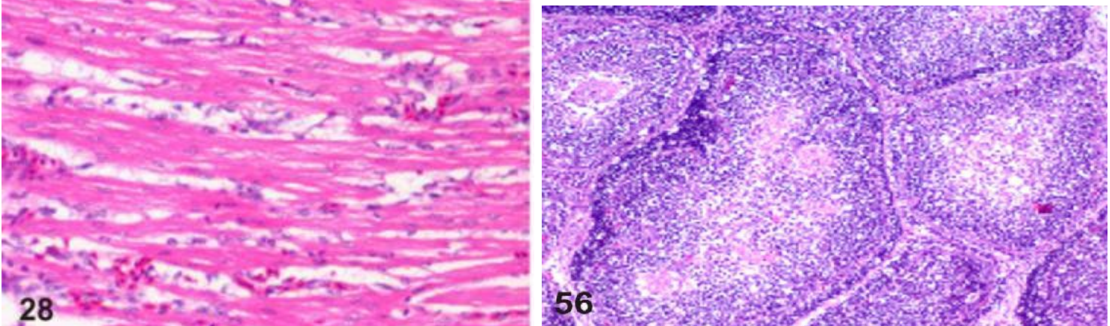
Indresh *et al.* (2013) studied the effects of Ochratoxin A(OA), T-2 toxin (T-2) and their combinations on the performance, biochemical and immune status of broiler chickens. 168 day-old Cobb broiler chicks, obtained from a commercial hatchery were divided by Complete Randomized Design into four groups of three replicates and fourteen chicks per replicate, with dietary treatments of 0.0 (control), 1 ppm OA, 2 ppm T-2 and their combination (1 ppm OA + 2.0 ppm T-2). The chicks were housed in deep litter independent conventional system with feed and water ad libitum throughout the experimental study. Body weight and feed intake were recorded weekly. At the end of the trial, blood was collected and was analyzed for total protein, serum albumin, uric acid and the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) and antibody titers against ND and IBD using ELISAtechnique. The toxin treated birds exhibited a significant decrease in the body weights and weight of lymphoid organs. A significant reduction in serum total protein, albumin and increase in serum uric acid levels were observed in toxin treated birds. The serum alanine amino transferase (ALT) and gamma glutamyl transferase (GGT) levels were decreased and antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) were decreased significantly. It was concluded that the presence of OA and T-2 in the diet showed depressing effects on performance, biochemical and immunological parameters indicating their adverse effects on the general health of broilers.

Jayaramu *et al.* (2013) conducted a study to evaluate the effect of feeding ochratoxin A and citrinin either alone or in combination in broiler chicken. Two hundred broiler chicks were divided into four groups of 50 chicks each with the following treatment viz. Control diet, (group I), OA 1 ppm, (group II), CTN 12.5 ppm (group III) and combination 1 ppm OA plus 12.5 ppm CTN (group IV) up to 35 days of the trial. The experimental and the control birds were sequentially sacrificed and examined at 7, 14, 21, 28 and 35th day of the experiment. On post-mortem examination grossly, the toxin fed birds showed congestion, enlargement, pallor or yellowish discoloration of liver with distended gall bladder, swollen and congested kidneys. In addition, congestion of heart with prominent vasculature, pale, dehydrated and shrunken

skeletal muscles, presence of small quantity of semisolid ingesta with slight mucous in crop and proventriculous, dry and shrunken gizzard, congested appearance of intestine with small quantity of mucous and congested pancreas was observed in all the toxin fed groups throughout the period of experimentation. Microscopically degenerative changes in hepatocytes, periportal fibrosis, periductular mononuclear cell infiltration, fatty degeneration, focal necrosis in the liver, degeneration and necrotic changes in the tubular epithelial cells in kidneys, myocardial degeneration, hyaline degeneration of muscle, mucosal hyperplasia of crop, proventriculitis, ventriculitis, catarrhal enteritis, pancreatitis, lymphoid depletion in the spleen, bursa of Fabricius and thymus were the prominent lesions observed when both the toxins were fed to birds from second to fifth week of age. Severity of these lesions was found to be enhanced and suggested the additive or synergistic effect of these toxins in the broiler chicken.



1. Section of Liver from OA and CTN fed bird at 28 days of age showing focal areas of hydrophic degeneration, fatty change and necrosis with infiltration of lymphoid cells. **2**. Section of Kidney from OA fed bird at 28 days showing congestion, haemorrhages, swollen and vacuolated tubular epithelialium, loss of brush border, desquamation of epithelial cells into the tubular lumen and presence of proteinaecious casts in the lumen. **Jayaramu *et al.* (2013)**



3. Section of heart from OA fed bird at 28 days of age showing edema, haemorrhage, separation and disruption of cardiac fibres with loss of cross striation. 4. Section of bursa of Fabricius from CTN fed bird at 28 days of age showing severe lymphocytolysis with histiocytosis giving starry sky appearance. **Jayaramu *et al.* (2013)**

Nedeljković-Trailović ***et al.* (2013)** performed a study on a total number of 48 Hybro broilers divided into four groups. After the pre-experimental period of 14 days, 3 experimental groups of broilers (n=12) were formed and fed diets that contained 0.5, 1.0 and 1.5 mg/kg ochratoxin A (OTA) during the next seven consecutive days. In the same period, the control group of broilers was fed a diet with no toxin added. After the period of toxin addition, blood samples were taken from 6 animals in each group. The remaining animals (n=6) from the control and experimental groups were fed diets without OTA until the 42nd day of the study, when the blood samples were taken again. The total level of blood serum proteins was affected by treatment with different doses of OTA, but a significant and dose dependent increase of albumins together

with a decrease of -globulin fraction was established. A/G ratio (Albumine/Globuline) suggested that the globulins were the dominant protein fraction in the blood serum samples obtained from all the broilers included in this study. The concentrations of - and - globulin in the serum were within physiological limits, but the concentration of - globulins significantly decreased. It can be concluded that the increasing dietary OTA levels (0.5, 1.0 and 1.5 mg/kg) had dose-dependent cumulative effect on blood serum proteins status in broilers, and the effect lasts even after the withdrawal of OTA from the feed.

Pozzo *et al.* (2013) divided thirty-six 1-day-old male broiler chicks into two groups, a control (basal diet) and an OTA (basal diet + 0.1 mg OTA/kg) group. The OTA concentration was quantified in serum, liver, kidney, breast and thigh samples. The thiobarbituric acid reactive substances (TBARS) content were evaluated in the liver, kidney, breast and thigh samples. The glutathione (GSH) content, and catalase (CAT) and superoxide dismutase (SOD) activity were measured in the liver and kidney samples. Histopathological traits were evaluated for the spleen, bursa of Fabricius and liver samples. Moreover, the chemical composition of the meat was analysed in breast and thigh samples. In the OTA diet-fed animals, a serum OTA concentration of 1.15 ± 0.35 ng/ml was found, and OTA was also detected in kidney and liver at 3.58 ± 0.85 ng OTA/g f.w. and 1.92 ± 0.21 ng OTA/g f.w., respectively. The TBARS content was higher in the kidney of the ochratoxin A group (1.53 ± 0.18 nmol/mg protein vs. 0.91 ± 0.25 nmol/mg protein). Feeding OTA at 0.1 mg OTA/kg also resulted in degenerative lesions in the spleen, bursa of Fabricius and liver. The maximum tolerable level of 0.1 mg OTA/kg, established for poultry feeds by the EU, represents a safe limit for the final consumer, because no OTA residues were found in breast and thigh meat. Even though no clinical signs were noticed in the birds fed the OTA-contaminated diet, moderate histological lesions were observed in the liver, spleen and bursa of Fabricius.

Yohannes *et al.* (2013) evaluated and recorded the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

Fareed *et al.* (2014) analyzed 186 samples comprising of poultry feed ingredients (n=114) and finished poultry feeds (n=72) for the detection of total aflatoxin (TA) and

ochratoxin A (OTA). The concentrations of TA and OTA in the samples were determined using direct competitive Enzyme-Linked Immunosorbent Assay (ELISA). Overall incidence of TA was recorded as 80.64% (n=150/186); whereas, in the feed ingredients, it was 86.84% (n=99/114), and in the finished feeds, the incidence of TA was 70.83% (n=51/72). Corn, cotton seed meal, sunflower meal, and cotton gluten meal were found to be highly (100%) contaminated with TA. The OTA was determined in 63.15% (n=72/114) and 29.17% (n=21/72) feed ingredients, and finished feed samples, respectively, with an overall incidence of 50% (n=93/186). Maximum level of OTA contamination (100%) was recorded in corn gluten meal. However, no feed contained OTA above the acceptable level as set by the European Union on OTA contamination in poultry finished feed. On the other hand, a number of samples contained TA above the acceptable limit. Thus, immediate control measures should be taken to ensure safe poultry for human consumption.

Iqbal *et al.* (2014) analyzed aflatoxins (AFs), ochratoxin A (OTA) and zearalenone (ZEN) in 115 chicken meat and 80 eggs samples, collected from central areas of Punjab, Pakistan. The study was carried out using reverse phase HPLC, equipped with fluorescence detector. The results revealed that 35% samples of chicken and 28% samples of eggs were found contaminated with AFs, and maximum level of AFB1 and total AFs was found in the liver part of chicken (layer) 7.86 and 8.01 mg/kg, respectively. Furthermore, 41% samples of chicken and 35% sample of eggs were found contaminated with OTA and maximum level 4.70 mg/kg was found in the liver part of chicken meat. However, 52% samples of meat and 32% samples of eggs were found contaminated with ZEN and maximum level 5.10 mg/kg was found in the liver part of chicken meat. The occurrence and incidence of AFs, OTA and ZEN in chicken meat and eggs are alarming and it may produce health hazards and urged the need of continuous monitoring for these toxins in chicken meat and eggs.

Khan *et al.* (2014) reviewed most significant scientific literature on ochratoxin and their possible detrimental effects on poultry birds and subsequent public health hazards. Recent studies have revealed that embryos, new born chicks and young poultry are more sensitive to ochratoxin A than adults. Ochratoxin-A has a high affinity for liver, kidneys, bursa of Fabricius and thymus. It causes an appreciable increase in the size of liver and kidneys where as the size of bursa and thymus is reduced. It also causes nephrotoxicity and hepatotoxicity with carcinogenic effect. In embryo,it causes teratologic defects in the form of anophthalmiafollowed by man dibular hypoplasia, micropthalmia, maxillary retrognathism, reduced body size, everted viscera, spina bifida and exencephaly. Biochemically it causes hypoproteinemia, hypoalbuminemia, hypoglobulinemia and hypoglycaemia. Similarly, it also causes increased levels of blood urea nitrogen (BUN), serum creatinine, uric acid, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and serum triglycerides. In order to prevent and reduce implications of these mycotoxins in poultry feed, there is needs for both global and national strategic programs to reduce the residual accumulation of mycotoxins in grain, to use advanced analytic techniques and to establish new limits concerning the maximum amount of mycotoxins allowed in poultry feed and products from poultry for human consumptions.

Armorini *et al.* (2015) evaluated the levels of ochratoxin (OTA) in kidney, liver and bile of laying hens, forty-five laying hens were enrolled in this study and divided into

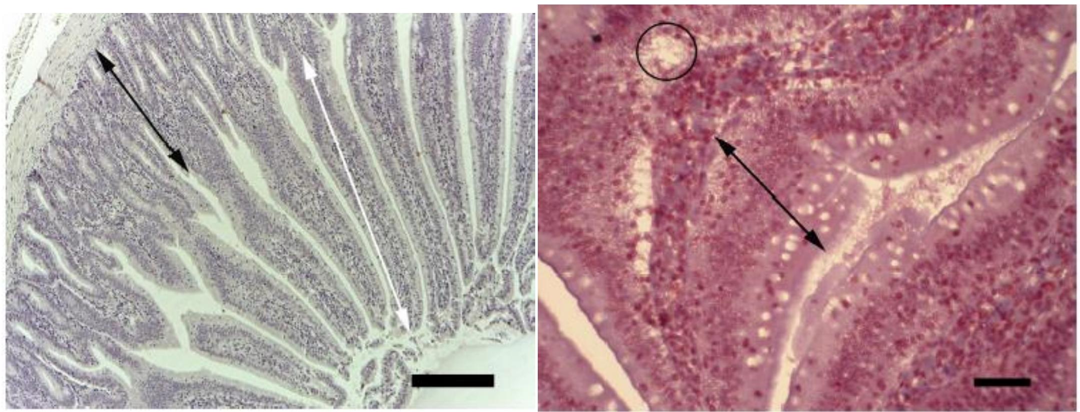
three equal groups: a control group D_0, and two experimental groups, D_1 fed with 10 µg/kg OTA diet and D_2 fed with 200 µg/kg OTA diet for 6 weeks. Kidneys, livers, and bile from all hens were collected and analyzed by HPLC method for the presence of OTA. Eggs collected 2 days before the start of the experiment and 2 days after its end were also analyzed for the presence of OTA. Results show a relevant biliary excretion of the mycotoxin, with high levels of OTA in the bile after administration of the toxin. OTA level in eggs was below the limit of detection (LOD). These results suggest the suitability of using bile as a matrix for screening measurements of OTA in laying hens.

Hanif *et al.* (2015) evaluated the effects of dietary ochratoxin A (OTA) in the presence and absence of a toxin deactivator on the organ weights, humoral immune response of broiler birds vaccinated against Newcastle disease (ND), hydropericardium syndrome (HPS) and infectious bursal disease (IBD). This was investigated in a 42 day completely randomized trial consisting of 9 dietary treatments with 1 negative control group. Birds were vaccinated against ND at 5 and 21, IBD at 11 and 24 and HPS at 15 days of age. The serum samples were collected at 14, 28 and 42 day of age were then assayed using haemagglutination inhibition (HI) for ND and indirect haemagglutination Inhibition test (IHA) for HPS and IBD, respectively. The exposure of broiler birds to two levels (500 and 1000ppb) of OTA reduced their humoral immune response against ND, HPS and IBD vaccines significantly (p in dose dependent manner. At day 14, only titers for ND were significantly elevated in group supplemented with 2kg/ton of toxin deactivator. While on day 28 and 42 of age the supplementation with 2kg/ton toxin deactivator significantly (p suppression of humoral immune system, even if the birds adequately vaccinated and predisposed to ND, HPS and IBD on challenge.

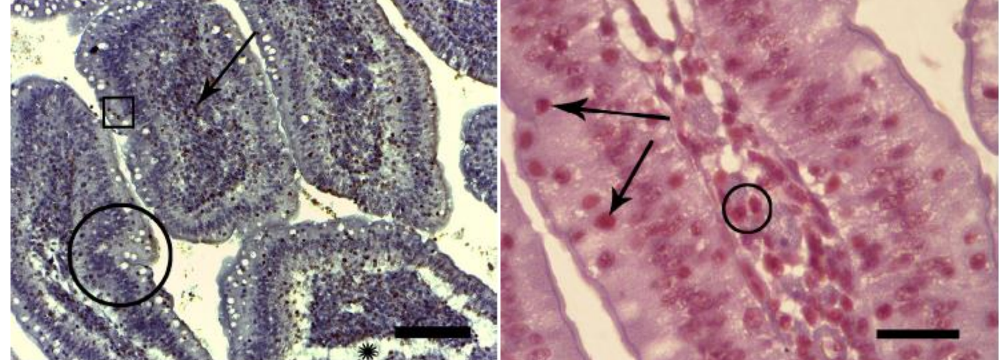
Heussner and. Bingle (2015) mentioned that ochratoxins are a group of mycotoxins produced by a variety of moulds. Ochratoxin A (OTA), the most prominent member of this toxin family, was first described by van der Merwe et al. in Nature in 1965. Dietary exposure to OTA represents a serious health issue and has been associated with several human and animal diseases including poultry ochratoxicosis, porcine nephropathy, human endemic nephropathies and urinary tract tumours in humans. More than 30 years ago, OTA was shown to be carcinogenic in rodents and since then extensive research has been performed in order to investigate its mode of action, however, this is still under debate. OTA is regarded as the most toxic family member, however, other ochratoxins or their metabolites and, in particular, ochratoxin mixtures or combinations with other mycotoxins may represent serious threats to human and animal health. This review summarised and evaluated current knowledge about the differential and comparative toxicity of the ochratoxin group.

SOLCAN *et al.* (2015) studied the immunotoxic effect of ochratoxin A (OTA) on the intestinal mucosaassociated lymphoid tissue and its cytotoxic action on the intestinal epithelium in broiler chickens experimentally treated with the toxin. From the 7th day of life, 80 male broiler chickens (Ross 308) were randomly divided into four groups of 20 birds each. The three experimental groups (E1–3) were treated with OTA for 28 days (E1: 50 µg/kg body weight [bw]/day; E2: 20 µg/kg bw/day; E3: 1 µg/kg bw/day) and the fourth group served as control. Histological examination of the intestinal mucosa and immunohistochemical staining for identification of CD4+, CD8+, TCR1 and TCR2 lymphocytes in the duodenum, jejunum and ileocaecal junction were performed, and CD4+/CD8+ and TCR1/TCR2 ratios were calculated. OTA toxicity

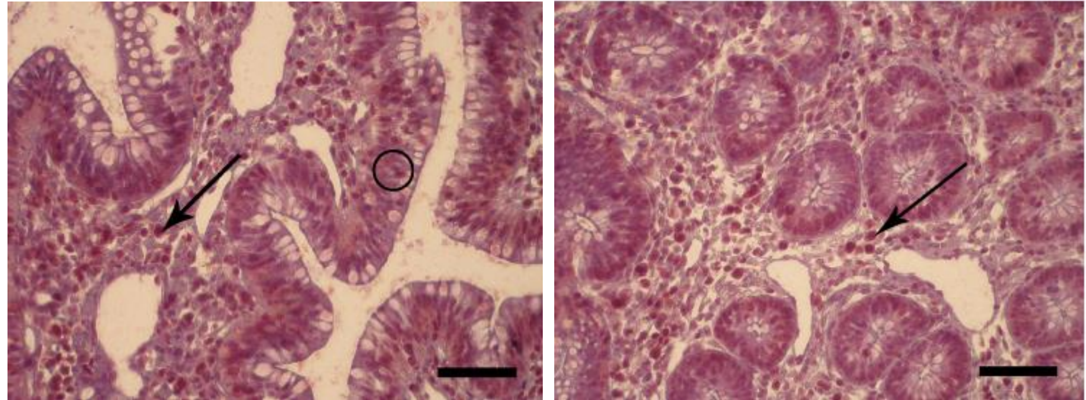
resulted in decreased body weight gain, poorer feed conversion ratio, lower leukocyte and lymphocyte count, and altered intestinal mucosa architecture. After 14 days of exposure to OTA, immunohistochemistry showed a significant reduction of the lymphocyte population in the intestinal epithelium and the lamina propria. After 28 days of exposure, an increase in the CD4+ and CD8+ values in both the duodenum and jejunum of chickens in Groups E1 and E2 was observed, but the TCR1 and TCR2 lymphocyte counts showed a significant reduction. No significant changes were observed in Group E3.



Duodenum of a control chicken on day 28. Normal structure, height of villi (white arrow) and depth of glands (black arrow). Gömöri trichrome stain. Bar = 200 µm, Duodenum of a chicken exposed to OTA for 28 days (E1).CD4+ lymphocytes. Mucosal epithelium with giant cells, without brush border (black arrow) and numerous small vacuoles (circle). Immunohistochemical (IHC) staining for LT CD4+. Bar 200 µm **SOLCAN *et al.* (2015)**

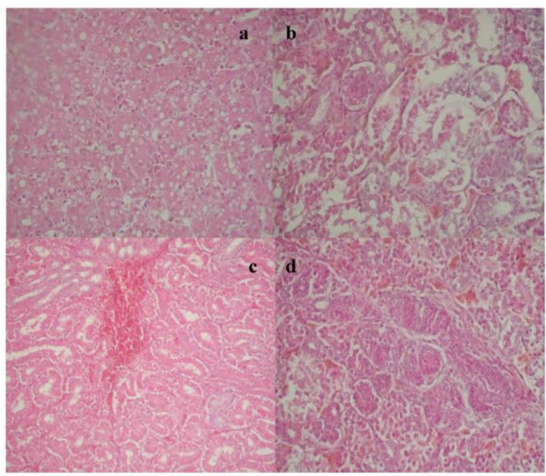


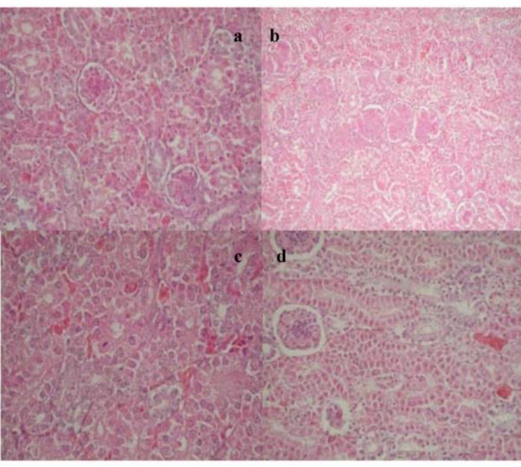
Jejunal villi of a chicken exposed to OTA for 21 days (E1). Mucosal epithelium with giant cells (black circle), without brush border and numerous small vacuoles (black star). Epithelium detached from the basal membrane; lymphocytes in the lamina propria (black arrow) and in intraepitheliallocation (black square). IHC staining for LT CD8+. Bar = 50 µm, Jejunal villi of a chicken exposed to OTA for 21 days (E3). Mucosal epithelium without brush border. CD8+ lymphocytes in intraepithelial location (black arrows) and in the lamina propria (black circle). IHC staining for LT CD8+. Bar = 100 µm **SOLCAN *et al.* (2015)**



Villi of the ileocaecal junction of a chicken exposed to OTA for 28 days (E2). TCR1 lymphocytes in intraepithelial location (black circle) and in the lamina propria (black arrow). IHC staining for LT TCR1. Bar = 100 μm Lamina propria of the mucosa at the ileocaecal junction in a chicken exposed to OTA for 28 days (E2). TCR2 intraepithelial lymphocytes of the lamina propria (black arrow). IHC staining for TCR2. Bar = 100 μm **SOLCAN *et al.* (2015)**

Nedeljković-Trailović *et al.* (2015) performed a study was to determine the efficacy of three different adsorbents, inorganic (modified zeolite), organic (esterified glucomannans) and mixed (inorganic and organic components, with the addition of enzymes), in protecting broilers from the toxic effects of ochratoxin A in feed. Broilers were fed diets containing 2 mg/kg of ochratoxin A (OTA) and supplemented with adsorbents at the recommended concentration of 2 g/kg for 21 days. The presence of OTA led to a notable reduction in body weight, lower weight gain, increased feed conversion and induced histopathological changes in the liver and kidneys. The presence of inorganic, organic and mixed adsorbents in contaminated feed only partially reduced the negative effects of OTA on the broiler performances. Broilers that were fed with adsorbent-supplemented feed reached higher body weight (17.96%, 19.09% and 13.59%), compared to the group that received only OTA. The presence of adsorbents partially alleviated the reduction in feed consumption (22.68%, 12.91% and 10.59%), and a similar effect was observed with feed conversion. The applied adsorbents have also reduced the intensity of histopathological changes caused by OTA; however, they were not able to prevent their onset. After the withdrawal of the toxin and adsorbents from the feed (21–42 days), all previously observed disturbances in broilers were reduced, but more remarkably in broilers fed with adsorbents.





(**a**) Hepatocyte vacuolation due to the accumulation of lipid droplets, Experimental Group I (E-I); (**b**) Necrotic foci localized in tubulocytes, E-I group; (**c**) Hemorrhagic areas with massive effusion of red blood cells, E-I group; (**d**) Renal tubular cell proliferation and formation of adenoma-like structures, E-I group.
(**a**) Edema of the renal proximal tubule cells with partial tubule lumen stenosis, dystrophic changes with the appearance of apoptotic bodies, E-II group; (**b**) Proliferation of mesangial cells and capillary endothelial cells in the glomeruli, E-II group; (**c**) Regenerative changes in the tubulocytes, E-I group after the withdrawal period; (**d**) Regenerative changes in the tubulocytes and sclerotic changes in the glomeruli, E-I group after the withdrawal period.

Hanif (2016), in a comprehensive review on ochratoxicosis, mentioned that ochratoxin A (OTA) is a mycotoxin produced by several fungi of the genera Aspergillus and Penicillium, principally P. verrucosum in temperate climate and A. ochraceous in warm regions. In poultry feed materials, mycotoxins are found most commonly in cereals and to a lesser degree, in meals. The presence of OTA in animal feed contributes significantly to health disorders and decreased production. In addition to aflatoxins, which is an ubiquitously distributed toxin, OTA is one of the reasons for economic losses in the poultry industry due to poor performance and immunosuppression. Moreover, OTA has also been noted for the carryover effect in meat and tissues. This review provides the information regarding the nephrotoxic and hepatotoxic effects of OTA in monogastric animals. Histopathological studies revealed a depletion of lymphoid tissues, granular degeneration in the epithelial and mononuclear proliferation and activation of capillary endothelium cells in the kidney and liver tissue of monogastric animals. Elevated liver enzymes and blood biochemical parameters related to kidney were also observed. For the first time, this article revealed that the reduced Newcastle Disease (ND), Infectious Bursal Disease (IBD) and Hydropericardium Syndrome (HPS) vaccine titers were noticed in broilers intoxicated with OTA. There are various possible ameliorating strategies that exist; however, deactivation of OTA is more convenient as compared to adsorption techniques. In brief, to overcome the implications of toxins on animal health, there is a need of good management practices to reduce the contamination in cereals, the usage of advanced analytical techniques and establishment of guidelines for OTA in animal feed and products.

Kongkapan *et al.* (2016) developed an analytical method using LC-ESI-MS/MS to quantify nine mycotoxins, consisting of aflatoxin B1 (AFB1), AFB2, AFG1, AFG2, T-2 toxin, deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and ochratoxin A (OTA) in broiler feeds. In total, 100 samples of broiler feeds were collected from poultry farms in Central Thailand. The survey found that AFB1 and ZEA were the most prevalent mycotoxins in the feed samples at percentages of 93% and 63%, respectively. The limit of detections (LODs) of investigated mycotoxins was 0.20-0.78 ng/g. AFB2, DON, AFG1, NIV and T-2 toxin were also detectable at low contamination levels with percentages of 20%, 9%, 7%, 5% and 1%, respectively, whereas OTA and AFG2 were not detected in any of the feed samples. These results suggest that there is a very low level of risk of the exposure to mycotoxins in feeds obtained from broiler farms in Central Thailand.

Sumbal *et al.* (2016) investigated the efficacy of ultra-violet irradiation for decontamination of ochratoxin A (OTA) in spiked and naturally contaminated poultry feed samples. Spiked and naturally contaminated feed samples were irradiated with ultra-violet light (UV) at distance of 25 cm over the feed samples. In vitro, the effect

of UV intensity (0.1 mW cm(-2) at 254 nm UV-C) on different types of poultry feeds contaminated with OTA was evaluated. The same samples were also irradiated with sunlight and analysed for OTA by an indirect enzyme linked immunosorbent assay method. Poultry feed samples containing 500 µg kg(-1) were 100% decontaminated in 180 min with UV radiation while OTA was decreased to 70-95 µg kg(-1) using the same poultry feed samples after 8 h sunlight irradiation. Therefore, UV light was found to be more effective. Only 1 h of UV irradiation was found to be sufficient to bring the OTA level to the maximum regulatory limit suggested for poultry feeds (100 µg kg(-1)), while 8 h were needed to obtain this level using sunlight radiations.

References

1. Abarca, M.L., M.R. Bragulat, G. Castella and F.J. Cabanes. 1994. Ochratoxin A production by strains of Aspergillus niger var. niger. Appl Environ Microbiol 60:2650-2652.
2. Armorini S, Al-Qudah KM, Altafini A, Zaghini A, Roncada Biliary ochratoxin A as a biomarker of ochratoxin exposure in laying hens: An experimental study after administration of contaminated diets. Res Vet Sci. 2015 Jun;100:265-70.
3. Awaad, M.H.H. , A. M. Atta , Wafaa A. Abd El-Ghany , M. Elmenawey , K. Ahmed ; A. A. Hassan , A. A. Nada and G. A. Abdelaleem . Effect of a Specific Combination of Mannan-Oligosaccharides and β-Glucans Extracted from Yeast Cell Wall on the Health Status and Growth Performance of Ochratoxicated Broiler Chickens . Journal of American Science, 2011;7(3).82-96
4. Ayed IA, Dafalla R, Yagi AI, Adam SE. Effect on ochratoxin A on Lohmann-type chicks. Vet Hum Toxicol. 1991 Dec;33(6):557-60.
5. Bayman, P., J.L. Baker, M.A. Doster, T.J. Michailides and N.E. Mahoney. 2002. Ochratoxin production by the Aspergillus ochraceus group and Aspergillus alliaceus. Appl Environ Microbiol 68:2326-2329.
6. Beg MU, Al-Mutairi M, Beg KR, Al-Mazeedi HM, Ali LN, Saeed T (2006). Mycotoxin in poultry feed in Kuwait. Archives of Environmental Contamination and Toxicology, 50: 595-602.
7. Biro, K., L. Solti, I. Barna-Vetro, G. Bago, R. Glavits, E. Szabo and J. FinkGremmels. 2002. Tissue distribution of ochratoxin A as determined by HPLC and ELISA and histopathological effects in chickens. Avian Pathol 31:141- 148.
8. Bodnarchuk AI, Kaspruk IM. Ochratoxicosis in ducks. Veterinarya, Moscow 1984; 2: 67–68 (Poult Abs 1984 10: no. 1092).
9. Bozzo, G., E. Ceci,1 E. Bonerba, S. Desantis, and G. Tantillo. Ochratoxin A in Laying Hens: High-Performance Liquid Chromatography Detection and Cytological and Histological Analysis of Target Tissues. 2008 J. Appl. Poult. Res. 17:151–156
10. Campbell, M.L., Jr., J.D. May, W.E. Huff and J.A. Doerr. 1983. Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. Poult Sci 62:2138-2144.
11. Carmen,s., I. Coman, Gh. Solcan, L. Miron, O.Z. Oprean HISTOLOGICAL AND ULTRASTRUCTURAL LESIONS OF THE KIDNEY IN EXPERIMENTAL OCHRATOXICOSIS OF BROILER CHICKENS. Bulletin UASVM, Veterinary Medicine 65(1)/2008 pISSN 1843-5270; eISSN 1843-5378
12. Chang, C.F.; Doerr, J.A. and Hamitton, P.B. (1981): "Experimental ochratoxicosis in turkey poults." Poult. Sci., 60(1):114-119.
13. Choudhury, H. and C. W. Carlson The Lethal Dose of Ochratoxin for Chick Embryos*Poultry Science* (1973) 52 (3): 1202-1203
14. Ciegler, A.; Fenell, D.I.; Sansing, G.A.; Detroy, R.W.; Bennett, G.A. Mycotoxin-producing strains of *Penicillium viridicatum*: Classification into subgroups. *Appl. Microbiol.* **1973**, *26*, 271–278.

15. Dalcero, A., Magnoli, C., Luna, M., Ancasi, G., Reynoso, M.M., Chiacchiera, S., Miazzo, R. and Palacio, G. (1998) Mycoflora and naturally ocurring mycotoxins in poultry feeds in Argentina. Mycopathologia 141, 37–43.
16. Denli M., Blandon J.C., Guynot M.E., Salado S., Perez J.F. (2008): Efficacy of a new ochratoxin-binding agent (OcraTox) to counteract the deleterious effects of Ochratoxin A in laying hens. Poultry Science, 87: 2266–2272.
17. Doerr, J.A.; Huff, W.E.; Hamilton, P.B. and Lillehoj, E.B. (1981): "Severe coagulopathy in young chickens produced by OA." Toxicol. Appl. Pharmacol., 59: 157- 163.
18. Doupnik, B., Jr., and J. Peckham. 1970. Mycotoxicity of Aspergillus ochraceus to chicks. Appl. Microbiol. 19:594- 597.
19. Dwivedi, P. and R.B. Burns. 1984. Pathology of ochratoxicosis A in young broiler chicks. Res Vet Sci 36:92-103.
20. Dwivedi P; R.B. Burns; M.H. Maxwell; 1984- Ultrastructural study of the liver and kidney in ochratoxicosis A in young broiler chicks. Res Vet Sci. Jan;36(1):104-16.
21. Elaroussi, M. A.; Mohamed, F. R.; Barkouky, E. M.; Atta, A. M.; Abdou, A. M.; Hatab, M. H., 2006. Experimental ochratoxicosis in broiler chickens. Avi. Pathol., 35: 263-9.
22. Elaroussi, M. A, F.R. Mohamed , M.S. Elgendy , E.M. El Barkouky , A.M. Abdou and M.H. Hatab. Ochratoxicosis in Broiler Chickens: Functional and Histological Changes in Target Organs. International Journal of Poultry Science 7 (5): 414-422, 2008
23. Elling, F.; Hald, B.; Jacobson, C. and Krogh, P. (1975): Sp-ontaneous cases of toxic nephro-pathy in poultry associated with ochratoxin A. Acta. Pathol.
24. Microbiol. Scand. Sec. A. 83: 739-741.
25. Elwan, I.F.; Shalaby, S.I. and Moustafa, A.M. PATHOLOGICAL STUDIES ON MYCOTOXINS IN OSTRICH. SCVMJ, X IV (2) 2009 177
26. Fareed,G., Sohail Hassan Khan* , Muhammad Ashraf Anjum and Naveed Ahmed. Determination of Aflatoxin and Ochratoxin in poultry feed ingredients and finished feed in humid semi-tropical environment. J. Adv. Vet. Anim. Res., 1(4): 201-207,2014
27. Frisvad, J.C.; Smedsgaard, J.; Larsen, T.O.; Samson, R.A. Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Stud. Mycol.* 2004, *49*, 201–241
28. Frisvad, J.C.; Filtenborg, O. Terverticillate penicillia: Chemotaxonomy and mycotoxin production. *Mycologia* 1989, *81*, 837–861.
29. Frisvad, J.C,J. Mick Frank , Jos A.M.P. Houbraken , Angelina F.A. Kuijpers and Robert A. Samson. New ochratoxin A producing species of Aspergillus section Circumdati. STUDIES IN MYCOLOGY 50: 23–43. 2004.
30. Frisvad, J. C. and Robert A. Samson Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. *Stud. Mycol.* **2004**, *49*, 1–173
31. Frye, C.E. and F.S. Chu. 1978. Distribution of ochratoxin A in chicken tissues and eggs. J. Food Safety 1:147-159.
32. Galtier, P., Alvinerie, M. & Charpenteau, J.L. (1981). The pharmacokinetic profiles of ochratoxin A in pigs, rabbits and chickens. Food and Cosmetic Toxicology, 19, 735–738.
33. Garcia A.R., Avila E., Rosiles R., Petrone V.M. Evaluation of two mycotoxin binders to reduce toxicity of broiler diets containing ochratoxin A and T-2 toxin contaminated grain. Avian Dis. 2003;47:691–699
34. Gentles A., Smith E.E., Kubena L.F., Duffus E., Johnson P., Thompson J., Harvey R.B., Edrington T.S. Toxicological evaluations of cyclopiazonic acid and ochratoxin A in broilers. Poult. Sci. 1999;78:1380–1384.
35. Ghimpeţeanu, O. M., Andreea Tolescu, Manuella Militaru. AFLATOXIN AND OCHRATOXIN CONTAMINATION IN POULTRY -A REVIEW Veterinary Medicine, Vol. LVIII 3, 1222-5304, 308-317.
36. Gibson, R. M., Baily, C. A., Kubena, L. F., Huff, W. E., and Harvey, R. B. (1989). Ochratoxin A and dietary protein. 1. Effect on body weight, feed conversion, relative organ weight, and mortality in three-week-old broiler. Poult Sci 68, 1658–63.

37. Gilani, S. H., Bancroft, J., and Reily, M. (1978). Teratogenisity of ochratoxin A in chick embryos. Toxicol Appl Pharmacol 46, 543–6.
38. Gupta S, Jindal N, Khokhar RS, Gupta AK, Ledoux DR, Rottinghaus GE. Effect of ochratoxin A on broiler chicks challenged with Salmonella gallinarum. Br Poult Sci. 2005 Aug;46(4):443-50.
39. Gupta S., Jindal N., Khokhar R.S., Asrani R.K., Ledoux D.R., Rottinghaus G.E. Individual and combined effects of ochratoxin A and Salmonella enterica serovar Gallinarum infection on pathological changes in broiler chickens. Avian Path. 2008;37:265–272.
40. Haazele F.M., Guenter W., Marquardt R.R., Frohlich A.A. Beneficial effects of dietary ascorbic acid supplement on hens subjected to ochratoxin A toxicosis under normal and high ambient temperatures. Can. J. Anim. Sci. 1993;73:149–157.
41. Hameed MR, MZ Khan, A Khan and I Javed, 2013. Ochratoxin induced pathological alterations in broiler chicks: effect of dose and duration. Pak Vet J, 33(2): 145-149.
42. Hameed, M.R. (2012). Ochratoxicosis In Chicken Pathological, Biochemical Alterations And Tissue Residues. Thesus. Department Of Pathology, Faculty Of Veterinary Science / University Of Agriculture, Faisalabad
43. Hamilton, P.B., W.E. Huff, J.R. Harris and R.D. Wyatt. 1982. Natural occurrences of ochratoxicosis in poultry. Poult Sci 61:1832-1841.
44. Hanif, N. Q. OCHRATOXICOSIS IN MONOGASTRIC ANIMALS – A REVIEW. J. Bioresource Manage. (2016) 3(1):3-24
45. Hanif NQ, Muhammad G, Siddique M, Khanum A, Ahmed T, Gadahai JA, Kaukab G (2008). Clinico-pathomorphological, serum biochemical and histological studies in broilers fed ochratoxin A and a toxin deactivator. British Poul Sci J. 49, 632-642.
46. Hanif, N. Q. and.G. Muhammad. Immunotoxicity of Ochratoxin A and Role of Trichosporon mycotoxinivorans on the Humoral Response to Infectious Viral Disease Vaccines in Broilers. Pakistan J. Zool., vol. 47(6), pp. 1683-1689, 2015
47. Harvey, R.B., L.F. Kubena, S.A. Naqi, J.E. Gyimah, D.E. Corrier, B. Panigrahy and T.D. Phillips. 1987. Immunologic effects of low levels of ochratoxin A in ovo: utilization of a chicken embryo model. Avian Dis 31:787-791.
48. Heussner A. H. and Lewis E. H. Bingle. Review comparative ochratoxin toxicity: A Review of the Available Data. Toxins 2015, 7, 4253-4282; 2 1
49. Huff, W.E. and J.A. Doerr. 1981. Synergism between aflatoxin and ochratoxin A in broiler chickens. Poult Sci 60:550-555.
50. Huff W.E; L.F. Kubena; R.B. Harvey; 1988- Progression of ochratoxicosis in broiler chickens. Poult Sci.Aug;67(8):1139-46.
51. Huff, W.E., R.D. Wyatt, T.L. Tucker and P.B. Hamilton. 1974. Ochratoxicosis in the broiler chicken. Poult Sci 53:1585-1591.
52. Huff, W.E., R.D. Wyatt and P.B. Hamilton. 1975. Nephrotoxicity of dietary ochratoxin A in broiler chickens. Appl Microbiol 30:48-51.
53. Iqbal, Shahzad Zafar, , Sonia Nisar , Muhammad Rafique Asi c , S. Jinap b,d. Natural incidence of aflatoxins, ochratoxin A and zearalenone in chicken meat and eggs. Food Control 43 (2014) 98e103
54. Indresh HC and Umakantha B (2013) Effects of ochratoxin and T-2 toxin combination on performance, biochemical and immune status of commercial broilers, Veterinary World 6(11): 945-949
55. Jayaramu, G. M., M. L. Satyanarayana, P. Ravikumar, V. T. Shilpa, H. D. Narayanaswamy & Suguna Rao. OCHRATOXIN A AND CITRININ INDUCED PATHOMORPHOLOGICAL CHANGES IN BROILER CHICKEN. G.J.B.B., VOL.2 (2) 2013: 230-234
56. Joo , Yang Don , Chang Won Kang , Byoung Ki An , Jong Sung Ahn , Radka Borutova. EFFECTS OF OCHRATOXIN A AND PREVENTIVE ACTION OF A MYCOTOXIN-DEACTIVATION PRODUCT IN BROILER CHICKENS. VETERINARIJA IR ZOOTECHNIKA (Vet Med Zoot). T. 61 (83). 2013

57. Kalorey, D.R., N. V. Kurkure*, J. S. Ramgaonkar, P. S. Sakhare, Shubhangi Warke and N. K. Nigot. Effect of Polyherbal Feed Supplement "Growell" during Induced Aflatoxicosis, Ochratoxicosis and Combined Mycotoxicoses in Broilers. Asian-Australasian Journal of Animal Sciences 2005;18(3): 375-383.
58. Khan, S. A., Venancio, E. J., Hirooka, E. Y., Rigobello, F., Ishikawa, A., Nagashima, L. A., Oba, A., & Itano, E. N. (2014). Avian ochratoxicosis: A review. African Journal of Microbiology Research, 8(35), 3216-3219.
59. Kongkapan, Jutamart , Saranya Poapolathep, Supaporn Isariyodom, Susumu Kumagai, Amnart Poapolathep. Simultaneous detection of multiple mycotoxins in broiler feeds using a liquid chromatography tandem-mass spectrometry *Journal of Veterinary Medical Science 2016 March 1, 78 (2): 259-64*
60. Kozaczynski, W. (1994). Experimental ochratoxicosis A in chickens. Histopathological and histochemical study. Archivum Veterinarium Polonicum, 34, 205– 219.
61. Koynarski, V.; Stoev, S.; Grozeva, N.; Mirtcheva, T.; Daskalov, H.; Mitev, J.; Mantle, P. Experimental coccidiosis provoked by Eimeria acervulina in chicks simultaneously fed on ochratoxin A diet. *Res. Vet. Sci.* **2007**, *82*, 225–231.
62. Krogh, P.; Elling, B.; Hold, B.; Syllin, V.E.; Skadhange, E. and Svensen, C.K. (1976a): “Experimental avian nephropathy- changes of renal function and structure induced by OAcontaminated feed." Acta Pathol. Microbiol. Scand., 84: 215- 221.
63. Kubena, L.F., T.D. Phillips, C.R. Creger, D.A. Witzel and N.D. Heidelbaugh. 1983. Toxicity of ochratoxin A and tannic acid to growing chicks. Poult Sci 62:1786-1792
64. Kumar ,A ., Naresh Jindal, Chhote L. Shukla , Rajesh K. Asrani , David R. Ledoux and George E. Rottinghaus. Pathological changes in broiler chickens fed ochratoxin A and inoculated with Escherichia coli. Avian Pathology (August 2004) 33(4), 413/417
65. Larsen, T.O.; Svendsen, A.; Smedsgaard, J. Biochemical characterization of ochratoxin A-producing strains of the genus *Penicillium*. *Appl. Environ. Microbiol.* **2001**, *67*, 3630–3635.
66. Manning, R.O. and R.D. Wyatt. 1984. Toxicity of Aspergillus ochraceus contaminated wheat and different chemical forms of ochratoxin A in broiler chicks. Poult Sci 63:458-465.
67. Masood; M. Z. A. Yousaf; T. Ahmad; and G. Muhammad. EFFECT OF OCHRATOXIN A ON LIVER ENZYMES AND HUMORAL RESPONSE TO HYDROPERICARDIUM SYNDROME VACCINE IN BROILERS. 5thInternational Poultry Conference 10-13 March 2009. Taba –Egypt
68. Micco, C., Miraglia, M., Onori, R., Ioppolo, A. & Mantovani, A. (1987). Long-term administration of low doses of mycotoxins in poultry. 1. Residues of ochratoxin A in broilers and laying hens. Poultry Science, 66, 47–50.
69. Micco, C., Miraglia, M., Benelli, L., Onori, R., Ioppolo, A. & Mantovani, A.L. (1988). Long term administration of low doses of mycotoxins in poultry. 2. Residues of ochratoxin A and aflatoxins in broilers and laying hens after combined administration of ochratoxin A and aflatoxin B1 . Food Additives and Contaminants, 5, 309–314.
70. Milicevic, D.; Jovanovic, M.; Matekalo-Sverak, V.; Radicevic, T.; Petrovic, M.; Lilic, S. A survey of spontaneous occurrence of ochratoxin A residues in chicken tissues and concurrence with histopathological changes in liver and kidneys. *J. Environ. Sci. Heal. C* **2011**, *29*, 159–175.
71. Moura, MA, Machado, CH, Porfírio, LC, & Freire, RB. (2004). Effects of ochratoxin a on broiler leukocytes. *Revista Brasileira de Ciência Avícola*, *6*(3), 187-190.
72. Munro, I.B. (1981). Field observations on possible mycotoxicoses. In G.A. Pepin, D.S.P. Patterson & D.E. Gray (Eds.), Proceedings of 4th Meeting on Mycotoxins in Animal Disease (p. 51).
73. Nedeljković-Trailović Jelena, Jovanović N., Sinovec **Zlatan Effects of exposure time and dietary ochratoxin A level on broiler performance. Acta** veterinaria 2004 Volume 54, Issue 5-6, Pages: 419-426

74. NEDELJKOVIC-TRAILOVIC JELENA, Stefanović S, Trailović SM.. *In vitro* investigation three different adsorbents against ochratoxin A in broilers. *Br. Poult. Sci.* **2013**, *54*, 515–523.
75. NEDELJKOVIC-TRAILOVIC JELENA, TRAILOVIC S, DIMITRIJEVIC MIRJANA and ILIC V. BLOOD SERUM PROTEIN STATUS IN BROILERS FED WITH INCREASING CONCENTRATIONS OF OCHRATOXIN A. Acta Veterinaria (Beograd), Vol. 63, No. 1, 77-88, 2013
76. Nedeljković-Trailović , Jelena, Saša Trailović , Radmila Resanović , Dragan Milićević , Milijan Jovanovic and Marko Vasiljevic . Comparative Investigation of the Efficacy of Three Different Adsorbents against OTA-Induced Toxicity in Broiler Chickens. *Toxins* **2015**, *7*(4), 1174-1191
77. Niemiec, J., W. Borzemska, P. Golinski, E. Karpinska, T. Szeleszczuk and P. Celeda. 1994. The effect of ochratoxin A on egg quality development of embryos and the level of toxin in egg and tissue of hens and chicks. J. Animal and Feed Sci. 3:309-316.
78. Page R.K., Stewart G., Wyatt R., Buch P.. Fletcher O.J., Brown J., 1980. Influence of low levels of ochratoxin A on egg production, egg-shell strains and serum uric acid levels in Leghorn-type hens. Avian Dis. 24, 777-780
79. PECKHAM, JOHN C. BEN DOUPNIK, JR., AND OSCAR H. JONES, JR. Acute Toxicity of Ochratoxins A and B in Chicks'**.** APPLIED MICROBIOLOGY, Mar. 1971, p. 492-494
80. Piskorska-Pliszczyńska J, Juszkiewicz T. Tissue deposition and passage into eggs of ochratoxin A in Japanese quail. J Environ Pathol Toxicol Oncol. 1990 Jan-Apr;10(1-2):8-10.
81. *Piskorska-Pliszczynska, J.Juszkiewicz, T. (Instytut Weterynarii, Pulawy* Tissue deposition and passage into eggs of ochratoxin A in Japanese quails [1984]
82. Pitt, J.I. *Penicillium viridicatum*, *Penicillium verrucosum* and production of ochratoxin A. *Appl. Environ. Microbiol.* **1987**,*53*, 266–269.
83. Pitt, J.I. *The genus Penicillium and Its Teleomorphic States Eupenicillium and Talaromyces*; Academic Press: New York, NY, USA, 1979.
84. Samson, R.A.; Stolk, A.C.; Hadlok, R. Revision of the Subsection *Fasciculata* of *Penicillium* and some Allied Species.*Stud. Mycol.* **1976**, *11*, 1–47.
85. Pitt, J.I. 1987. Penicillium viridicatum, Penicillium verrucosum, and production of ochratoxin A. Appl Environ Microbiol 53:266-269. Pitt, J.I., J.C. Basilico, M.L. Abarca and C. Lopez. 2000. Mycotoxins and toxigenic fungi. Med Mycol 38 Suppl 1:41
86. Pozzo, L., Cavallarin, L., Antoniazzi, S., Guerre, P., Biasibetti, E., Capucchio, M. T. and Schiavone, A. (2013), Feeding a diet contaminated with ochratoxin A for broiler chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 2. Effects on meat quality, oxidative stress, residues and histological traits. Journal of Animal Physiology and Animal Nutrition, 97: 23–31.
87. Prior, M.G. 1976. Mycotoxin determinations on animal feedstuffs and tissues in Western Canada. Can J Comp Med 40:75-79.
88. Prior, M.G., J.B. O'Neil and C.S. Sisodia. 1980. Effects of ochratoxin A on growth response and residues in broilers. Poult Sci 59:1254-1257.
89. Prior, M.G. and C.S. Sisodia. 1978. Ochratoxicosis in White Leghorn hens. Poult Sci 57:619-623. 217
90. Prior, M.G. and C.S. Sisodia. 1982. The effects of ochratoxin A on the immune response of Swiss mice. Can J Comp Med 46:91-96
91. Raju M.V.L.N., Devegowda G. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin) Brit. Poult. Sci. 2000;41:640–650. doi: 10.1080/713654986.
92. Raper, K.B.; Thom, C. *A Manual of Penicillia*; The Williams & Wilkins Company: Baltimore, MD, USA, 1949.

93. Reichmann, K.G., Blaney, B.J., Connor, J.K. (1982) The significance of aflatoxin and ochratoxin in the diet of Australian chickens, Aust. Vet.J. 58:211-212.
94. Rosa C.A.R., Ribeiro J.M.M., Fraga M.J., Gatti M., Cavaglieri L.R., Magnoli C.E., Dalcero A.M., Lopes C.W.G. Mycoflora of poultry feeds and ochratoxin-producing ability of isolated Aspergillus and Penicillium species. Vet. Microb. 2006;113:89–96
95. Rotter R.G., Frohlich A.A., Marquardt R.R. Influence of dietary charcoal on ochratoxin A toxicity in Leghorn chicks. Can. J. Vet. Res. 1989;53:449–453.
96. Sakhare P.S., Harne S.D., Kalorey D.R., Warke S.R., Bhandarker A.G., Kurkure N.V. Effect of toxiroak polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicosis in broilers. Vet. Arch. 2007;77:129–146.
97. Sakthivelan, M. S., Sakthivelan and Ganne Venkata Sudhakar Rao, "Effect of Ochratoxin A on Body Weight, Feed Intake and Feed Conversion in Broiler Chicken," Veterinary Medicine International, vol. 2010, Article ID 590432, 4 pages, 2010. doi:10.4061/2010/590432
98. Samson,R.A., Jos A.M.P. Houbraken1 , Angelina F.A. Kuijpers , J. Mick Frank and Jens C. Frisvad. New ochratoxin A or sclerotium producing species in Aspergillus section Nigri. STUDIES IN MYCOLOGY 50: 45–61. 2004.
99. Santin, E., A.C. Paulillo, P.C. Maiorka, A.C. Alessi, E.L. Trinder, P., 1969. Enzymatic colorimetric method for the Krabbe and A. Maiorka, 2002. The effects of ochratoxin/aluminosilicate interaction on the tissues and humoral immune response of broilers. Avian Pathol., 31: 73-79
100. Sawale G.K., Gosh R.C., Ravikanth K., Maini S., Rekhe D.S. Experimental Mycotoxicosis in Layer Induced by Ochratoxin A and its Amelioration with Herbomineral Toxin Binder 'Toxiroak' Int. J. Poult. Sci.2009;8:798–803.
101. Sawarkar, R., P.M. Sonkusale, N.V. Kurkure, C.R. Jangade, S. Maini and K. Ravikanth, 2011. Experimental Afla and Ochratoxin Induced Mixed Mycotoxicosis in Broilers and its Amelioration with Herbomineral Toxin Binder 'Toxiroak Gold'. *International Journal of Poultry Science, 10: 560-566.*
102. Shlosberg, A., Elkin, N., Malkinson, M., Orgad, U., Hanji, V., Weisman, Y., Meroz, M. & Bock, R. (1997). Severe hepatopathy in geese and broilers associated with ochratoxin in feed. Mycopathologia, 138, 71–76.
103. Scudamore KA, Patel S. Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom. Food Addit Contam. 2000 May;17(5):407-16.
104. Shah, H.U., T.J. Sinson, S. Alam and K.F. Khattak. 2008. Mould incidence and aflatoxin B1 and Ochratoxin A contamination of maize kernels in Swat Valley, North West Frontier Province, Pakistan. *International Grain Quality and Technology Congress,* July 15-18, 2008. Chicago
105. Singh, G. S.; Chauhan, H. V.; Jha, G. J.; and Singh, K. K., 1990. Immunosuppression due to chronic ochratoxicosis in broilers. J. Comp. Pathol., 103: 399-410.
106. SOLCAN, Carmen, Mihaela GOGU (Panagachi), Gheorghe SOLCAN. Protective Effect of Hypophae Rhamnoides Oil Against Ochratoxicosis in Chickens. Bulletin UASVM, Veterinary Medicine 68(1)/2011 pISSN 1843-5270;
107. SOLCAN, Carmen , Geta PAVEL , Viorel Cezar FLORISTEAN , Ioan Sorin Beschea CHIRIAC , Bogdan Gabrie ŞLENCU and Gheorghe SOLCAN.EFFECT OF OCHRATOXIN A ON THE INTESTINAL MUCOSA AND MUCOSA-ASSOCIATED LYMPHOID TISSUES IN BROILER CHICKENS. Acta Veterinaria Hungarica volume 63, 1, pp. 30–48, 2015,
108. Sreemannarayana, O.; Marquardt, R.R.; Frohlich, A.A.; Vitti, G.T.; Abramson, D. Organ weights, liver constituent and serum components in growing chicks fed ochratoxin A. *Arch. Environ. Contam. Toxicol.* **1989**, *18*, 404–408.
109. Stander, M.A., Sleyn, P.S., Liibben, A., Miljkovic, A., Manlle, P.O. & Marais, G.J., 2000b. Influence of halogen sails on Ihe production of the ochratoxins by Aspergillus ochraceus Wilh. J. Agric. and Food Chem. 48, 1865-1871.

110. Stander, M.A., P.S. Steyn, A. Lu bben, A. Miljkovic, P.G. Mantle and G.J. Marais. 2000. Influence of halogen salts on the production of the ochratoxins by Aspergillus ochraceus J. Agri. Food Chem. 48:1865-1871.
111. Stewart, , R.K/G., Wyatt, R., Buch, P., Fletcher, O.J. & Brown, J. (1980). Influence of low levels of ochratoxin A on egg production, egg shell stains and serum uric-acid levels in leghorn type hens. Avian Diseases, 24, 777–780.
112. Stoev S.D.;2002 – Spontaneaus mycotoxic nephropathy in Bulgarian chickens with unclarified mycotoxic aetiology. Vet. Res. 33; 83-93.
113. Stoev,S., Hristo Daskalov, Boˇzica Radic, Ana-Marija Domijan, Maja Peraica. Spontaneous mycotoxic nephropathy in Bulgarian chickens with unclarified mycotoxin aetiology. Veterinary Research, BioMed Central, 2002, 33 (1), pp.83-93.
114. Stoev SD; D. Djuvinov; T. Mirtcheva; D. Pavlov; P. Mantle; 2002- Studies on some feed additives giving partial protection against ochratoxin A toxicity in chicks. Toxicol Lett. Sep 5;135(1-2):33-
115. Sumbal, G.A. Zahid Hussain Shar, Syed Tufail Hussain Sherazi, Sirajuddin, Shafi Muhammad Nizamani, Safaraz Ahmed Mahesar Decontamination of poultry feed from ochratoxin A by UV and sunlight radiations. *Journal of the Science of Food and Agriculture 2016, 96 (8): 2668-73*
116. Szeleszczuk, P , J. Niemiec [2] , E. Karpińska [1] and W. Bielecki Influence of different levels of ochratoxin A in the diet on morphological changes in immunological system and humoral and cellular immunity in hens and their progeny. Arch.Geflügelk., **71** (1). S. 19-24 , 2007
117. **Trenk HL**, **Butz ME**, **Chu FS**. Production of ochratoxins in different cereal products by Aspergillus ochraceus. **Appl Microbiol.** 1971 Jun;21(6):1032-5.
118. Thirumala-Devi K[1], Mayo MA, Reddy G, Reddy DV. Occurrence of aflatoxins and ochratoxin A in Indian poultry feeds. J Food Prot. 2002 Aug;65(8):1338-40.
119. Tohala, S. H. ochratoxin A on egg production, egg-shell strains and serum uric acid levels in Leghorn-type hens. Avian Dis. 24, 777-780
120. Van der Merwe KJ, Steyn PS, Fourie L, Scott, De B, Theron LL (1965). Ochratoxin A, a toxic metabolite produced by Aspergillus ochraceus Wilh. Nature 205: 1112–1113.
121. van Egmond, H.P. (1991). Worldwide regulations for ochratoxin A. In M. Castegnaro, R. Plestina, G. Dirheimer, I.N. Chernozemsky & H. Bartsch (Eds.), Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours (pp. 331–336). Lyon: International Agency for Research on Cancer.
122. van Egmond, H.P. (1994). Survey of data on the incidence and levels of ochratoxin A in food and animal feed worldwide. Journal of Natural Toxins, 2, 125–144.
123. Verma, J., T.S. Johri, B.K. Swain and S. Ameena, 2004. Effect of graded levels of aflatoxin, ochratoxin and their combination on the performance and immune response of broilers. Br. Poult. Sci., 45: 512-518
124. Vesela D., Vesely D., Jelinek R., 1983. Toxic effects of ochratoxin A and citrinin, alone and in combination, on chicken embryos. Appl. Environ. Microbiol. 45, 91-93
125. Visagie, C.M., J. Varga, J. Houbraken, M. Meijer, S. Kocsube, N. Yilmaz, R. Fotedar, K.A. Seifert, J.C. Frisvad, and R.A. Samson. Ochratoxin production and taxonomy of the yellow aspergilli (Aspergillus section Circumdati), Studies in Mycology 78: 1–61.1,2014
126. Visconti A. & Bottalico, A. (1983). High levels of ochratoxins A and B in mouldy bread responsible for mycotoxicosis in farm animals. Journal of Agricultural and Food Chemistry, 31, 1122–1123.
127. Yohannes,T., A. Sharma, S. Singh and V. Sumi, "Experimental Haematobiochemical Alterations in Broiler Chickens Fed with T-2 Toxin and Co-Infected with IBV," *Open Journal of Veterinary Medicine*, Vol. 3 No. 5, 2013, pp. 252-258..
128. Wang GH, Xue CY, Chen F, Ma YL, Zhang XB, Bi YZ, Cao YC. Effects of combinations of ochratoxin A and T-2 toxin on immune function of yellow-feathered broiler chickens. Poult Sci. 2009 Mar;88(3):504-10. doi: 10.3382/ps.2008-00329.

129. Wangikar, P. B., Dwivedi, P., Sinha, N., Sharma, A. K., and Telang, A. G. (2005). Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects. Toxicol 215, 37–47.
130. Wei X, Sulik KK. Pathogenesis of caudal dysgenesis/sirenomelia induced by ochratoxin A in chick embryos. Teratology. 1996 Jun;53(6):378-91.
131. Xue , C.Y., G. H. Wang ,† F. Chen ,† X. B. Zhang ,† Y. Z. Bi ,† and Y. C. Cao. Immunopathological effects of ochratoxin A and T-2 toxin combination on broilers. Poultry Science (2010) 89 (6): 1162-1166.
132. Zaghini, A., M. Simioli, P. Roncada & L. Rizzi.. Effect of Saccharomyces cerevisiae and esterified glucomannan on residues of Ochratoxin A in kidney, muscle and blood of laying hens. Italian Journal of Animal Science.6, 737-739. 2007
133. Zahoor-ul-Hassan, Khan, M. Z., Khan, A., and Javed, I. (2010). Pathological responses of white leghorn breeder hens kept on ochratoxin Acontaminated feed. Pak Vet J 30, 118–23.
134. Zahoor-ul-Hassan, Khan, M. Z., Khan, A., Hassan, I. J., and Saleemi, M. K. (2011). Immunological status of progeny of hens kept on ochratoxin A contaminated feed. J Immuotox 8, 122–30.
135. ZAHOOR-UL-HASSAN1 , MUHAMMAD ZARGHAM KHAN2 , MUHAMMAD KASHIF SALEEMI2 , AHRAR KHAN2 , IJAZ JAVED3 , AND SHERAZ AHMED BHATTI. Toxico-Pathological Effects of In Ovo Inoculation of Ochratoxin A (OTA) in Chick Embryos and Subsequently in Hatched Chicks. Toxicologic Pathology, 40: 33-39, 2012

4.3. Sterigmatocystin (STC)

Sterigmatocystin is a toxic metabolite structurally closely related to the aflatoxins (compare general fact sheet number 2), and consists of a xanthone nucleus attached to a bifuran structure. Sterigmatocystin is mainly produced by the fungi *Aspergillus nidulans* and *A. versicolor*. It has been reported in mouldy grain, green coffee beans and cheese although information on its occurrence in foods is limited. It appears to occur much less frequently than the aflatoxins, although analytical methods for its determination have not been as sensitive until recently, and so it is possible that small concentrations in food commodities may not always have been detected. Although it is a potent liver carcinogen similar to aflatoxin B1, current knowledge suggests that it is nowhere near as widespread in its occurrence. If this is the true situation it would be justified to consider sterigmatocystin as no more than a risk to consumers in special or unusual circumstances. A number of closely related compounds such as o-methyl sterigmatocystin are known, and some may also occur naturally.Sterigmatocystin (STC) is a polyketide mycotoxin that is produced by several fungal species, including:

1. *Aspergillus flavus,*
2. *Aspergillus parasiticus,*
3. *Aspergillus versicolor*
4. *Aspergillus togoensis*
5. *Aspergillus ochraceoroseus*
6. *Aspergillus rambellii*
7. *Aspergillus asperescens,*
8. *Aspergillus aureolatus,*
9. *Aspergillus eburneocremeus,*

10. *Aspergillus protuberus,*
11. *Aspergillus tardus,*
12. *Aspergillus astellatus (= Emericella astellata)*
13. *Aspergillus olivicola (= Emericella olivicola)*
14. *Aspergillus venezuelensis (= Emericella venezuelensis)*
15. *Penicillium inflatum*
16. *Aschersonia coffeae*
17. *Aschersonia marginata*
18. *Aschersonia species*
19. *Bipolaris species*
20. *Botryotrichum species*
21. *Chaetomium cellulolyticum,*
22. *Chaetomium longicolleum,*
23. *Chaetomium malaysiense*
24. *Chaetomium virescens*
25. *Humicola species*
26. *Moelleriella species*
27. *Monicillium species*
28. *Podospora anserina*

- Aspergillus versicolor is the most common source.

- Members of Aspergillus section Flavi, which includes the major aflatoxin producers, efficiently convert sterigmatocystin through 3-methoxysterigmatocystin to aflatoxins (Rank et al. 2011; Fig. 4). An exception in this section is A. togoensis, which is able to produce both aflatoxins and sterigmatocystin (**Wicklow et al. 1989; Rank et al. 2011**)

- Sterigmatocystin is a penultimate precursor of aflatoxin biosynthesis and also a toxic and carcinogenic substance produced by many **Aspergillus species** belonging mainly to sections Versicolores, Usti, Aenei, Ochraceorosei, Cremei and Nidulantes of the Aspergillus genus (**Varga *et al*. 2010a; Rank *et al.* 2011)**

- Owing to the structural similarities, AFs and STC share prominent toxic effects, including genotoxicity and carcinogenicity (**Miller and Trenholm, 1994)**

Chemical structure and properties of sterigmatocystin

- Sterigmatocystin is (3aR,12cS)-3a,12c-dihydro-8-hydroxy-6-methoxy-7H-furo[3',2':4,5]furo[2,3-c]xanthen-7-one;

- Sterigmatocystin is methylated at the C8-hydroxyl group by methyl sulphate or methyl iodide. Methanol or ethanol in acid, form dihydroalkoxysterigmatocystin from STC (**Lou et al., 1994; Versilovskis and De Saeger, 2010).**

- **Formula**:C18H12O6,
- **molecular mass** 324.28 g/mol;
- **melting point**: 245-246 °C.
- **Ultraviolet (UV) spectrum** (in ethanol): 235 nm (extinction coefficient (ε) = 24 500), 249 nm (ε = 27 500), and 329 nm (ε = 13 100) (**Cole and Schweikert, 2003).**
- Sterigmatocystin is characterised by a weak fluorescence (**Maness *et al.*, 1976).**
- Sterigmatocystin is readily soluble in chloroform, as well as other organic solvents, such as methanol, ethanol and acetonitrile (**Septien *et al.*, 1993; Versilovskis and De Saeger, 2010).**

Toxicokinetics in rats

- The maximal rate of absorption from the gastrointestinal system was not higher than 30 % of the applied dose. **(Walkow *et al,* 1985)**
- The highest concentration of radioactivity in serum appeared three hours after treatment and the half-life was calculated to be 30 minutes. Radioactivity was concentrated mainly in liver, stomach, kidney, duodenum and lung and to a lesser extent in body fat, muscle tissue, testis, bone and in rectum tissue. An initially high accumulation of radioactivity was also found in stomach (**Purchase and van der Watt, 1970**)
- Gastric erosions observed after orally feeding with STC (**Kusunoki *et al.,* 2011**).
- The most conclusive evidence for the formation of a reactive STC epoxide was obtained**,** when STC was incubated under cell-free conditions with DNA in the presence of rat liver microsomes. Subsequent isolation and degradation of the DNA followed by HPLC analysis revealed the presence of a covalent adduct, which was purified and identified by nuclear magnetic resonance spectroscopy and mass spectrometry as 1,2-dihydro-2-(N7 -guanyl)-1-hydroxy-STC (**Essigmann *et al.,*1979**).
- Most of the orally given STC given to rats was eliminated in the faeces (64–92 %), and about 10 % via urine. The calculated elimination half-life in these

Sprague-Dawley rats varied between 61.5 h in immature females and 130 h in mature male rats and the cumulative total elimination rate (urine and faeces) after 96 h exceeded 99 % in immature and mature males, and varied between 71.5 and 77.4 % in immature and mature females, respectively **Walkow et al. (1985**).

Toxicokinetics *in* poultry

- Sterigmatocystin feeding study, newly-hatched male Warren chicks (treatment group: n = 15; control: n = 10) received normal feed for 3 days and on day 4, 5 and 6 feed contaminated with STC in concentrations of 1.6, 1.6 and 0.8 g/kg feed, respectively. (**Sayed, 1993**)
    - This resulted in 60 % mortality within eight days of feeding.
    - The surviving chicks from this group were fed a control diet without STC for an additional seven weeks.
    - After this period, grey spots on the liver surface, an increase in the kidney mass, elevated serum alkaline phosphatase and decreased triglycerides were observed.

- **Chickens (n = 20) received a diet with an increasing sterigmatocystin** concentration (control diet first week, 20 mg STC/kg feed in the second week, 40 mg STC/kg feed in the third week and 50 mg STC/kg feed until seven weeks; equivalent to 40, 61 and 73 mg/kg b.w. per day respectively based on rough calculations): (**Sayed, 1993**)
    - The chickens displayed after seven weeks, severe liver cirrhosis and fatty degeneration.
    - Cellular necrosis and intercellular inflammation were diagnosed,
    - B.W. gains and feed conversion rates were reduced in comparison with the control group.
    - Serum biochemical parameters were altered, such as increased AST, γ-GT and creatine kinase, and reduced total protein, triglycerides, and cholesterol in serum.
    - ALT, LDH, GLDH, alkaline phosphatase, urea, creatinine and uric acid were not affected.
    - Symptoms of anaemia were recorded in haematological findings

- **Effects of five successive intra-abdominal injections of sterigmatocystin** (STG), administered at 11, 13, 15, 17, and 19 days of age, on the growth pattern of chicks and their organs, and on the concentration of certain blood and liver constituents (**Sreemannarayana *et al.*, 1988a**):.
    - The STG, when administered at dosages of .5 and .7 mg per injection, markedly reduced chick growth and affected organ weights.
    - In general, there was an increase in the relative size of the crop, proventriculus, gizzard, large intestine, kidney and pancreas and a decrease in relative size of the bursa of Fabricius.
    - Liver, heart, and spleen size was not affected. Peritonitis was observed in chicks given the high dosage STG.
    - The STG elevated the activity of serum aspartate aminotransferase and the number of circulating granulocytes and depressed concentrations of

total serum proteins, albumin, potassium, and the total number of circulating white blood cells and agranulocytes.
  - The STG treatment decreased the concentration of dry matter, DNA, RNA, and protein in the liver, affected glycogen concentration differentially, and had no effect on lipid concentration.
  - Liver and kidneys also showed degenerative changes as detected histopathologically.

- **Effects of a single oral dose of sterigmatocystin (stg) in chicks** on body and organ weights, the concentration of different blood constituents, and the histology of certain organs **(Sreemannarayana *et al.*,1988b)**
  - The LD50 as determined in experiment 1 was 41 mg/kg bodyweight for chicks weighing 93 g.
  - All deaths occurred within 18 to 35 hours after stg administration.
  - The body and organ weights in the surviving chicks were depressed 5 days after the administration of stg.
  - Concentrations of serum total protein, albumin, creatine kinase, and potassium were also depressed in the 4 mg stg-treated chicks.

Description of main sterigmatocystin producing fungi

1. ***Aspergillus versicolor*** (Vuill.) Tirab., (1908).

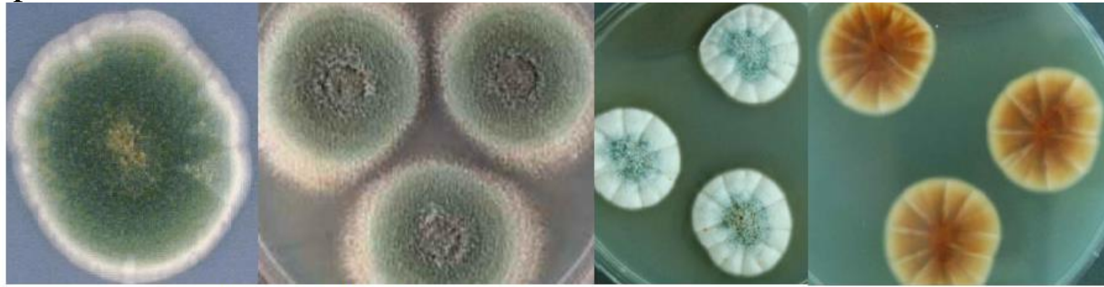
Synonyms:

- *Aspergillus amoenus* Roberg, 1931
- *Aspergillus versicolor var. fulvus* Nakaz. et al., 1932
- *Aspergillus versicolor var. rutilobrunneus* J.N. Rai, S.C. Agarwal & J.P. Tewari, 1971
- *Sterigmatocystis versicolor* Vuill., 1903

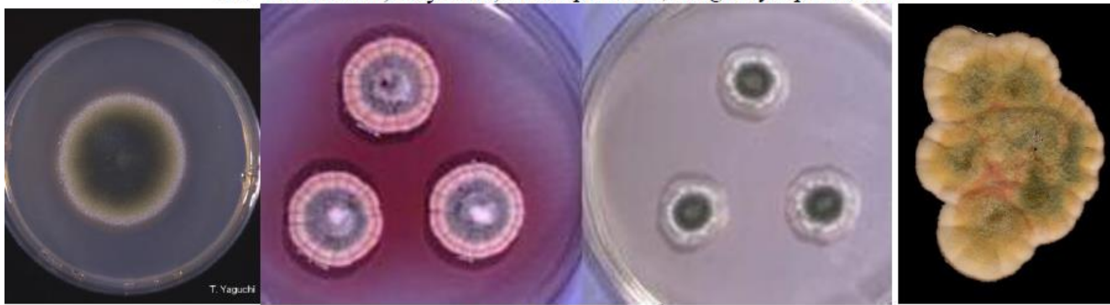
Colonies on CYA 16-25 mm diam, plane or lightly sulcate, low to moderately deep, dense; mycelium white to buff or orange; conidial heads sparse to quite densely packed, greyish green; pink to wine red exudate sometimes produced; reverse orange or reddish brown. Colonies on MEA 12-25 mm diam, low, plane, and dense, usually velutinous; mycelium white to buff; conidial heads numerous, radiate, dull or grey green; reverse yellow brown to orange brown. Colonies on G25N 10-18 mm diam, plane or umbonate, dense, of white, buff or yellow mycelium;

reverse pale, yellow brown or orange brown. No growth at 5°C. Usually no growth at 37°C, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from surface or aerial hyphae, stipes 300-600µm long, with heavy yellow walls, vesicles variable, the largest nearly spherical, 12-16µm diam, fertile over the upper half to two-thirds, the smallest scarcely swollen at all and fertile only at the tips, bearing closely packed metulae and phialides, both 5-8µm long; conidia mostly spherical, very small, 2.0-2.5µm diam, with walls finely to distinctly roughened or spinose, borne in radiate heads.

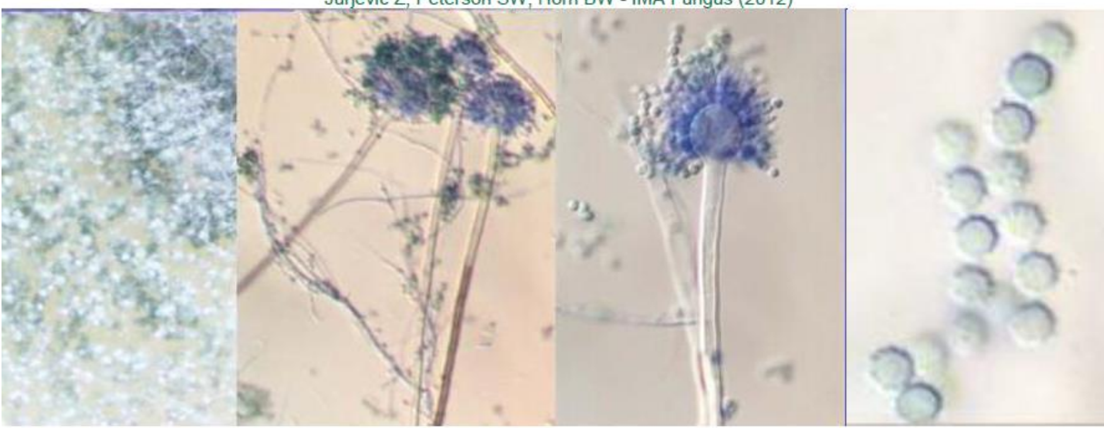


A. versicolor, Mycota, Mold-pro.com, fungi myospecies inf.



www.tamagawa.ac.j

Jurjevic Z, Peterson SW, Horn BW - IMA Fungus (2012)



Aspergillus versicolor, www.tamagawa.ac.jp

2. Aspergillus togoensis (Henn.) Samson & Seifert, The ascomycete genus Penicilliopsis and its anamorphs: 419 (1985)

Synonyms:
≡Stilbothamnium togoense Henn., Botanische Jahrbücher für Systematik Pflanzengeschichte und Pflanzengeographie 23: 542 (1897) [MB#374610]
≡Stilbothamnium togoënse Henn. (1897) [MB#283268]
=Stilbothamnium nudipes Hauman, Bulletin de la Société Botanique Belgique 69: 123 (1936)

Aspergillus togoensis (CBS 272.89)., www.researchgate.net

Reports:

Scott *et al.* (1972) analysed 27 grain samples in Canada (wheat, oats, barley and rye) which were associated with lung problems in farmers and elevator operators from farm storage bins. The authors found STC in one (approximately 300 µg/kg) out of 20 wheat samples and no STC was found in the other grains. This was the first published report of STC occurring in an agricultural product. In the United States of America (USA),

Schroeder and Kelton (1975) produced sterigmatocystin by 59% of *Aspergillus flavus* cultures and by 16% of *A. parasiticus* cultures. All sterigmatocystin-producing cultures of the *A. flavus*group also simultaneously produced aflatoxin or *O*-methylsterigmatocystin. Sterigmatocystin was produced by *A. chevalieri, A. ruber*, and *A. amstelodami*, species not previously reported to produce the compound. In 5-day-old chicken embryos, the no-effect level of toxicity of sterigmatocystin was between 1 and 2 µg/egg; the mean lethal dose was 5 to 7 µg; and 90 to 100% of the embryos were killed with 10 µg. Teratogenic effects and weight reduction were generally associated with nonlethal doses.

Stoloff (1976) reported a Food and Drug Administration (FDA) survey of 457 samples of small grains over a two-year period in which STC could not be detected in any of the samples. No recent surveys on the occurrence of STC in North America could be identified.

Sugimoto *et al.* (1977) investigated mycotoxin contamination of mould-infected brown rice during long-term storage in warehouses. They found sterigmatocyetin in two samples of contaminated rice with levels of 50 and 450 µg/kg simultaneously with ochratoxin A (OTA) (430 and 230 µg/kg) and citrinin (1130 and 700 µg/kg).

Devi and Polasa (1982) screened samples of maize samples used in poultry feed for mycotoxins in India. Out of 50 samples, three samples were contaminated with STC at concentrations varying from traces to 150 µg/kg

Thurm *et al.* (1979) examined a total of 142 samples of vegetable foods for the occurrence of sterigmatocystin. The samples examined were fruits and vegetables which had spontaneously gone mouldy or begun to rot under natural conditions on the one hand, and organoleptically impeccable fruit juices and maize specimens on the other hand. The samples were taken at the manufacturing plant or procured on the market in the framework of operative controls. Sterigmatocystin was detected in none of the samples under investigation. From this it may be concluded that the risk of its occurrence in vegetable foods is not very great in our country. Nevertheless, due to its cancerogenic and toxic properties, sterigmatocystin should remain included in the examination for mycotoxins in the framework of food control.

Bartos and Matyas (1983) examined seventy-four samples (24 samples of wheat, 19 samples of barley, 16 samples of maize, 10 samples of oats and 5 samples of rye) coming from the South Moravian, West Slovakian and East Slovakian regions from the 1980 and 1981 harvests. Only two barley samples and two maize samples were found to be positive: the maize samples contained about 50 micrograms and the samples of barley 200 and 400 micrograms of sterigmatocystine per 1 kg. One sample of wheat had a trace amount of the substance.

Buckle (1983) examined during the period 1976--1979, just over 400.0 samples of animal feedstuffs comprising cereals, compound feeds, hay and silage for moulds and mycotoxins. Examination was carried out in the course of routine advisory and invastigational work undertaken by the Agricultural Development and Advisory Service (Ministry of Agriculture, Fisheries and Food) Microbiology Laboratories in England and Wales in connection with livestock health and production problems and

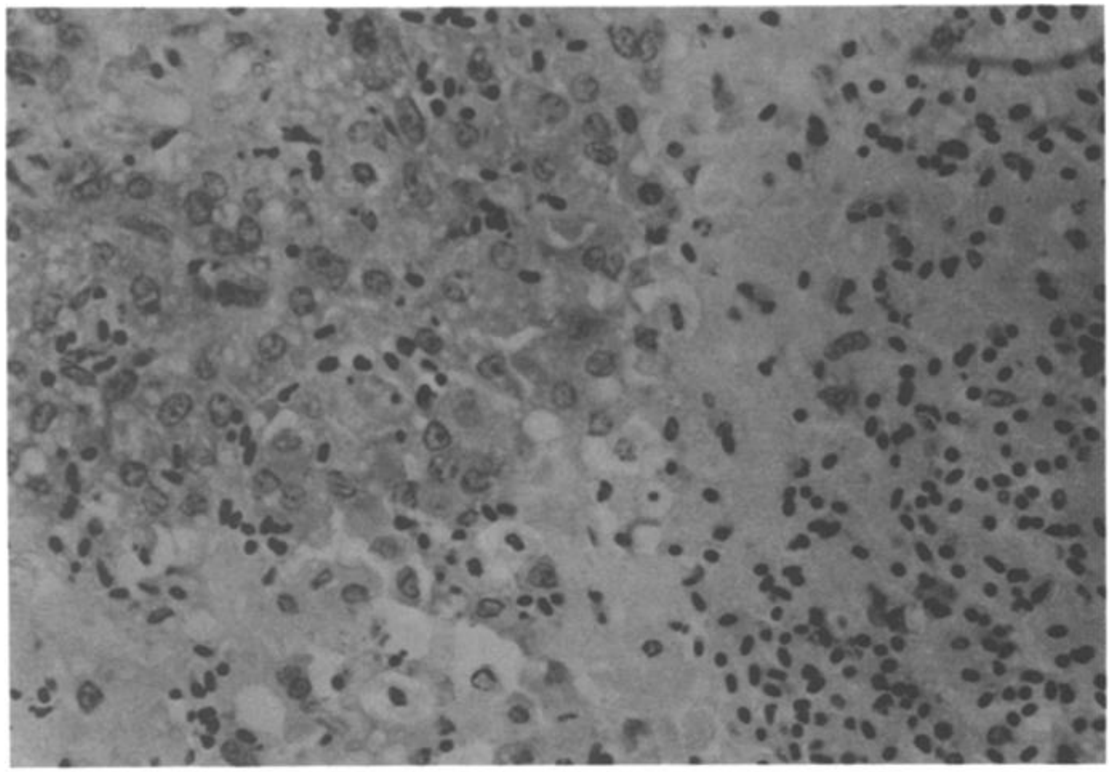
defects in grain storage. Mouldy cereals, mostly invaded by Penicillium and Aspergillus species, were often found contaminated with ochratoxin A (12.8% positive) and also with citrinin, sterigmatocystin and zearalenone to a lesser extent. Aflatoxin B1 was detected in barley which had been inadequately treated with propionic acid in 3 instances.

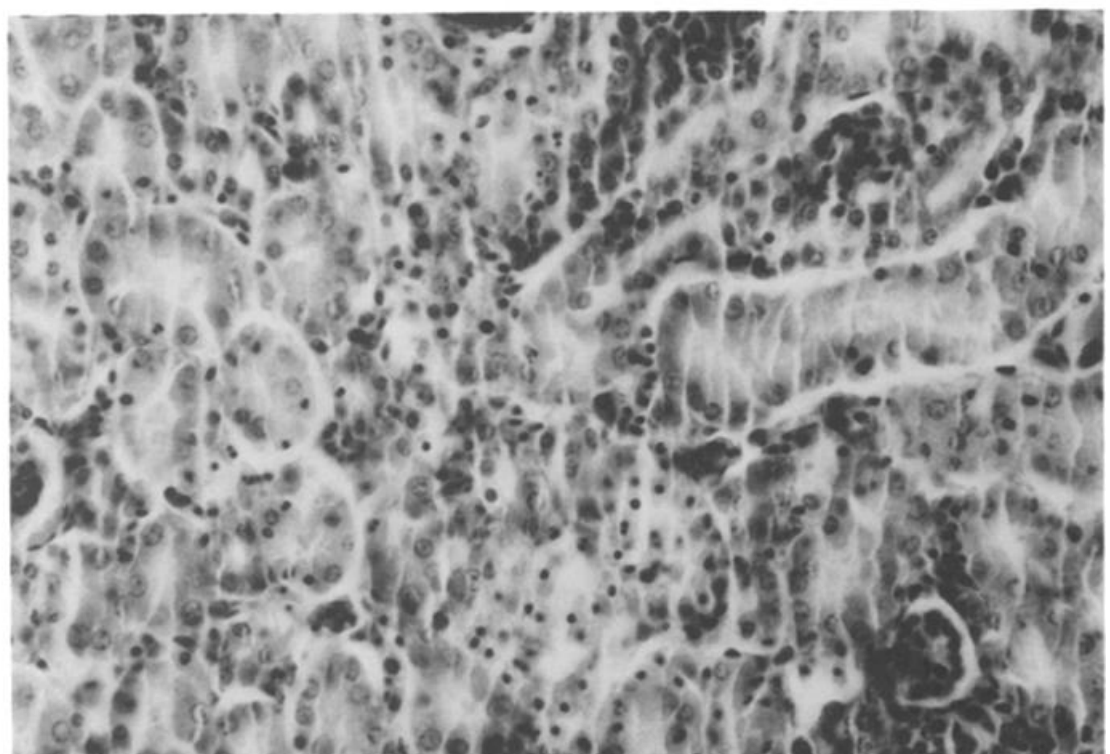
Salam and Shanmugasundaram (1983) reported observations of adverse effects following a single oral dose of 50, 100, 250 or 500 µg STC/chick (n = 5 per dose group), but this study is poorly reported and as such it is not possible to draw any conclusions. Birds given 100 µg STC were reported to die on day two and histopathological examination showed fatty changes in the liver and in the distal tubules and glomerular endothelium of the kidneys, but these changes were not attributed to any dose level.

Takahashi *et al*. (1984) used TLC to study brown rice grains, infected by A. versicolor, which had been stored in a warehouse for two to three years after harvest. They found that the concentration of sterigmatocystin in the milled rice plus bran fraction was in the range of 3800–4300 µg/kg.

Sreemannarayana *et al.* (1986). gave a group of 40 ten-day-old chicks 4.0 mg STC/kg b.w. by i.p. injection and compared with 32 age-matched birds acting as controls receiving the carrier (olive oil) alone. Deaths occurred between 18 and 35 hours after dosing. Blood was collected from all chicks at 24 hours and from the 16 surviving dosed chicks at 36 hours. The dosed birds had increased AST, ALT and LDH, and decreased serum albumin and total protein.

Sreemannarayana *et al.* (1987) carried out 4 experiments with 10 to 12 day old leghorn chicks weighing approximately 93 to 101 g. The chicks were injected intraperitoneally with sterigmatocystin (STG) dissolved in olive oil. The LD50 values as established in the first two experiments were 10.0 and 14.0 mg/kg body weight with most of the deaths occurring between 9 and 21 h following injection. Histopathological studies demonstrated that there was hemorrhage, foci of degeneration and necrosis with fibroblastic proliferation in sinusoids of the liver while the kidneys showed tubular degeneration and necrosis. Biochemical analysis of blood sera demonstrated that STG caused a marked elevation in the activities of lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase, and a depression of creatine kinase, but no effects on -r-glutamyl transferase, amylase and lipase. Free and conjugated bilirubin were elevated in the sera while total protein, albumin, glucose, potassium, chloride and phosphorous concentrations were depressed. In addition, total white blood cells and circulating agranulocytes were depressed while circulating granulocytes were elevated. STG did not significantly affect the concentration of uric acid, cholesterol, triglycerides, calcium, magnesium and sodium in blood.





Microphotographs of the liver (top) and the kidney (bottom) of a chick 24 h after intraperitoneal injection of I mg of stg showing hemorrhage, degeneration, vacuolation and fibroblastic proliferation in the liver and tubular degeneratiopyknotic cells in the kidney. X320 H&E. **Sreemannarayana *et al.* (1987)**

Sreemannarayana *et al.* (1988a) carried out a study to establish the effects of five successive intra-abdominal injections of sterigmatocystin (STG), administered at 11, 13, 15, 17, and 19 days of age, on the growth pattern of chicks and their organs, and on the concentration of certain blood and liver constituents. The STG, when administered at dosages of .5 and .7 mg per injection, markedly reduced chick growth and affected organ weights. In general, there was an increase in the relative size of the crop, proventriculus, gizzard, large intestine, kidney and pancreas and a decrease in relative size of the bursa of Fabricius. Liver, heart, and spleen size was not affected. Peritonitis was observed in chicks given the high dosage STG. The STG elevated the activity of serum aspartate aminotransferase and the number of circulating granulocytes and depressed concentrations of total serum proteins, albumin, potassium, and the total number of circulating white blood cells and agranulocytes. The STG treatment decreased the concentration of dry matter, DNA, RNA, and protein in the liver, affected glycogen concentration differentially, and had no effect on lipid concentration. Liver and kidneys also showed degenerative changes as detected histopathologically. The results of these studies suggest that STG affects several tissues including the digestive system, liver, kidney, pancreas, and the immunological system.

Sreemannarayana *et al.* (1988b) carried out 2 experiments to determine LD50 in chicks and the effects of a single oral dose of sterigmatocystin (stg) on body and organ weights, the concentration of different blood constituents, and the histology of certain organs. The LD50 as determined in experiment 1 was 41 mg/kg bodyweight for chicks weighing 93 g. All deaths occurred within 18 to 35 hours after stg administration. The body and organ weights in the surviving chicks were depressed 5 days after the administration of stg. Concentrations of serum total protein, albumin, creatine kinase, and potassium were also depressed in the 4 mg stg-treated chicks. In experiment 2, treated (4 mg stg/chick) as compared to control chicks had altered serum concentrations or activities of the following constituents: aspartate aminotransferase, 306%; alanine aminotransferase, 963%; lactate dehydrogenase, 283%; amylase, 115%; lipase, 300%; γ-glutamyltransferase,-10%; total proteins,-25%; albumin,-27%; potassium,-10%; magnesium,-12%; calcium,-2%; phosphorus,-39%; chloride, 6%; triglycerides,-51%; uric acid,-1%; conjugated bilirubin, 460%; total WBC,-13%; circulating monomorphonuclear leukocytes,-25%, and granulocytes, 42%. Histopathologic examination revealed mild to severe degenerative changes in the liver, pancreas, kidney, and lymphoid tissue, namely, the bursa of Fabricius. Overall, the results would suggest that stg has a pronounced effect on the liver, kidneys, pancreas, lymphoid tissue, and probably certain sections of the gastrointestinal tract and that these effects persist in the liver and the kidneys over a 5-day period.

Ozay and Heperkan (1989) obtained a total of 167 corn samples, including imported and locally grown corn, from various regions and store houses in Turkey and surveyed for mould occurrence and mycotoxin content. The mould contamination level was 10(5) - 10(6) colonies/g.A. flavus, A. niger, F. oxyporum, P. variable, andRhizopus spp. However, the dominant flora showed significant differences between the imported and domestic corns. Afiatoxin B1 was found in 16 % of the samples ranging from 2-74μg/kg. Ochratoxin A and sterigmatocystin were found at

minimum detection levels. Mycotoxin production characteristics of mould isolates were also determined.

Valente Soarez and Rodriguez-Amaya (1989) purchased 60 rice samples at random in retail stores and used a TLC method for the analysis (LOD = 15 µg/kg, LOQ = 35 µg/kg). Sterigmatocystin was not detected in any sample.

Pande *et al.* (1990) screened 30 samples each of wheat and rice and 22 of maize qualitatively and quantitatively for the presence of mycotoxins. Among 30 wheat samples, two were positive for sterigmatocystin with levels of 110 and 145 µg/kg, from 30 rice samples, three were positive with a concentration between 108 and 157 µg/kg and no sterigmatocystin was found in any of the 22 maize samples analysed.

Scudamore *et al.* (1992) carried out examination of 330 samples of animal feed ingredients for the presence of a number of mycotoxins has been carried out. These samples were drawn from 186 animal feed mills in the United Kingdom. Aflatoxin B1 was the mycotoxin found most frequently, occurring in most samples of rice bran, maize products, palm kernels and cottonseed, but not in only 3 out of 20 samples of sunflower, in 1 out of 20 samples of soya and in no samples of peas, beans or manioc. Analytical difficulties were met with some combinations of commodity and mycotoxin and all results are uncorrected for recovery. The highest level was detected in a sample of maize gluten: 41 micrograms/kg of aflatoxin B1 (47 micrograms/kg total aflatoxins). Maize products also frequently contained fumonisins B1 and B2 at levels up to nearly 5,000 micrograms/kg in total and zearalenone up to a maximum level of 500 micrograms/kg. Ochratoxin A and citrinin were found in approximately 20% of wheat and barley samples. One sample of barley contained ochratoxin A at a level of 102 micrograms/kg and citrinin at a level of 8 micrograms/kg. Low levels of ochratoxin A also occurred in a few samples of other ingredients: rice bran, palm kernel and beans. Sterigmatocystin at 18 micrograms/kg was found in one sample of organically grown wheat and a trace amount of zearalenone in one sample of manioc. Multi-mycotoxin contamination also occurred, particularly in some samples of maize for which 19 out of 50 samples contained both aflatoxins and fumonisins.

Jesenská *et al.* (1994) examined the effect of 11 mycotoxins on the ciliary movement of tracheal epithelium from one-day-old chicks in vitro. Sterigmatocystin and diacetoxyscirpenol were most ciliostatically active in vitro; the ciliostatic effect was observed after 2 d if the amount concentration was 30 micrograms/L. In contrast, patulin stopped the movement of cilia after 2 d only if its concentration was 20 mg/L.

Pozzi *et al.* (1995) in Brazil, analysed a total of 130 samples of maize (10 post-harvest and 120 stored for one year) using TLC, but sterigmatocystin was not found in any of the samples. The samples were collected from the region characterised by humid tropical weather, with hot and rainy summers and dry winters.

Scudamore and Hetmanski (1995) found STC in 8 (17 %) out of 46 samples of poorly stored cereals (wheat, barley and oats). In another study, Scudamore et al. (1997) monitored the occurrence of mycotoxins in grains intended for use as animal feed, again using HPLC, with an LOD of 15 µg/kg. Out of 45 samples of barley and

50 samples of wheat, STC was found in one organically grown wheat sample at a concentration of 18 µg/kg. STC was not detected in any of the 122 samples of cereals (barley, maize, wheat, oats, rice and rye) using HPLC, with an LOD of 3 µg/kg and LOQ of 6 µg/kg (MAFF, 1998). Negative results for STC were also observed in a survey by the Food Standards Agency (FSA, 2002a), in which a total of 100 samples of rice were monitored. Using an LC–MS/MS method with an LOD of 0.15 µg/kg and an LOQ of 0.30 µg/kg,

Pieckova and Jesenská (1997) evaluated the ciliostatic activity of exo- and endometabolites of 243 filamentous fungal strains by in vitro bioassay using tracheal organ cultures of 1-d-old chicks. Chloroform-extractable metabolites produced in the cultivation medium (25 degrees C/10 d) by 30 out of 72 (41%) investigated strains displayed the ciliostatic activity as did metabolites from the biomass of the spores and the mycelium of 46 other strains (26%). This result could contribute to the clarification of the correlation between fungi and respiratory disorders in some working places and in damp dwellings.

Scudamore et al. (1998a) analysed sterigmatocystin with a multi-mycotoxin method for 22 toxins in 40 samples of maize gluten (LOD = 100 µg/kg) and in 27 samples of other maize products such as maize germs/brans, baby maize, maize meals, flaked maize and maize screens (LOD = 20 µg/kg). No sterigmatocystin was found in any of these feed samples.

Scudamore *et al.* (1998b) tested 40 rice bran samples used in animal feed for the presence of 20 mycotoxins in the UK using HPLC (LOQ = 50 µg/kg). No sterigmatocystin was found in any sample.

El-Shanawany *et al.* (2005) tested 40 Egyptian silage samples for mycotoxins using TLC and found STC in two samples. Forty-three species and 2 species varieties belonging to 17 genera were isolated using glucose Czapek's and Sabouraud's dextrose agar media at 28 degrees C. The most prevalent genera were Aspergillus (57.5 and 100 of the samples), Penicillium (100 and 55%) on the two mentioned media, respectively.. Mycotoxin profiles were also determined in these samples: Aflatoxins showed the highest incidence rates of occurrence, it occurred in 22.5% of all samples analyzed. Other mycotoxins were detected from all samples (T2 toxins and sterigmatocystin at incidence of 7.5 and 5%, respectively). The screening of the characteristics mycotoxins of different isolates of Aspergillus isolated from silage samples was tested. The results clarified that some mycotoxins (aflatoxins-aspergillic acid-beta nitro propionic acid-cyclopiazonic acid-kojic acid and sterigmatocystin) were produced by some isolates of A. flavus. Some isolates of A.fumigatus could produce gliotoxin and verrucologen. All of A. niger isolates tested were able to produce kojic acid. One isolate of A. ochraceous formed ochratoxin A and other isolate produced penicillic acid. Concerning A. terreus isolates, the results showed that 5 isolates were able to produce citrinin and 4 isolates had ability to produce patulin. A. versicolor isolates showed the ability to produce ochratoxin A.

Tanaka et al. (2007), analysed 48 brown rice samples using HPLC-UV and none of them were contaminated with sterigmatocystin.

Versilovskis *et al.* (2008) analysed 95 samples of different Latvian grains from 2006 (wheat, oats, ryes, barley and buckwheat samples) and 120 samples from 2007. STC was found in 14 % of the samples from 2006, with concentrations ranging from 0.7 to 83 µg/kg. In 35 % of the samples from 2007, STC concentrations ranging from 1 to 47 µg/kg were found. The highest concentrations have been detected in wheat and barley, medium concentrations in buckwheat and quite low concentrations in oats and rye samples. In total, for both years 26 % of the 215 analysed samples contained STC above the LOD. .

Monbaliu *et al.* (2010) developed and validated a multi-mycotoxin LC–MS/MS method, accredited according to ISO17025, for the simultaneous detection of 23 mycotoxins, including STC, and used this method for the analysis of feed samples from Belgium. A total of 78 wheat and maize samples taken in 2008, intended for use as animal feed were analysed and STC was not found in any of the samples. Among 367 additional grain samples intended for use as animal feed from the period 2008-2011, 11 samples of wheat, maize and barley showed STC concentrations between 6.9 and 574 µg/kg. Co-occurrence with other mycotoxins was reported for all these 11 STC positive samples (i.e. above 4.75 µg/kg,).

Versilovskis and De Saeger (2010) described occurrence of STC in foodstuffs. The toxin has been reported in grains, nuts, green coffee beans, spices, beer and cheese. It should be noted that not all studies reported LOD and LOQ values, meaning that negative findings must be interpreted with caution.

Kovalenko *et al.* (2011) in Russia, analysed samples of wheat (n = 93), corn (n = 111) and barley (n = 146) intended for use as animal feed between 2006 and 2009 and using ELISA (LOD/LOQ not reported). The percentage of samples above 100 µg/kg varied depending on the year between 0 and 21 %, 5 and 8 % and 0 and 23 % for wheat, corn and barley, respectively

The European Food Safety Authority (EFSA) (2013) was asked by the European Commission to deliver a scientific opinion on sterigmatocystin (STC) in food and feed. STC is a polyketide mycotoxin that shares its biosynthetic pathway with aflatoxins. Following an EFSA call for data, analytical results from 247 food and 334 feed samples were submitted. In food, analytical results on STC were reported to be all below the limit of detection or limit of quantification. In feed, only four quantified results were reported. Therefore, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that the available occurrence data are too limited to carry out a reliable human and animal dietary exposure assessment. Acute oral toxicity of STC is relatively low, and liver and kidneys are the target organs. STC is mutagenic in both bacterial and mammalian cells after metabolic activation and forms DNA adducts. Tumourigenicity has been observed after oral, intraperitoneal, subcutaneous and dermal administration resulting in hepatocellular carcinomas, haemangiosarcomas in the liver, angiosarcomas in brown fat and lung adenomas. Since no exposure data were available, the margin of exposure approach for substances that are genotoxic and carcinogenic could not be applied for STC, and thus the CONTAM Panel could not characterise the risk for human health. Regarding animals, the Panel noted that STC is hepatotoxic in poultry and pigs, and nephrotoxic in poultry and toxic in several fish species. However, in the absence of exposure data

for livestock, fish and companion animals, and given the limited knowledge on the adverse effects of STC, the CONTAM Panel could not characterise the risk for animal health. More occurrence data on STC in food and feed need to be collected to allow dietary exposure assessment. For food, methods with a limit of quantification of less than 1.5 μg/kg should be applied.

References:

1. Bartos J and Matyas Z, 1983. Study of the presence of sterigmatocystin in Czechoslovak grain. Veterinarni Medicina (Praha), 28, 189-192.
2. Buckle AE, 1983. The occurrence of mycotoxins in cereals and animal feed-stuffs. Veterinary Research Communications, 7, 171-186.
3. Devi GR and Polasa H, 1982. Mycotoxins frum fungi on maize. Current Science, 51, 751-751
4. El-Shanawany AA, Mostafa ME and Barakat A, 2005. Fungal populations and mycotoxins in silage in Assiut and Sohag governorates in Egypt, with a special reference to characteristic Aspergilli toxins. Mycopathologia, 159, 281-289.
5. Essigmann JM, Barker LJ, Fowler KW, Francisco MA, Reinhold VN and Wogan GN, 1979. Sterigmatocystin-DNA interactions: identification of a major adduct formed after metabolic activation in vitro. Proceedings of the National Academy of Sciences of the United States of America, 76, 179-183.
6. János Varga, Nikolett Baranyi1 , Muthusamy Chandrasekaran2 , Csaba Vágvölgyi1,2, Sándor Kocsubé. Mycotoxin producers in the Aspergillus genus: an update. Acta Biol Szeged 59(2):151-167 (2015)
7. Jesenská Z and Bernát D, 1994. Effect of mycotoxins on in vitro movement of tracheal cilia from oneday-old chicks. Folia Microbiologica, 39, 155-158.
8. Kovalenko AV, Soldatenko NA, Fetisov LN and Strel'tsov NV, 2011. More Accurate Determination of the Minimum Allowable Level of Sterigmatocystin in Piglet Feed. Russian Agricultural Sciences, 37, 504-507.
9. Kusunoki M, Misumi J, Shimada T, Aoki K, Matsuo N, Sumiyoshi H, Yamaguchi T and Yoshioka H, 2011. Long-term administration of the fungus toxin, sterigmatocystin, induces intestinal metaplasia and increases the proliferative activity of PCNA, p53, and MDM2 in the gastric mucosa of aged Mongolian gerbils. Environmental Health and Preventive Medicine, 16, 224-231
10. Miller JD and Trenholm L, 1994. Mycotoxins in Grain: Compounds Other Than Aflatoxin. Eagan Press, 552 pp
11. Ozay G and Heperkan D, 1989. Mould and mycotoxin contamination of stored corn in Turkey. Mycotoxin Research, 5, 81-89.
12. Pande N, Saxena J and Pandey H, 1990. Natural occurrence of mycotoxins in some cereals. **Mycoses, 33, 126-128.**
13. **Panel on Contaminants in the Food Chain**. Scientific Opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed**.** EFSA Journal 2013;11(6):3254 [81 pp.]
14. Pieckova E and Jesenská Z, 1997. Ciliostatic activity in day old chicks indicates microscopic fungi toxicity in vitro. Annals of Agricultural and Environmental Medicine, 4, 35-36.
15. Purchase IF and van der Watt JJ, 1970. Carcinogenicity of sterigmatocystin. Food and Cosmetics Toxicology, 8, 289-295.
16. Pozzi CR, Correa B, Gambale W, Paula CR, Chacon-Reche NO and Meirelles MC, 1995. Postharvest and stored corn in Brazil: mycoflora interaction, abiotic factors and mycotoxin occurrence. Food Additives and Contaminants, 12, 313-319
17. Rank C, Nielsen KF, Larsen TO, Varga J, Samson RA, Frisvad JC (2011) Distribution of sterigmatocystin in filamentous fungi. Fungal Biol 115:406-420.

18. Salam SBA and Shanmugasundaram ERB, 1983. Acute toxicity of sterigmatocystin in chicks. Current Science, 52, 1068-1070..
19. Sayed A, 1993. Clinical, haematological and biochemical studies on the effect of sterigmatocystin in sheep. Doctoral thesis. University of Veterinary Medicine Vienna, Austria.
20. Schroeder HW, Kelton WH: Production of sterigmatocystin by some species of the genus *Aspergillus* and its toxicity to chicken embryos. Applied Microbiol 30:589-591, 1975
21. Scott PM, Van Walbeek W, Kennedy B and Anyeti D, 1972. Mycotoxins (ochratoxin A, citrinin, and sterigmatocystin) and toxigenic fungi in grains and other agricultural products. Journal of Agricultural and Food Chemistry, 20, 1103-1109.
22. Soares C, Rodrigues P, Peterson SW, Lima N, Venâncio A (2012) Three new species of Aspergillus section Flavi isolated from almonds and maize in Portugal. Mycologia 104:682-697
23. Sreemannarayana O, Frohlich AA and Marquardt RR, 1986. Serum biochemical studies in chicks injected intraperitoneally with sterigmatocystin. Poultry Adviser, 19, 53-58.
24. Sreemannarayana O, Frohlich AA and Marquardt RR, 1987. Acute toxicity of sterigmatocystin to chicks. Mycopathologia, 97, 51-59.
25. Sreemannarayana O, Frohlich AA and Marquardt RR, 1988a. Effects of repeated intra-abdominal injections of sterigmatocystin on relative organ weights, concentration of serum and liver constituents, and histopathology of certain organs of the chick. Poultry Science, 67, 502-509.
26. Sreemannarayana O, Marquardt RR, Frohlich AA and Juck FA, 1986b. Some acute biochemical and pathological-changes in chicks after oral-administration of sterigmatocystin. Journal of the American College of Toxicology, 5, 275-287
27. Stoloff L, 1976. Report on mycotoxins. Journal of the Association of Official Analytical Chemists, 59, 317-323.
28. Scudamore KA, Hetmanski MT, Chan HK and Collins S, 1997. Occurrence of mycotoxins in raw ingredients used for animal feeding stuffs in the United Kingdom in 1992. Food Additives and Contaminants, 14, 157-173.
29. Scudamore KA, Nawaz S and Hetmanski MT, 1998a. Mycotoxins in ingredients of animal feeding stuffs: II. Determination of mycotoxins in maize and maize products. Food Additives and Contaminants, 15, 30-55.
30. Scudamore KA, Nawaz S, Hetmanski MT and Rainbird SC, 1998b. Mycotoxins in ingredients of animal feeding stuffs: III. Determination of mycotoxins in rice bran. Food Additives and Contaminants, 15, 185-194.
31. Sugimoto T, Minamisawa M, Takano K, Sasamura Y and Tsuruta O, 1977. Detection of ochratoxin A, citrinin and sterigmatocystin from storaged rice by natural occurrence of Penicillium viridicatum and Aspergillus versicolor. Journal of the Food Hygienic Society of Japan, 18, 176-181.
32. Takahashi H, Yasaki H, Nanayama U, Manabe M and Matsuura S, 1984. Distribution of sterigmatocystin and fungal mycelium in individual brown rice kernels naturally infected by Aspergillus versicolor. Cereal Chemistry, 61, 48-52
33. Tanaka K, Sago Y, Zheng Y, Nakagawa H and Kushiro M, 2007. Mycotoxins in rice. International Journal of Food Microbiology, 119, 59-66.
34. Thurm V, Koch CE and Paul P, 1979. Hygienic significance of sterigmatocystin in vegetable foods. 3. Occurrence of sterigmatocystin. Nahrung, 23, 121-123. Poland (Szebiotko et al., 1981)
35. Valente Soares LM and Rodriguez-Amaya DB, 1989. Survey of aflatoxins, ochratoxin A, zearalenone, and sterigmatocystin in some Brazilian foods by using multi-toxin thin-layer chromatographic method. Journal - Association of Official Analytical Chemists, 72, 22-26.

36. Versilovskis A and De Saeger S, 2010. Sterigmatocystin: occurrence in foodstuffs and analytical methods - an overview. Molecular Nutrition and Food Research, 54, 136-147.
37. Walkow J, Sullivan G, Maness D and Yakatan GJ, 1985. Sex and age differences in the distribution of 14C-sterigmatocystin in immature and mature rats: a multiple dose study. Journal of the American College of Toxicology, 4, 45-51.

4.4. Cyclopiazonic acid (CPA) mycotoxicosis

Cyclopiazonic acid (α-cyclopiazonic acid; CPA, Figure 1) is an indole-tetramic acid mycotoxin produced by the ubiquitous genera of molds *Aspergillus* and *Penicillium*.

CPA and aflatoxins often co-contaminate crops. The source of these mycotoxins is complicated by the fact that both *aspergilli* and *penicillia* are often found in the same crop and as well as after grain storage

Chemical properties

Molecular Formula: $C_{20}H_{20}N_2O_3$

Molecular Weight: 336.3844 g/mol

Melting point: 245-246 °C

When heated to decomposition it emits toxic fumes of /nitrogen oxides

H H H N O O HN HO

Cyclopiazonic acid producing fungi

Cyclopiazonic acid is a mycotoxin that was first isolated from a culture of Penicillium cyclopium during a screening for toxigenic moulds [**Holzapfel, 1968]**

- Cyclopiazonic acid is named after the strain, *Penicillium cyclopium* Westling from which it was originally isolated. [**Hermansen *et al.,* 1984],**
- *Penicillium cyclopium* or its synonym *P. aurantiogriseum* have not been found to make CPA, and the CPA-producing strain originally isolated (CSIR 1082) was later identified as *P. griseofulvum* Dierckx [**Frisvad *et al.,* 1989**].

- *P. chrysogenum*, *P. nalgiovense,P. crustosum*, *P. hirsutum* and *P. viridicatum* also have been reported to produce CPA [**El-Banna *et al*., 1987**]
- *P. griseofulvum*, *P. camemberti*, *P. urticae* and *P. commune* have been reported to consistently produce CPA [**Burdock *et al*., 2000**].
- *Aspergillus* species such as *A. flavus*, *A. oryzae*, *A. fumigatus, A. versicolor,* and *A. tamarii* also produce CPA [**Dorner, 1983**,**1987**].
- 30% of the *A. fumigatus* and *A. phoenicis* strains were able to produce CPA [**Vinokurova *et al.*, 2007**]
- Only one of 21 *A. versicolor* strains was able to produce CPA [**Vinokurova *et al.*, 2007**].
- *A. versicolor* Tiraboschi originally reported to produce CPA [25] was later identified as *A. oryzae* [**Ohmomo *et al*., 1973, Domsch *et al*., 1980**].

- Cyclopiazonic acid is produced by several other fungal species of the genera Penicillium and Aspergillus, including Penicillium camemberti (**Nishe *et al*., 1985).**
- **Based on all these authors the following species have been reported to produce CPA**
  1. *Aspergillus flavus*
  2. *Aspergillus versicolor*.
  3. *Aspergillus minisclerotigenes*
  4. *Aspergillus parvisclerotigenus*
  5. *Aspergillus pseudocaelatus*
  6. *Aspergillus pseudotamarii*
  7. *Aspergillus mottae*
  8. *Aspergillus sergii*
  9. *Aspergillus fumigatus*
  10. *Aspergillus phoenicis*
  11. *Aspergillu oryzae*
  12. *Aspergillu tamarii*
  13. *Penicillium cyclopium*
  14. *Penicillium griseofulvum*
  15. *Penicillium camemberti*
  16. *Penicillium commune*,
  17. *Penicillium nalgiovense,*
  18. *Penicillium crustosum*,
  19. *Penicillium hirsutum*
  20. *Penicillium viridicatum*
  21. *Penicillium urticae*

Toxicokinetics

Studies to evaluate the avian toxicity of CPA have been conducted in chickens (Dorner et al., 1983; Norred et al., 1988; Kubena et al., 1994; Balachandran and Parthasarathy, 1996a; Gentles et al., 1999; Kamalavenkatesh et al., 2005, Venkatesh et al., 2005; Kumar and Balachandran, 2009; Malekinejad et al., 2010)

1. **In an acute toxicity study a single dose of CPA** at 0.5, 5.0, or 10.0 mg/kg body weight administered to 4-week-old chickens (**Norred *et al*., 1988**):

   - significant reduction in body weight gain at the two lower doses and actual body weight loss in the 10-mg/kg dosing group, and these effects were seen within 24 h of dosing in each group.

   - Recovery of normal body weight gain was dose dependent,

     - with the 0.5-mg/kg group recovering within 48 h of dosing,

     - the 5.0-mg/kg group recovering within 96 h,

     - the 10 mg/kg group continuing to show significantly reduced body weights vs. controls at the final, 96 h, sampling time.

- This study suggested that the acute NOEL in young chickens is less than 0.5 mg/kg body weight/day.

2. **In an acute toxicity in which laying hens were orally dosed with CPA** at 2.5, 5.0, or 10.0 mg/kg body weight/day for 9 consecutive days (**Dorner *et al*., 1994**):

   i. All hens in the 10-mg/kg group and 80% of hens in the 5-mg/kg group died before the end of the study

   ii. egg production ceased 1 and 4 days after the initiation of dosing in the 10-mg/kg and 5-mg/kg groups, respectively.

3. In a study using multiple dose levels, (**Malekinejad *et al*., 2010**)

   - significant effects in liver and kidney of broiler chickens after 28 days' exposure to CPA at dosages of 0.01, 0.025, and 0.050 mg/kg body weight/day, though no significant reductions in body weight gain or other clinical symptoms were observed.

   - Increased liver weights and liver/body weight ratios were observed in chickens dosed at 0.025 or 0.050 mg CPA/kg body weight/day.

   - Pathological abnormalities indicative of inflammation were observed in liver and kidney at all dose levels tested.

   - Changes in numerous biochemical markers in blood serum which are associated with oxidative stress were observed in the two higher dose

levels, and many of these changes were already evident after only 2 weeks of dosing.

This study suggests that the NOEL is less than 0.01 mg/kg body weight and establishes a LOEL of 0.01 mg/kg body weight/day for CPA in chickens, much lower than previous studies.

4. **In studies in chickens, CPA was added to feed at a single, fixed concentration** (ranging from 10 to 50 ppm in feed) and chickens were allowed to consume this feed *ad libitum* for periods of 21 to 28 days **(Dorner et al., 1983; Kubena et al., 1994; Balachandran and Parthasarathy, 1996a; Gentles et al., 1999; Kamalavenkatesh et al., 2005, Venkatesh et al., 2005; Kumar and Balachandran, 2009).**

- Effects observed in these studies included
    - body weight reductions, where feed contained 25 ppm CPA or higher,
    - gross damage to liver, kidney, crop, and proventicular mucosa, with associated histopathological damage.
    - damage to thymus and spleen, with increased apoptosis in splenocytes and reductions in lymphocytes, including helper and cytotoxic T cell populations, when chickens were fed *ad libitum* with feed containing CPA at 10 or 20 ppm.

  These findings suggest an immunosuppressive potential for CPA which may be the result of direct toxicity of CPA to lymphoid organs and endoplasmic reticulum (ER) stress.

Transfer of CPA to meat and eggs

- In 4-week-old chickens **dosed** a single oral dose, (**Norred *et al*., 1988**).
    - In high doses at 0.5, 5.0, or 10.0 mg/kg body weight CPA was shown to distribute rapidly into breast and thigh muscle of chickens with the peak concentration of CPA in the meat seen at 3 h after dosing
    - In lower doses (0.5 and 5.0 mg/kg body weight), CPA was eliminated from the meat within 24 to 48 h
- In laying hens orally dosed with CPA at 2.5, 5.0, or 10.0 mg/kg body wei*ght/day* for 9 consecutive days.
    - CPA began to appear in eggs from dosed hens within 24 h of the initial dose, accumulating almost exclusively in egg whites.
    - In the group dosed at 2.5 mg/kg, the only dosing level in which egg production continued for the duration of the study, the CPA concentration in egg whites gradually increased over the first 6 days of the trial, with some variability thereafter. Concentration of CPA in

pooled egg whites from this dosing group was 313 ng/g and 350 ng/g on day 6 and day 9, respectively.

- In paired subchronic exposure study in which laying hens were dosed for 28 days at dosages of 1.25 and 2.5 mg CPA/kg body weight/day (**Dorner et al., 1994**),
    - most of the CPA in eggs accumulated in the whites, with variable concentrations over the course of the study, with concentrations in the range of
        - 60-160ng/g (mean =105 ng/g) in the 1.25 mg/kg/day dosing group
        - 18-193 ng/g (mean = 97 ng/g) in the 2.5 mg/kg/day dosing group.

Occurrence of CPA in feeds

- **Gallagher *et al.* (1978)** reported the natural occurrence of CPA in maize, estimating the CPA concentration in one of the tested samples at 10 µg/g.
- **Widiastuti *et al.* (1988)** detected CPA in 21 of 26 corn samples collected from a poultry feed mill in Indonesia over the course of a year, with concentrations ranging from 0.03 to 9 µg/g, half with levels above 1 µg/g. The levels of CPA fairly consistently exceeded the levels of total aflatoxin, often by more than 100-fold, and all CPA-contaminated samples were co-contaminated with aflatoxin.
- **Lee and Hagler (1991)** found aflatoxin contamination in all seven loads, with concentrations ranging from 3 to 508 ng/g. They also found co-contamination with CPA in four samples, with concentrations ranging from <25 ng/g (the limit of determination) to 250 ng/g.
- **Urano *et al.* (1992)** found that 51% of maize samples had measurable levels of CPA (limit of determination 25 ng/g), with the highest concentration measured at 2.8 µg/g and an average concentration of 467 ng/g. All of these samples were co-contaminated with aflatoxin, while 16 samples (36%) contained only aflatoxin, and 6 samples (13%) were not measurably contaminated with either CPA or aflatoxin.
- **Balachandran and Parthasarathy (1996)** examined multiple feed items including 20 randomly collected lots of maize and six lots known to be contaminated with aflatoxin. Nine of the 20 randomly sampled lots and one of the six aflatoxin-contaminated lots contained measurable amounts of CPA, with estimated levels ranging from 0.4 to 12 µg/g.
- **Abbas *et al.* (2008)** reported at-harvest CPA levels of 61 and 72.2 ng/g in maize.. All plots were co-contaminated with aflatoxin, and total aflatoxin levels were 104 and 200 ng/g.

- **<u>Mansfield *et al.* (2008</u>)** identified CPA in samples of maize silage in Pennsylvania (though at relatively low concentrations. This was thought to be the result of colonization by *Penicillium spp.*

Toxicokinetics of CPA combined with other mycotoxins

Individual and combined effects of aflatoxin (AF) and cyclopiazonic acid (CPA) in day-old Petersen x Hubbard broiler chickens to 3 wk of age. Treatments were arranged in a 2 x 2 factorial with levels of 0 and 3.5 mg AF/kg of feed, and 0 and 50 mg CPA/kg of feed. **Smith *et al.* (1992)**

- Body weight gain was significantly (P less than .05) reduced by AF, CPA, and the AF-CPA combination at the end of 3 wk.
- Aflatoxin significantly increased the relative weight of the kidney and serum concentration of blood urea nitrogen and decreased serum concentrations of protein, albumin, cholesterol, phosphorus, and the activity of lactate dehydrogenase.
- The toxicity of CPA was expressed through
  - increased relative weights of the liver, kidney, and proventriculus,
  - increased levels of uric acid and cholesterol, and decreased serum phosphorus.
- The activity of AF-CPA combination was characterized by
  - increased relative weight of the liver, kidney, pancreas, and proventriculus,
  - decreased concentrations of serum albumin and phosphorus,
  - increased concentrations of serum glutamic oxalacetic transaminase and blood urea nitrogen,
  - decreases in the relative weight of the bursa of Fabricius.
- Post-mortem examination revealed that the chickens fed CPA and the AF-CPA combination had thickened mucosa and dilated proventricular lumens, hard fibrotic spleen, and atrophy of the gizzard.
- The data from the present study demonstrate that both AF and CPA alone and the AF-CPA combination can limit broiler performance and adversely affect broiler health. In most cases the effects of AF and CPA were additive.

Individual and combined effects of ochratoxin A (OA) and cyclopiazonic acid (CPA) in Petersen × Hubbard broiler chickens from 1 d to 3 wk of age. The experimental design was a 2 × 2 factorial with treatments of 0 and 2.5 mg OA/kg feed and 0 and 34 mg CPA/kg feed **GENTLES *et al.* (1999)**.

- Body weight gain was reduced ($P < 0.05$) by OA, CPA, and OA-CPA in combination at the end of 3 wk.
- Ochratoxin A significantly increased the relative weight of the kidney and serum concentrations of uric acid and triglycerides and decreased total protein, albumin, and cholesterol.
- CPA induced primarily increased relative weights of the pro- ventriculus and increased activity of creatine kinase.
- OA-CPA induced increased relative weights of the liver, kidney, pancreas, and proventriculus; decreased concentrations of serum albumin, total protein, and

cholesterol; increased activity of creatine kinase; and increased concentrations of triglycerides and uric acid.

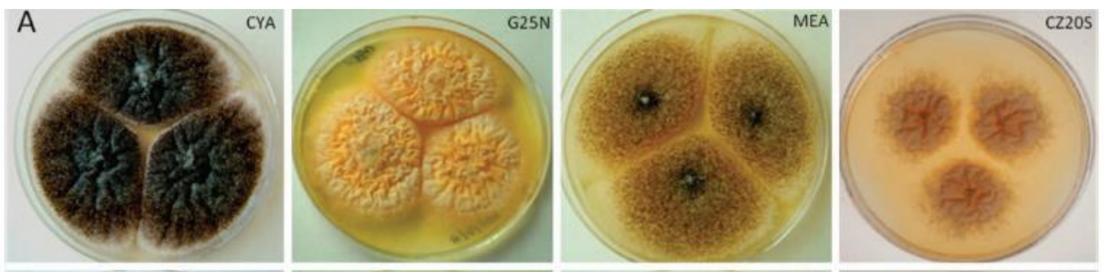
- Postmortem examination revealed that the chickens fed CPA or OA-CPA had thickened mucosa and dilated proventricular lumen.
- Data from this study demonstrate that OA, CPA, and the OA-CPA combination can limit broiler performance and adversely affect broiler health. The interaction of the compounds was primarily additive or less than additive in the parameter in which the interaction occurred.

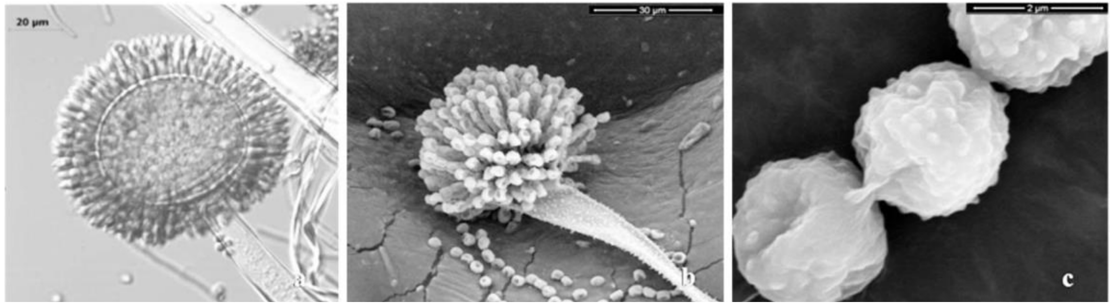
Description of some cyclopiazonic acid producers

1. Aspergillus mottae **C. Soares, S.W. Peterson et A.Venaˆncio sp. nov.**

Colonies on CYA attained . 70 mm diam in 7 d at 25 C, 50–54 mm diam at 37 C and 20–40 mm diam at 42 C; colonies on MEA attained . 70 mm diam at 25 C, 50–53 mm diam at 37 C and 20–40 mm diam at 42 C; colonies on G25N attained 40 mm diam at 25 C, 30 mm diam at 37 C and 17–20 mm diam at 42 C; colonies on CZ20S attained 35–40 mm diam at 25 C, . 70 mm diam at 37 C; and 17–20 mm diam at 42 C; no growth occurred at 5 C. Colony surface plane, mycelia white, yellow-green conidia heads scarce at 25 C, numerous dark brown small sclerotia, 249– 371 mm diam, covering the plate on CYA and MEA, sclerotia yellow and fewer on G25N and CZ20S media (FIG. 5A), conidial heads more plentiful with growth at 42 C. Conidial heads normally biseriate but uniseriate heads also occur. Vesicles globose to subglobose 36–43 mm diam; metulae 9.0–11.8 3 3.6– 5.4 mm; phialides 5.8–8.2 3 2.8–4.8 mm; stipes hyaline, smooth; conidia globose to subglobose, smooth to finely rough, 3.3–4.3 mm diam

A. mottae resembles A. flavus, A. nomius, A. bombycis, A. arachidicola and A. minisclerotigenes in having yellow-green biseriate conidial heads. A. arachidicola and A. bombycis are not known to produce sclerotia, whereas A. mottae produces numerous small dark sclerotia, such as A. minisclerotigenes, A. parvisclerotigenus and some strains of A. flavus. A. flavus isolates are variable for sclerotium production, producing dark sclerotia when present, and A. flavus vesicles are up to 85 mm diam, whereas the vesicles of A. mottae are 36–43 mm diam. Conidia of A. mottae appear smooth to finely rough, similar to those of A. flavus.

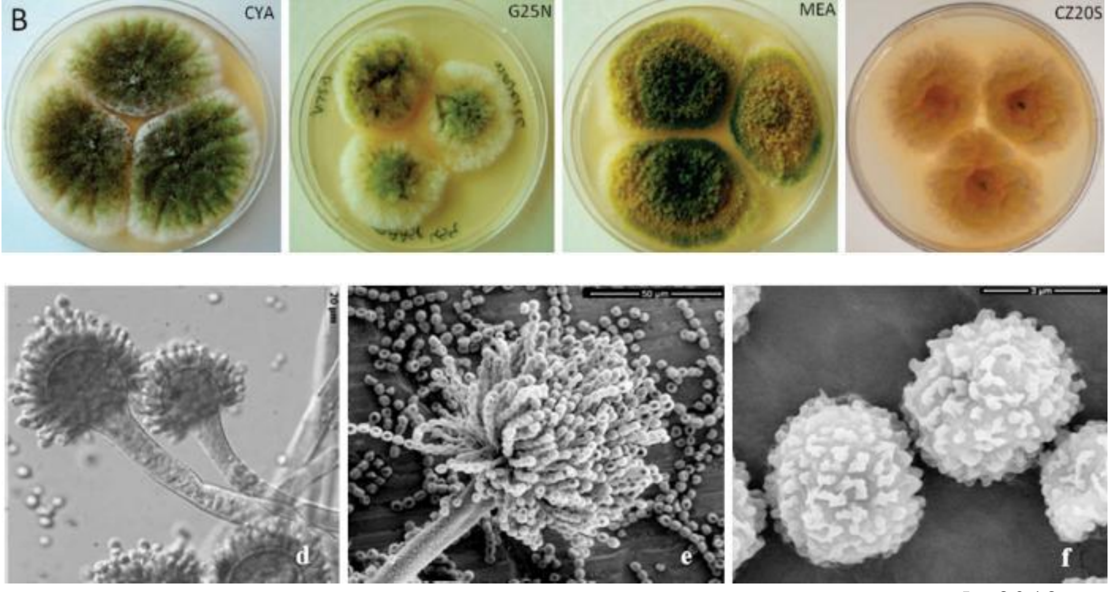




Aspergillus mottae MUM 10.231, conidiophores (a, b) and conidia (c)**, SOARES et al., 2012**

2. **Aspergillus sergii** P. Rodrigues, S.W. Peterson, A. Venaˆncio et N. Lima sp. Nov

Colonies after 7 d growth on CYA attained 55 mm diam at 25 C, 60 mm diam at 37 C and 15–25 mm diam at 42 C; colonies on MEA attain 55 mm diam at 25 C, 55 mm diam at 37 C and 15–25 mm diam at 42 C, colonies on G25N attained 37 mm diam at 25 C, 40 mm at 37 C and 10–20 mm at 42 C; colonies on CZ20S attained 40 mm diam at 25 C, . 70 mm diam at 37 C and 15–25 mm diam at 42 C; no growth at 5 C. Colony surface is plane, velvety and dense; conidial heads in a uniform, dense layer but sparse in the areas of sclerotium production showing a color between those of A. flavus and A. parasiticus (FIG. 5B); sclerotia brown, type L, 513–551 mm diam. Conidial heads uniseriate; vesicles globose, 26–36 mm diam; phialides 5.5–6.8 3 2.5–3.1 mm; stipes hyaline, smooth; conidia globose to subglobose, rough, greenish, 3.3–4.3 mm diam Aspergillus sergii most closely resembles A. parasiticus because of the rough conidia and the production of predominantly uniseriate conidial heads. The two species differ in colony color, which is a lighter green in A. sergii, and on phialide and conidial sizes. A. sergii phialides are 5.5–6.7 3 2.5–3.1 mm, while A. parasiticus phialides are 7–10 3 2.5–5; conidia of A. sergii are 3.3–4.2 mm diam and roughened, while those of A. parasiticus are 4–6 mm diam and roughened to echinulate. A. sergii also differs from A. parasiticus by CPA production

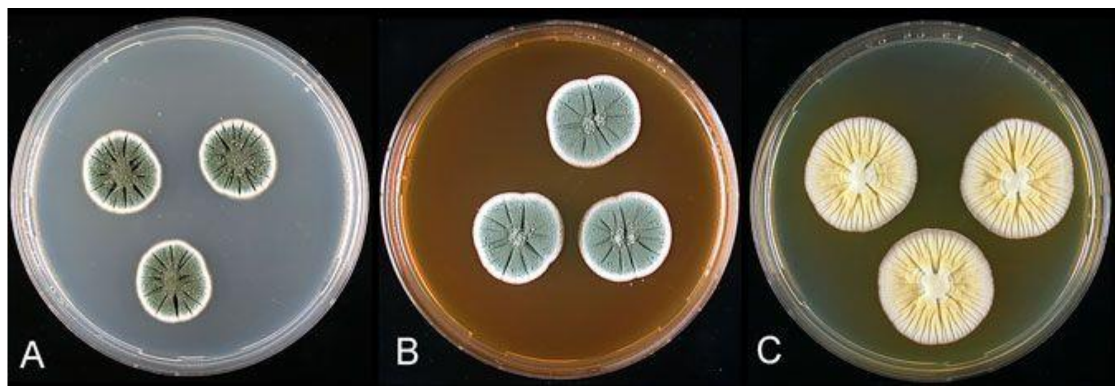


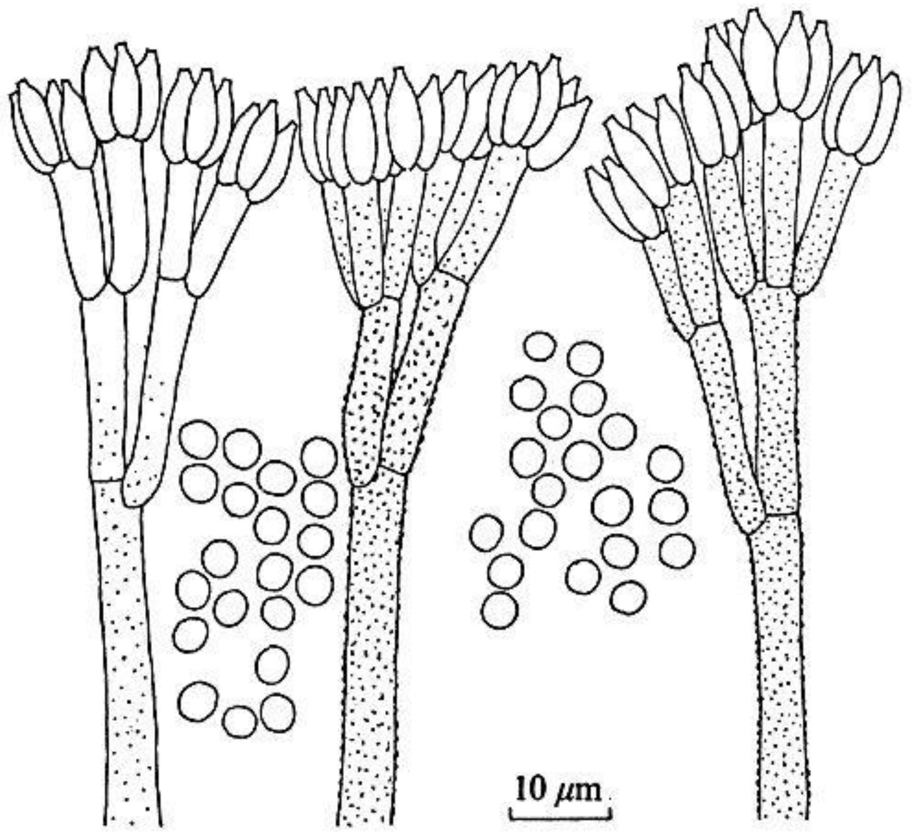
Aspergillus sergii MUM 10.219, conidiophores (d, e) and conidia (f). **SOARES et al., 2012**

3. **Penicillium cyclopium** Westling, Arkiv før Botanik 11 (1): 90 (1911)

≡Penicillium verrucosum var. cyclopium (Westling) Samson, Stolk & Hadlok, Studies in Mycology 11: 37 (1976)

Colonies on Czapek agar and CYA at 25°C growing restrictedly producing grey green conidia with a granular to fasciculate colony surface, often with exudate droplets. The colony reverse is orange to red or pinkish brown with the colour often diffusing into the agar medium or more rarely creamish yellow. On MEA the conidia are blue green with a strong blue element and colonies have a distinct yellow reverse, often with the yellow colour diffusing into the medium. On YES agar there is no sporulation and the colony mycelium if often strongly yellow, reverse colour distinct yellow. On CREA weak growth but strong acid production. Conidiophores two-stage branched (terverticillate) with all elements adpressed, stipes rough-walled. Conidia smooth-walled, globose to subglobose, 3-3.5 µm in diam.



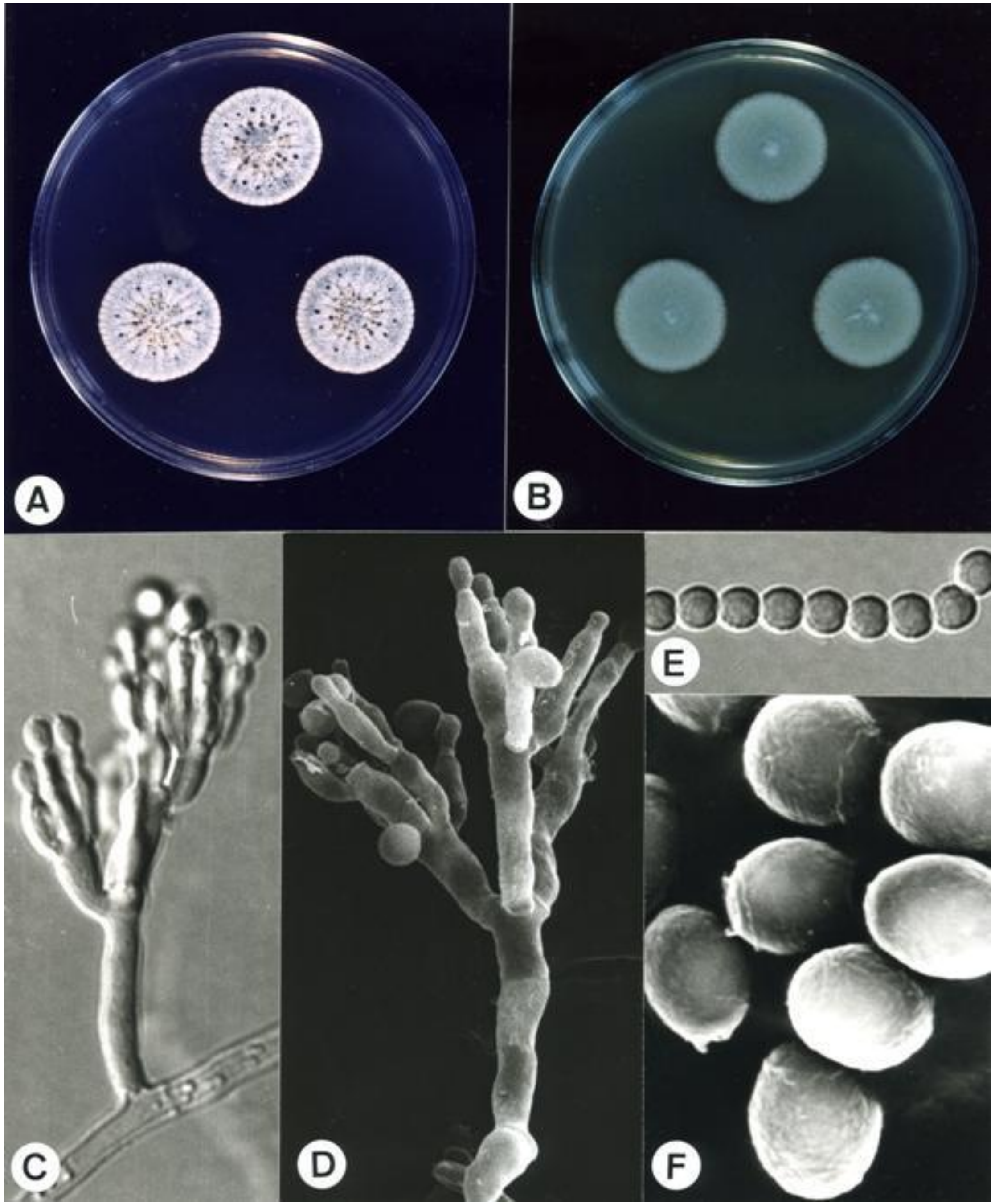


Penicillium cyclopium. Mycobank

4. **Penicillium griseofulvum** Dierckx, Annales de la Société Scientifique de Bruxelles 25 (1): 88 (1901)

=Penicillium patulum Bainier, Bulletin de la Société Mycologique de France 22: 208 (1906)
=Penicillium urticae Bainier, Bulletin de la Société Mycologique de France 23 (1): 15 (1907)
=Penicillium flexuosum E. Dale, Annales Mycologici 24: 137 (1924) [MB#265101]
=Penicillium maltum M. Hori & T. Yamam., Jap. J. Bacteriol.: 1105 (1954) [MB#335748]
=Penicillium duninii Sidibe, Mikol. Fitopatol.: 371 (1974)

Colonies (CzA) slowly growing, fasciculate to synnematal, greyish-green; soluble pigment reddish-brown. Microscopy. Conidiophore stipes of very variable length, smooth-walled, brownish; penicilli terverticillate to quaterverticillate. Metulae 7-10 µm long, sometimes apically inflated. Phialides closely packed, very short, ampulliform, 4.5-6.0 µm. Conidia ellipsoidal, smooth-walled, 3.0-3.5 µm long. Physiology. Intolerant to benomyl. No growth at 37°C.

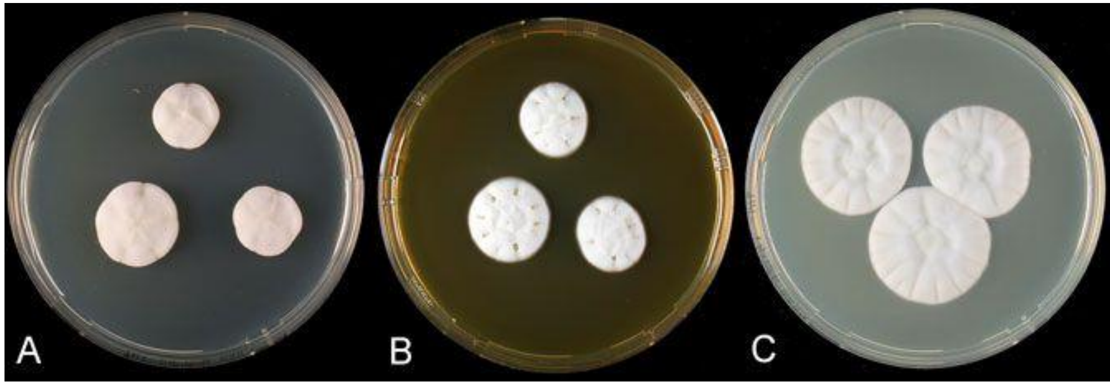


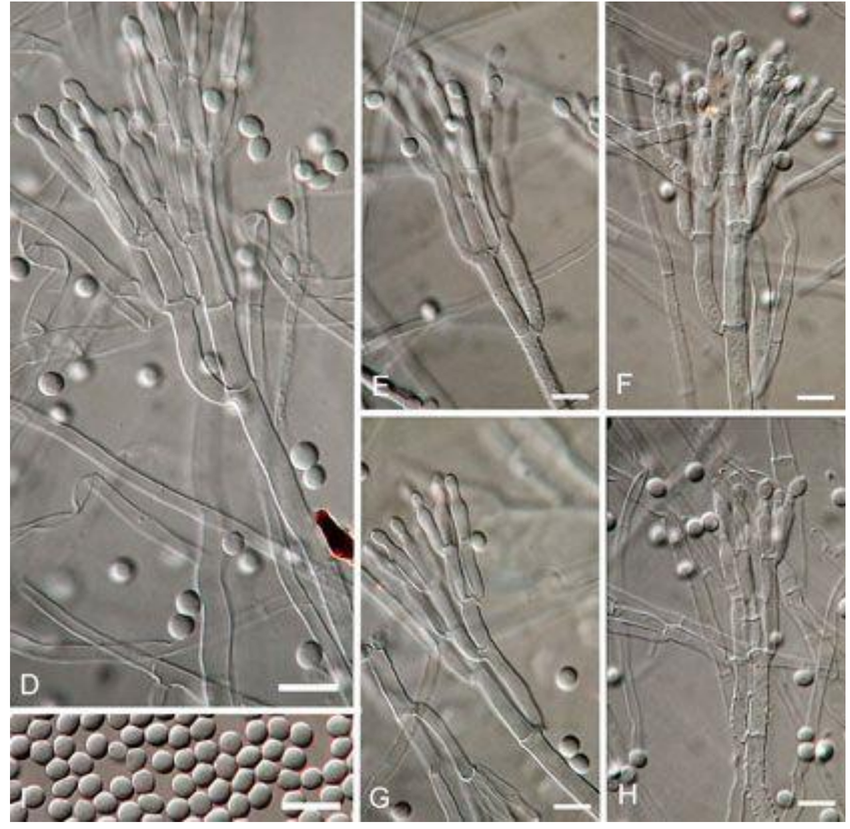
Tzean, SS et al. 1994. **Penicillium griseofulvum**

5. **Penicillium camemberti** Thom, U.S.D.A. Bureau of Animal Industry Bulletin 82: 33 (1906)

=Penicillium album Epstein, Ark. Hyg. Bakt.: 360 (1902) [MB#146877]
=Penicillium rogeri Wehmer, Hendb. Tech. Mykol.: 226 (1906) [MB#492645]
=Penicillium caseicola Bainier, Bulletin de la Société Mycol de France 23: 94 (1907)
=Penicillium biforme Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 54 (1910)
=Penicillium candidum Roger, La Cellule 33: 193 (1923) [MB#174737]
=Penicillium paecilomyceforme Szilvinyi, Zbl f Bakt. und Parasit. Abt 2 103: 156 (1941)

Colonies on Czapek's solution agar (growing more or less restrictedly, about 2-3 cm. in 10 days to two weeks at room temperature, loose-textured, floccose, cottony (fig. 110C), pure white at first, changing to pale gray-green near glaucous to greenish glaucous after 7 to 8 days, deeply lapse throughout, hyphae not tending to form ropes or fascicles; reverse uncolored or in cream to very pale yellow shades; odor pronounced, simulating that of potato peels; exudate not produced, or present as scattered, small, uncolored droplets largely submerged in the mycelial mass; penicilli fairly abundant, asymmetric, with conidial chains forming an irregular, tangled mass, commonly measuring from 50 to 80 µm in length, but with individual structures ranging from 30 to 100 µm in length, borne upon long conidiophores arising from the substratum or upon short branches from aerial hyphae; conidiophores more or less tangled, extremely variable in length, ranging from 250 to 600 µm by 2.5 to 3.5 µm when arising from the substratum, 40 to 200 µm in length when borne on aerial hyphae, with walls of conidiophores and fruiting branches commonly slightly roughened; spore-bearing apparatus ranging from 25.0 to 50.0 µm in length, irregularly branched, with branches and metulae often poorly differentiated; branches commonly 12.0 to 18.0 µm by 2.2 to 3.4 µm; metulae borne at different levels in the penicillus and usually in groups of 2 or 3, ranging in size from 9 to 14 µm by 2.2 to 3.2 µm; sterigmata in groups of 2 to 5, rarely more, 9.0 to 14.0 µm by 2.2 to 2.8 µm; conidia elliptical when first formed, becoming subglobose at maturity, commonly measuring 3.5 to 5.0 µm by 3.0 to 4.5 µm, smooth-walled, lightly colored in mass

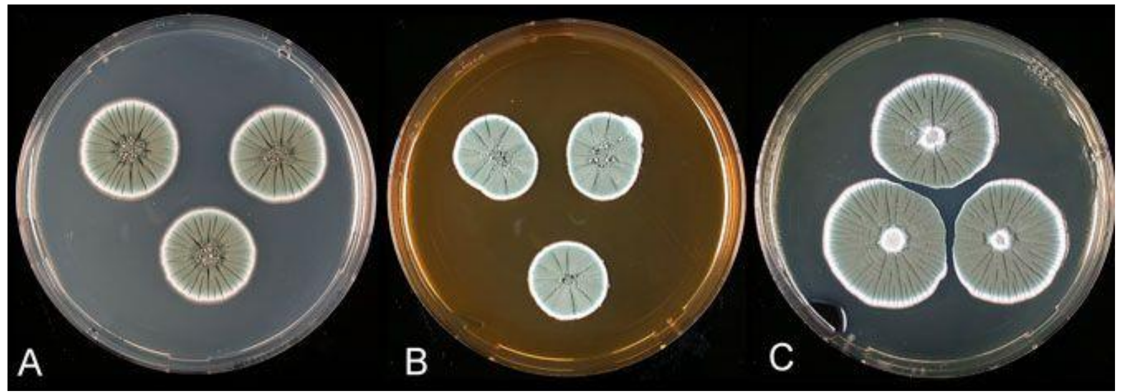


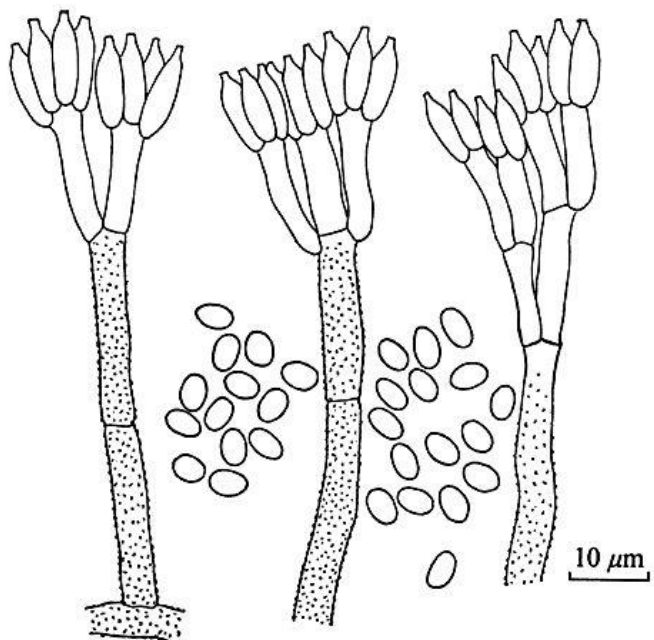


Penicillium camemberti, Mycobank

6. **Penicillium camemberti** Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 56 (1910)

=Penicillium flavoglaucum Biourge, La Cellule 33: 130 (1923)
=Penicillium fuscoglaucum Biourge, La Cellule 33: 128 (1923)
=Penicillium lanosogriseum Thom, The Penicillia: 327 (1930)
=Penicillium lanosoviride Thom, The Penicillia: 314 (1930)
=Penicillium psittacinum Thom, The Penicillia: 369 (1930)
=Penicillium ochraceum var. macrosporum Thom, The Penicillia: 310 (1930)
=Penicillium cyclopium var. album G. Sm., Trans Brit Mycol Soc 34 (1): 18 (1951)
=Penicillium roqueforti var. punctatum S. Abe, J Gen Appl Microbiol Tokyo 2 , 99 (1956)





Penicillium camemberti, Mycobank

Reports:

Gallagher *et al.* (1978) found twenty-eight of 54 isolates of Aspergillus flavus grown on autoclaved agricultural commodities such as wheat, rice and corn to produce the mycotoxin cyclopiazonic acid. Eighteen of the A. flavus isolates produced aflatoxin, and fourteen isolates produced both cyclopiazonic acid and aflatoxin. A preliminary screening of some aflatoxin-contaminated corn samples revealed for the first time the natural occurrence of cyclopiazonic acid in agricultural commodities, estimating the CPA concentration in one of the tested samples at 10 μg/g. estimating the CPA concentration in one of the tested samples at 10 μg/g.

Norred *et al.* (1987) developed a liquid chromatographic procedure for the determination of cyclopiazonic acid (CPA) in poultry meat. CPA was extracted from ground meat with chloroform-methanol (80 + 20), partitioned into 0.1N sodium hydroxide, acidified, and extracted into dichloromethane. An interfering component of meat was removed by transferring the dichloromethane extract to a minicolumn containing silica gel and washing the column with petroleum ether and chloroform. CPA was eluted with methanol-acetic acid (99 + 1), and subjected to ligand-exchange liquid chromatography. Recovery of CPA from 40 separate samples of meat spiked with CPA at levels from 0.016 to 15.6 ppm was 70.4 +/- 14.1%. Analysis of meat from a chicken orally dosed with 10 mg CPA/kg body weight revealed that 14.5% of the dose was in muscle 48 h after administration.

Dorner *et al.* (1983) purified cyclopiazonic acid (CPA) from cultures of Aspergillus flavus, and ca. 14 g of the toxin was collected for use in feeding studies. Chicken rations were artificially contaminated with purified CPA at concentrations of 10, 50, and 100 ppm (microgram/g) and fed ad libitum to eight groups of chickens for 7 weeks. Chickens receiving feed with 100 ppm of CPA had high mortality, decreased weight gain, and poor feed conversion when compared with birds receiving other doses. Postmortem examination showed that chickens fed the two greatest doses of

CPA had proventricular lesions characterized by mucosal erosion and hyperemia (100 ppm) and by thick mucosa and dilated proventricular lumens (50 ppm). Birds given 100 ppm of CPA in feed also had numerous yellow foci in their livers and spleens. Microscopic examination of tissues of birds that received 100 ppm of CPA revealed ulcerative proventriculitis, mucosal necrosis in the gizzard, and hepatic and splenic necrosis and inflammation. Birds given 50 ppm of CPA had hyperplasia of the proventricular mucosal epithelium. Birds given 10 ppm of CPA and control birds had no significant treatment-related lesions.

Stolz *et al.* (1988) reported an outbreak of disease in quail in Indonesia which was observed to have many of the characteristics of mycotoxicosis, and a sample of the feed involved was found to contain CPA at 6000 ng/g, along with lower levels of aflatoxins (465 ng/g) and ochratoxin A (500 ng/g). The clinical signs in affected birds, including opisthotonus, as well as the histopathological findings supported a diagnosis of CPA toxicity.

Widiastuti *et al.* (1988) detected cyclopiazonic acid (CPA) at concentrations as high as 9 ppm in 21 of 26 corn samples from a Bogor poultry feed mill. This is the first demonstration of the natural occurrence of CPA in Indonesia. CPA was always accompanied by other mycotoxins, especially aflatoxins, suggesting that the interactive toxicity of these mycotoxins to poultry should be investigated.

Chang-Yen and Bidasee (1990) developed an improved visible spectrophotometric method for detection of cyclopiazonic acid in poultry feed and corn. The method is based on the reaction of cyclopiazonic acid with Ehrlich reagent and detection at 580 nm. Reaction conditions were optimized with respect to reaction and measurement times and acid and Ehrlich reagent concentrations. Calibration curves were linear from 1 to 20 micrograms cyclopiazonic acid in 3 mL Ehrlich reagent, with a lower detection limit of 0.08 mg/kg for 50 g samples of poultry feed and corn. Recoveries from 50 g samples of poultry feed spiked with cyclopiazonic ranging from 0.16 to 1.20 mg/kg averaged 93.8%. Moldy corn and poultry feed samples analyzed by this method contained between 1 and 4 mg/kg cyclopiazonic acid.

Lee and Hagler (1991) tested seven truck-loads of maize for mycotoxin contamination. Aflatoxin was identified in all 7 at concentrations from 3 ng/g-501 ng/g (aflatoxin B_1+ B_2). Cyclopiazonic acid was identified in 4 loads with concentrations from 25-250 ng/g. Deoxynivalenol was found in 4 of 5 loads tested, over a range of 46-676 ng/g. Ninteeen isolates of *Aspergillus flavus* from the samples were tested for ability to accumulate cyclopiazonic acid and aflatoxin in liquid culture. Fourteen produced cyclopiazonic acid (0.5-135 µg/mL), 12 produced aflatoxin (0.01-0.70 µg/mL, aflatoxin B_1+ B_2), and one aflatoxin-producing isolate did not produce cyclopiazonic acid.

Smith *et al.* (1992) evaluated the individual and combined effects of aflatoxin (AF) and cyclopiazonic acid (CPA) in day-old Petersen x Hubbard broiler chickens to 3 wk of age. Treatments were arranged in a 2 x 2 factorial with levels of 0 and 3.5 mg AF/kg of feed, and 0 and 50 mg CPA/kg of feed. Production performance, serum biochemistry, and gross pathological observations were evaluated. Body weight gain was significantly (P less than .05) reduced by AF, CPA, and the AF-CPA combination at the end of 3 wk. Aflatoxin significantly increased the relative weight of the kidney

and serum concentration of blood urea nitrogen and decreased serum concentrations of protein, albumin, cholesterol, phosphorus, and the activity of lactate dehydrogenase. The toxicity of CPA was expressed through increased relative weights of the liver, kidney, and proventriculus, increased levels of uric acid and cholesterol, and decreased serum phosphorus. The activity of AF-CPA combination was characterized by increased relative weight of the liver, kidney, pancreas, and proventriculus, decreased concentrations of serum albumin and phosphorus, increased concentrations of serum glutamic oxalacetic transaminase and blood urea nitrogen, and decreases in the relative weight of the bursa of Fabricius. Post-mortem examination revealed that the chickens fed CPA and the AF-CPA combination had thickened mucosa and dilated proventricular lumens, hard fibrotic spleen, and atrophy of the gizzard. The data from the present study demonstrate that both AF and CPA alone and the AF-CPA combination can limit broiler performance and adversely affect broiler health. In most cases the effects of AF and CPA were additive.

Urano *et al.* (1992) found that 51% of maize samples had measurable levels of CPA (limit of determination 25 ng/g), with the highest concentration measured at 2.8 µg/g and an average concentration of 467 ng/g. All of these samples were co-contaminated with aflatoxin, while 16 samples (36%) contained only aflatoxin, and 6 samples (13%) were not measurably contaminated with either CPA or aflatoxin.

Dorner *et al.* (1994) detected cyclopiazonic acid residues in eggs from chickens that were given oral doses of the toxin in2 separate studies: an acute study ovedr 9 days with dose groups of 0.0,2.5, 5.0 and 10.0 mg/kg of live weight and a chronic study over 4 weeks with dose groups of 0.0, 1.25 and 2.5 mg/kg of live weight .Eggs from birds in all groups throuout both studies contained CPA. The concentrations were much higher in egg white than in egg yolk, averaging approximately 100 and 10 ng/g, respectively.

Kubena *et al.* (1994) studied the effects of feeding 6 mg T-2 toxin (T-2) and 34 mg cyclopiazonic acid (CPA)/kg of diet singly and in combination in male broiler chicks from 1 d to 3 wk of age. Body weights were depressed by T-2, CPA, and the combination of T-2 and CPA. There was a significant synergistic interaction between T-2 and CPA for relative liver and kidney weights and serum cholesterol and triglyceride concentrations and a significant interaction between T-2 and CPA for 3-wk body weights and relative bursa of Fabricius weights, which were less than additive. Neither the efficiency of feed utilization nor mortality was affected by dietary treatments. Oral lesions were present in a majority of the chicks fed diets containing T-2 with or without CPA. When compared with controls, other variables measured exhibited additive or less than additive toxicity. These data demonstrate that T-2 and CPA alone and in combination can cause reduced performance and adversely affect broiler health. The effects of these mycotoxins may be exacerbated by other factors when under field conditions; hence, the potential detrimental effects of these two mycotoxins when present alone or in combination cannot be dismissed.

Balachandran and Parthasarathy (1996a) fed three hundred and forty-eight Vencob broiler chickens diets containing Penicillium griseofulvum rice culture material with 0, 12.5, 25 and 50 ppm of the mycotoxin cyclopiazonic acid (CPA) for 28 days. Serum samples were collected from 9 birds in each group at weekly intervals to study the effect of sublethal doses of CPA on certain serum biochemical parameters. Significant reductions in weight gains ($p < 0.01$) and feed consumptions ($p < 0.05$) were observed at 25 and 50 ppm. Exocrine pancreas showed degenerative

and necrotic changes in CPA fed chickens. The CPA had significant ($p < 0.05$) influence on serum total protein, albumin, cholesterol, amylase and lipase levels. CPA did not affect serum glucose levels. There was a decline in levels of total serum protein and albumin in CPA fed groups. But serum cholesterol, amylase and lipase showed dose-dependent increases.

Balachandran and Parthasarathy (1996b) detected cyclopiazonic acid (CPA) activity in 40 out of 100 samples (67 randomly collected and 33 known to contain aflatoxin) of feeds and feedstuffs, i.e., maize 10/26, groundnut cake 10/20, sunflower seed cake 7/10, sorghum 4/10, wheat 1/1, little millet 1/1, rice 1/1, deoiled rice bran 0.1, fishmeal 0/1, chick mash 1/3, grower mash 0/7, layer mash 3/11, broiler mash 2/6 and cattle feed 0/2, concentrations ranged from 0.4 to 12, 0.5 to 20, 0.3 to 20, 0.3 to 20, 20, 10, 10, 1.5, 1 to 15, 8 and 15 ppm, respectively. Co-occurrence of CPA was found in 14 of 33 aflatoxin-containing samples.

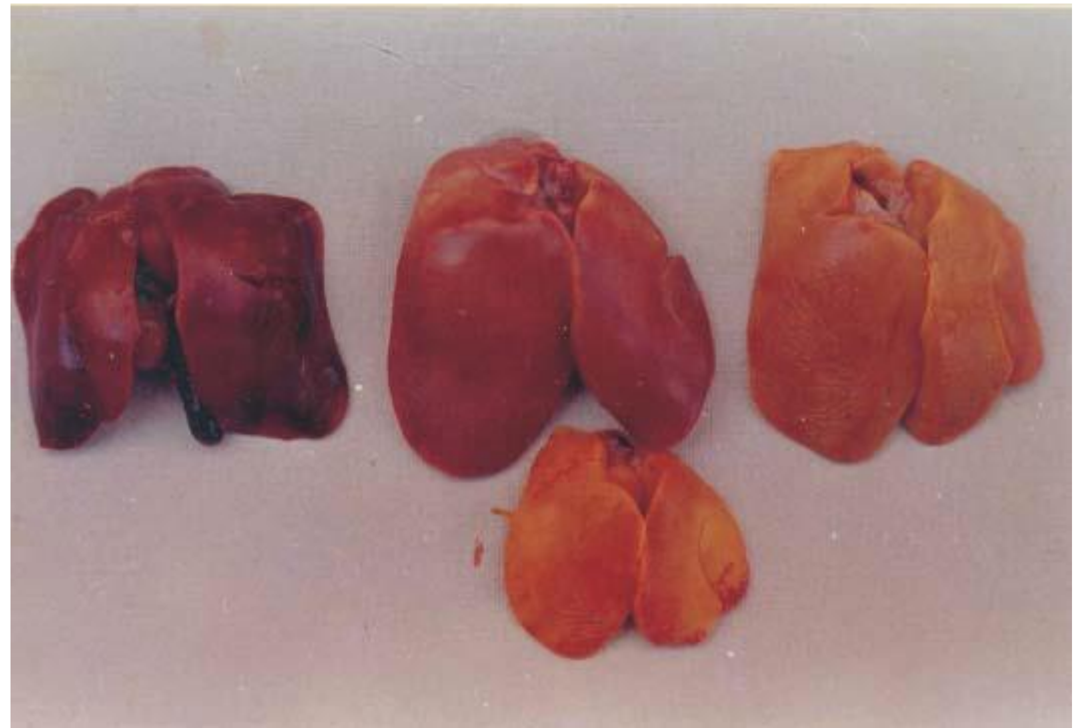
GENTLES *et al.* (1999) evaluated the individual and combined effects of ochratoxin A (OA) and cyclopiazonic acid (CPA) in Petersen × Hubbard broiler chickens from 1 d to 3 wk of age. The experimental design was a 2 × 2 factorial with treatments of 0 and 2.5 mg OA/kg feed and 0 and 34 mg CPA/kg feed. Production performance, serum biochemistry, and gross pathological observations were evaluated. Body weight gain was reduced ($P < 0.05$) by OA, CPA, and OA-CPA in combination at the end of 3 wk. Ochratoxin A significantly increased the relative weight of the kidney and serum concentrations of uric acid and triglycerides and decreased total protein, albumin, and cholesterol. The toxicity of CPA was expressed primarily through increased relative weights of the pro- ventriculus and increased activity of creatine kinase. Exposure to OA-CPA was characterized by increased relative weights of the liver, kidney, pancreas, and proventriculus; decreased concentrations of serum albumin, total protein, and cholesterol; increased activity of creatine kinase; and increased concentrations of triglycerides and uric acid. Postmortem examination revealed that the chickens fed CPA or OA-CPA had thickened mucosa and dilated proventricular lumen. Data from this study demonstrate that OA, CPA, and the OA-CPA combination can limit broiler performance and adversely affect broiler health. The interaction of the compounds was primarily additive or less than additive in the parameter in which the interaction occurred.

Vaamonde *et al.* (2003) screened Aspergillus section flavi strains isolated from peanuts, wheat and soybean grown in Argentina for aflatoxins (type B and G) and cyclopiazonic acid (CPA) production. Aspergillus flavus was the predominant species in all substrates, although there was almost the same proportion of A. flavus and Aspergillus parasiticus in peanuts. Aspergillus nomius was not found. Incidence of aflatoxigenic A. flavus strains was higher in peanuts (69%) than in wheat (13%) or soybeans (5%) while the ratio of CPA producers A. flavus isolated from all substrates was very high (94% in peanuts, 93% in wheat and 73% in soybeans). Isolates of A. flavus able to produce simultaneously aflatoxins type B and CPA were detected in all substrates, suggesting the possibility of co-occurrence of these toxins. Almost all isolates of A. parasiticus resulted aflatoxins (type B and G) producers but did not produce CPA. Five of sixty-seven strains isolated from peanuts showed an unusual pattern of mycotoxin production (aflatoxins type B and G simultaneously with CPA). These strains also produced numerous small sclerotia like S strains of A. flavus detected in cottonseed in Arizona and in soils of Thailand and West Africa.

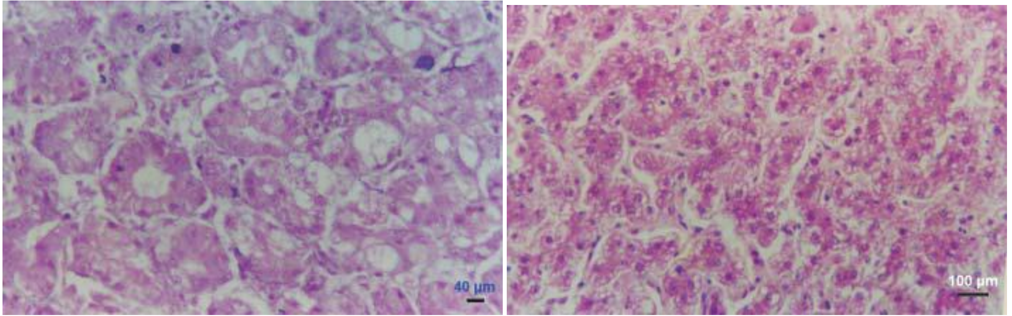
Kamalavenkatesh *et al.* (2005) fed 40, newly hatched, unsexed broiler chicks diets containing 10 ppm cyclopiazonic acid (CPA) and 1 ppm T-2 toxin (T2) either individually or in combination for 28 days to study the immunopathological effects. Lymphoid organs revealed lymphocytolysis and lymphoid depletion in all toxin fed birds. Thymic and splenic CD+4 and CD+8 lymphocytes decreased significantly ($p<0.01$) in toxin fed birds when compared to the control. Thymic CD+8 lymphocytes of T2 and CPA-T2 showed significant ($p<0.01$) decrease from that of CPA and control groups. Splenic CD+4 and CD+8 lymphocytes showed significant ($p<0.01$) decrease in CPA and CPA-T2 fed groups when compared to the control. The T2 group did not differ significantly from that of control. The stimulation index (SI) of splenocytes to concavalin A revealed significant ($p<0.01$) decrease in all toxin fed birds. Significant ($p<0.01$) decrease were observed for the haemagglutination inhibition (HI) titres to Newcastle disease virus vaccine F strain (NDV) of birds fed CPA, T2 and in combination. Significant ($p<0.01$) interaction was found for lymphocyte subsets, SI and HI titres to NDV. The study indicated the immunosuppressive effect of these toxins either alone or in combination in broiler chicks.

.

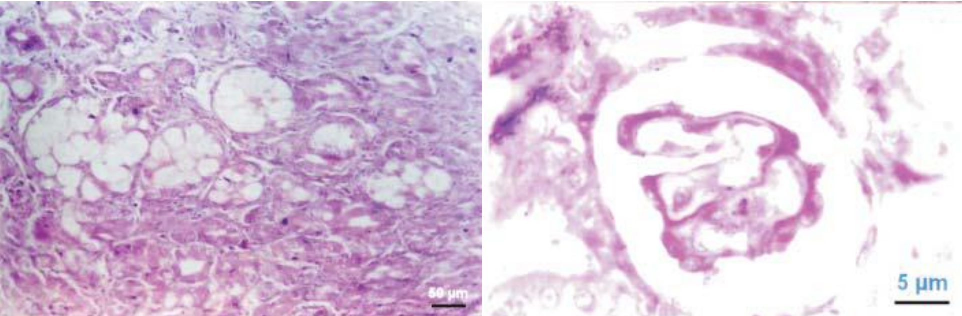
Kumar and Balachandran (2009) induced an experimental mycotoxicoses into **broiler chickens** by feeding 1 ppm aflatoxin (AF) and 20 ppm cyclopiazonic acid (CPA) from 0 to 28 days of age to evaluate the **gross and histopathological changes**. Grossly, AF and AF-CPA fed birds showed enlargement, yellowish discoloration of the liver while the CPA fed birds showed enlargement and congestion. The CPA and AF-CPA fed birds showed thickening of crop and necrosis and thickening of proventricular mucosa. Histopathologically, degenerative and necrotic changes were observed in the liver, kidneys, intestine, pancreas, heart, pectoral muscle, spleen and bursa of Fabricius of all toxin fed birds. Besides, hyperplastic changes were also observed in the crop, proventriculus and gizzard in the CPA fed birds. The lesions were more marked in the AF-CPA group. The study revealed that AF and CPA in combination could act cumulatively and adversely affect the health of broiler chicken.



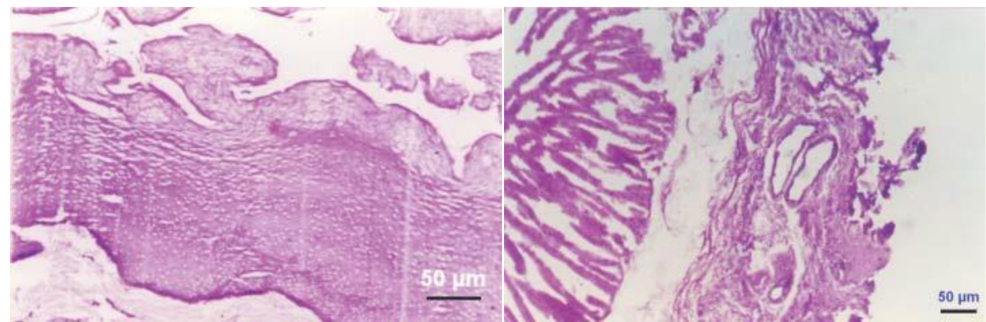
Liver CPA toxicosis. Congestion (upper left), aflatoxicosis-paleness and yellow discoloration (middle and upper right), AF-CPA toxicosis - yellow discoloration (bottom). **Kumar and Balachandran (2009)**



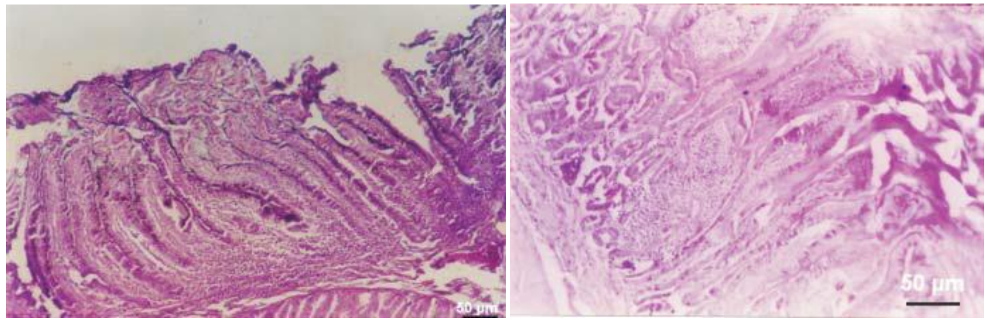
Aflatoxicosis. Liver showing acinar arrangement of regenerating hepatocytes. H&E, scale bar = 40 µm. CPA toxicosis. Liver showing microvesicular fatty degeneration of hepatocytes. H&E, scale bar = 100 µm. **Kumar and Balachandran (2009)**



AF-CPA toxicosis. Liver showing macrovesicular fatty degeneration and fatty cyst formation in the regenerating hepatocytes. H&E, scale bar = 50 µm. AF-CPA toxicosis. Kidney showing thickening of glomerular basement membrane and collapse of glomerulus .H&E, scale bar = 5 µm. **Kumar and Balachandran (2009)**



AF-CPA toxicosis. Crop mucosa showing epithelial hyperplasia and vacuolar degeneration. H&E, scale bar = 50 µm. Aflatoxicosis. Proventriculus showing partial necrosis of mucosa, dilated crypts and submucosal edema. H&E, scale bar = 50 µm. **Kumar and Balachandran (2009)**



CPA toxicosis. Proventriculus hyperplasia of mucosa with heavy infi ltration of lymphocytes. H&E, scale bar = 50 µm. AF-CPA toxicosis. Gizzard showing defective keratinoid membrane formation. H&E, scale bar = 50 µm.
Kumar and Balachandran (2009)

Malekinejad *et al.* (2011) studied the effect of cyclopiazonic acid (CPA) on oxidative stress markers in the liver and kidneys of broiler chicks. Ten-day-old male broiler chicks (Ross 308) were assigned into the control and test groups, which received normal saline and 10, 25, and 50 µg/kg CPA, respectively, for 28 days. Body weight gain, serum level of alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), uric acid, creatinine, and blood urea nitrogen (BUN) were measured after 2 and 4 weeks exposure. Moreover, the total thiol molecules (TTM) and malondialdehyde (MDA) content of the liver and kidneys were assessed. No significant differences ($p > 0.05$) were found in body weight gain between the control and test groups. Whereas, the hepatic weight increased significantly ($p < 0.05$) in animals that received 25 and 50 µg/kg CPA. Both ALP and GGT level in serum were elevated in comparison to the control group. CPA also resulted in uric acid, creatinine, and BUN enhancement in broilers. The MDA content of the liver and kidneys showed remarkable increase. By contrast, the TTM levels in the liver and kidneys were significantly ($p < 0.05$) attenuated. Histopathological findings confirmed the biochemical changes in either organ characterized by inflammatory cells infiltration along with severe congestion and cell swelling, suggesting an inflammatory response. These data suggest that exposure to CPA resulted in hepatic and renal disorders, which were reflected as biochemical markers alteration and pathological injuries in either organ. The biochemical alteration and pathological abnormalities may be attributed to CPA-induced oxidative stress.

References:

1. Balachandran C, Parthasarathy KR. Influence of dietary rice culture material containing cyclopiazonic acid on certain serum biochemical parameters of broiler chickens. Mycopathologia. 1996a;132:161–166.
2. Balachandran C, Parthasarathy KR. Occurrence of cyclopiazonic acid in feeds and feedstuffs in Tamil Nadu, India. Mycopathologia. 1996b;133:159–162
3. Burdock G.A., Flamm W.G. Review Article: Safety assessment of the mycotoxin cyclopiazonic acid. Int. J. Toxicol. 2000;19:195–218.
4. Chang-Yen, I., & Bidasee, K. (1990). Improved spectrophotometric determination of cyclopiazonic acid in poultry feed and corn.*Journal of the Association of Official Analytical Chemists*, *73*(2), 257-259.
5. Domsch K.H., Gams W., Anderson T.-H. Compendium of Soil Fungi. Academic Press; London, UK: 1980.
6. Dorner JW, Cole RJ, Lomax LG, Gosser HS, Diener UL. Cyclopiazonic acid production by Aspergillus flavus and its effects on broiler chickens. Appl Environ Microbiol. 1983;46:698–703.
7. Dorner J.W., Cole R.J., Diener U.L. The relationship of *Aspergillus flavus* and *Aspergillus parasiticus*with reference to production of aflatoxins and cyclopiazonic acid. Mycopathologia. 1984;87:13–15
8. Dorner, Joe W. , Richard J. Cole, Deborah J. Erlington, Sucheep Suksupath, Graham H. McDowell, Wayne L. Bryden. Cyclopiazonic Acid Residues in Milk and Eggs. *J. Agric. Food Chem.*, 1994, *42* (7), pp 1516–1518
9. El-Banna A.A., Pitt J.I., Leistner L. Production of mycotoxins by *Penicillium* species. Sys. Appl. Microbiol. 1987;1:42–46.
10. Frisvad J.C. The connection between the *Penicillia* and *Aspergilli* and mycotoxins with special emphasis on misidentified isolates. Arch. Environ. Contam. Toxicol. 1989;18:452–467.
11. GENTLES,.A E. E. SMITH, L. F. KUBENA, E. DUFFUS, PAUL JOHNSON, J. THOMPSON, R. B. HARVEY, and T. S. EDRINGTON. Toxicological Evaluations of Cyclopiazonic Acid and Ochratoxin A in Broilers. 1999 Poultry Science 78:1380–1384
12. Hermansen K., Frisvad J.C., Emborg C., Hansen J. Cyclopiazonic acid production by submerged cultures of *Penicillium* and *Aspergillus* strains. FEMS Microbiol. Lett. 1984;21:253–261.
13. Holzapfel C.W. The isolation and structure of cyclopiazonic acid, a toxic metabolite of *Penicillium cyclopium* Westling. Tetrahedron. 1968;24:2101–2119
14. Kamalavenkatesh P, Vairamuthu S, Balachandran C, Manohar BM, raj GD. Immunopathological effect of the mycotoxins cyclopiazonic acid and T-2 toxin on broiler chicken. Mycopathologia. 2005 Feb;159(2):273-9.
15. Kubena LF, Smith EE, Gentles A, Harvey RB, Edrington TS, Phillips TD, Rottinghaus GE. Individual and combined toxicity of T-2 toxin and cyclopiazonic acid in broiler chicks. Poult Sci. 1994;73:1390–1397.
16. KUMAR, R., C. BALACHANDRAN: Histopathological changes in broiler chickens fed afl atoxin and cyclopiazonic acid. Vet. arhiv 79, 31-40, 2009.
17. Malekinejad H, Akbari P, Allymehr M, Hobbenaghi R, Rezaie A. Cyclopiazonic acid augments the hepatic and renal oxidative stress in broiler chicks. Hum Exp Toxicol. 2011 Aug;30(8):910-9.
18. Norred WP, Cole RJ, Dorner JW, Lansden JA. Liquid chromatographic determination of cyclopiazonic acid in poultry meat. J Assoc Off Anal Chem. 1987 Jan-Feb;70(1):121-3.
19. Ohmomo S., Sugita M., Matazo A. Isolation of cyclopiazonic acid, cyclopiazonic acid imine and bissecodehydrocyclopiazonic acid from the cultures of *Aspergillus versicolor* (Vuill.) Tiraboschi. J. Agr. Chem. Soc. Jpn. 1973;47:57–63.
20. Smith EE, Kubena LF, Braithwaite CE, Harvey RB, Phillips TD, Reine AH. Toxicological evaluation of aflatoxin and cyclopiazonic acid in broiler chickens. Poult Sci. 1992 Jul;71(7):1136-44.

21. Vaamonde, Graciela, Andrea Patriarca, Virginia Ferna´ndez Pinto, Ricardo Comerio, Claudia Degrossi. Variability of aflatoxin and cyclopiazonic acid production by Aspergillus section flavi from different substrates in Argentina. International Journal of Food Microbiology 88 (2003) 79 – 84
22. Vinokurova N.G., Ivanushkina N.E., Khmel'nitskaia , II, Arinbasarov M.U. Synthesis of alpha-cyclopiazonic acid by fungi of the genus *Aspergillus*. Prikl. Biokhim. Mikrobiol. 2007;43:486–489
23. Widiastuti,R. , R. Maryam, B. J. Blaney, Salfina, D. R. Stoltz Cyclopiazonic acid in combination with aflatoxins, zearalenone and ochratoxin A in Indonesian corn Mycopathologia December 1988, Volume 104, Issue 3, pp 153–156

4.5. Citrinin mycotoxicosis

- Citrinin is a nephrotoxic mycotoxin produced by several species of the genera Aspergillus, Penicillium and Monascus.
- Citrinin is generally formed after harvest and occurs mainly in stored grains, but also in other plant products such as beans, fruits, fruit and vegetable juices, herbs and spices, and also in spoiled dairy products.
- Citrinin is known to occur also as an undesirable contaminant in Monascus fermentation products (generally described as red mould rice (RMR)), which have been used in Asia for centuries for meat preservation and food colouring
- Citrinin is a potent nephrotoxin with hepatic and teratogenic activity.
- Citrinin causes "Balkan nephropathy" and a form of "Cardiac Beriberi" often known as yellow rice fever in humans
- Citrinin causes free radical damage to DNA
- Citrinin causes disruption of mitochondrial membrane-bound enzymatic activities as well as structural integrity.
- Citrinin inhibits specifically the electron transport chain (ETC) of mitochondria by inhibiting the NADH dehydogenase activity. dermal contact
- Citrinin in poultry feeds causes major economic loss to poultry sector.

.

Co-occurrence of citrinin with other mycotoxins

Co-occurrence of citrinin with other mycotoxins was observed, especially with ochratoxin A in grains and grain-based products, and with patulin in fruits and fruit and vegetable juices

Co-occurrence of citrinin and aflatoxins affect the productivity of broiler chicken by producing lesion in many organs, lowering the growth rate, feed conversion and resistance to infectious diseases by impairing both the cellular and humoral immunity of chicken which also leads to vaccination failures (**Coulombe, 1993**).

Co-occurrence of citrinin and ochratoxin, the twin mycotoxins usually produced by the same the fungi, causes major economic loss to poultry sector , both **are** nephrotoxic at relatively high doses in poultry feeds, causing swelling and eventual necrosis of the kidneys and affecting the function of liver at a lesser extent

Natural levels of CTN in poultry feed

- 40 and 4800 ppb (**Ahamad and Vairamuthu, 2000**)
- 1 ppb to 12 ppm (**Natrajan et al., 1999; kumar et al., 2005**),

Chemical structre

- Chemical formula and exact mass of CTN H2 and CTN H1 are C H O and 224.1049g and C H O and 412.1522g,
- Citrinin is a heat sensitive and decomposes during heat treatment to form other complex compounds such as citrinin H2 and citrinin H1 with higher and weaker cytotoxicity than the original citrinin, respectively.

1. Citrinin

Chemical Formula: $C_{13}H_{14}O_5$
Exact Mass: 250.0841
Molecular Weight: 250.2473

(3*R*,4*S*)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3*H*-isochromene-7-carboxylic acid

3. Citrinin H_1

Chemical Formula: $C_{24}H_{26}O_7$
Exact Mass: 412.1522
Molecular Weight: 412.4325

(2*R*)-1-((2*R*,3*S*)-5-hydroxy-2,3,4,10-tetramethyl-8,11-dioxo-3,8,11,11b-tetrahydro-2*H*-pyrano[4,3,2-*kl*]xanthen-9-yl)propan-2-yl formate

2. Citrinin H_2

Chemical Formula: $C_{12}H_{16}O_4$
Exact Mass: 224.1049
Molecular Weight: 224.2530

(2*R*,3*S*)-3-(3,5-dihydroxy-2-methylphenyl)butan-2-yl formate

Physico-chemical charateristics

- Citrinin is a crystalline lemon yellow compound with maximum UV-absorption at 250 and 333 nm (in methanol) a
- Citrinin is a phenol derivative or quinonemethine mycotoxin
- Citrinin is insoluble in cold water or slightly soluble in water, but fairly soluble in aqueous sodium hydroxide, sodium carbonate, ethyl alcohol, methyl alcohol and most other polar organic solvents
- Citrinin melting point is 175°C.
- Citrinin crystallizes in a disordered structure, with the p-quinone and o-quinone tautomeric forms in a dynamic equilibrium in the solid state
- Citrinin decomposition occurs at temperature greater than 175°C under dry conditions and also at temperature greater than 100°C in the presence of water

Toxicokinetics .

- **Manning *et al.* (1985)** gave broiler chicks citrinin and/or ochratoxin A for 3 weeks at 300 mg/kg feed or 3 mg/kg feed, respectively. Both toxins alone caused growth depression, and haematolological and histopathological changes. However, not only was there no evidence of additive or synergistic effects in combination, the combination even ameliorated aspects of the toxicoses.

- **Kumar et al. (2008)** made an exclusively-immunological study in rabbits given 15 mg citrinin/kg feed or 0.75 mg ochratoxin A/kg feed, or in combination, for 60 days. Whereas citrinin alone evoked little effect on cell-mediated immunity, in combination with ochratoxin A, it appeared to add to the well-known immuno-supressive characteristic of ochratoxin A. The absence of any comment on the animal"s health through the experiment indicates absence of adverse clinical effects of citrinin at 15 mg/kg feed. Kitchen et al. (1977b), in their experiments with dogs given ochratoxin A alone or with high intraperitoneal doses of citrinin (5 or 10 mg/kg b.w.) daily for up to 14 days, specifically concluded that „whether or not the increased mortality caused by the administration of combined toxins represents true synergism or only additive toxicity was not established".

- **Vesela et al. (1983)**, in their studies on chick embryos found additive but not synergistic effects of combining citrinin with ochratoxin A

- Toxic effects were not described in broiler chicks fed a diet containing 65 mg citrinin/kg feed (**Carlton, 1980).**
- In a study administering 0, 33, 65, 130 or 260 mg citrinin/kg feed to broiler chicks (n = 5 per group of Cobb x Cobb colour-sexed male and female chicks) from one day old for 4-6 weeks, diarrhoea was observed at the two highest concentrations. At necropsy these chicks had haemorrhages in the jejunum as well as enlarged livers and kidneys. Chicks fed lower dose levels appeared normal macroscopically. All dietary levels resulted in lymphocyte and eosinophil infiltrations of the liver, kidneys and pancreas. The authors interpreted their qualitative observations of anaplastic areas of the kidney and pancreas, observed at the highest concentration of 260 mg/kg citrinin as of being suggestive that citrinin may be a carcinogen in chickens **(Roberts and Mora, 1978)**.
- Citrinin was fed to broiler chickens at concentrations of 300 mg citrinin/kg feed from one day of age until 3 weeks, and the birds were sacrificed on day 21. Compared to controls, birds fed citrinin had a significantly ($p \leq 0.05$) lower body weight on days 14 and 21 and an increased water consumption on days 7, 14 and 21. At the last day of the study, serum protein, albumin and globulin were significantly higher in the birds fed citrinin than in controls Post-mortem investigations of these animals revealed mild renal structure changes, associated with proximal tubular intra-nuclear membrane-bound inclusions, misshaped mitochondria, as well as an increase in size and number of peroxisomes and secondary lysosomes. (**Manning *et al.,* 1985**).
- Birds fed the citrinin containing diet only to day 7, showed similar but milder changes Citrinin fed to mature laying hens at concentrations of 0, 50 or 250 mg/kg diet for three weeks had no effect on body weight, feed consumption, egg production, egg weight, or quality of eggshell. Moderate diarrhoea, which subsided once the birds returned to their normal diet, was observed after approximately three weeks at the highest dose level. Diet containing 0, 62.5, 125, 250 or 500 mg citrinin/kg feed given to broiler chicks from hatching to three weeks, resulted in a statistically significant decrease in body weight at the highest dose level. All dose levels resulted in enlarged kidneys, and a slight dose related increase in liver weight (**Brown *et al.,* 1986**).
- **Glahn and Wideman (1987)** evaluated the dose/time-response effects of citrinin given at two concentrations of 200 and 400 mg/kg in two experiments (unilateral renal portal infusion for comparing a non-infusion with an infusion period or with systemic i.v. infusion together with parathyroid hormone) in 12 and respectively 4 immature Single Comb White Leghorns (about 1kg b.w.). Controls were treated with an ethanol solution only. Examining urethral urine after a maximum of 90 min, showed effects in the urine flow, free water clearance, fractional sodium excretion and urine osmolality but no effects on glomerular filtration rates, fractional potassium excretion or fractionated inorganic phosphate excretion.
- In a follow up study, ten week old Leghorn pullets (n = 5 per group) were examined for renal function 10 days after administering 6 mg citrinin/kg b.w. Although citrinin induced effects on renal function (diuresis evident by higher urine flow rates, flow/glomerular filtration rate values and decreased osmolality), the duration of the effects was short (**Glahn et al., 1989**).

- **Abdelhamid and Dorra (1990)** examined the effects of a dietary concentration of 100 µg citrinin/kg feed in a six weeks feeding study, followed by two weeks recovery of 13 months old laying hens (Egyptian breed Mamourah). Citrinin-fed hens (three hens were checked after 6 weeks and another three after eight weeks) had enlarged spleen and enlarged reproductive organs. Investigating a large number of parameters (relative organ weights, blood parameters, chemical and physical characteristics in carcass muscles, liver characteristics, and bone minerals) the observed alterations in the citrinin group compared to controls were confined to changes in the relative organ weight of the adrenal glands, and compositional changes (fat/protein) in the liver, red and white muscle fibres. No renal lesions were visible during post mortem investigations. Residues of citrinin were found in eggs and muscles ranging from 6.2 to 10.6 ppb on fresh weight basis. This low concentration suggests a specific sensitivity of chickens towards citrinin, but has not been reported in other studies.
- **Mehdi et al. (1983)** reported LD50 values of 56 mg/kg b.w. and 57 mg/kg b.w. for turkey poults and ducklings, respectively. Citrinin fed via the diet to ducklings (from one day old for 15 days) at 100, 250 and 500 mg/kg feed was observed to be nephrotoxic at 250 and 500 mg/kg feed, with the effects noted to be more severe in the highest dose group. Decreased weight gain and feed consumption was reported at both dose levels, and at autopsy the kidneys were observed to be „swollen, pale and friable" with the tubular epithelial cells noted to be necrotic

Citrinin producing fungi

Citrinin producing Aspergillus species

1. Aspergillus alabamensis
2. Aspergillus carneus
3. Aspergillus niveus
4. Aspergillus ochraceus
5. Aspergillus. oryzae
6. Aspergillus terreus

Citrinin producing Monascus species

7. Monascus aurantiacus
8. Monascus floridanus
9. Monascus lunisporas
10. Monascus pallens
11. Monascus pilosus
12. Monascus purpureus

13. Monascus. ruber
14. Monascus sanguineus

Citrinin producing Penicillium species

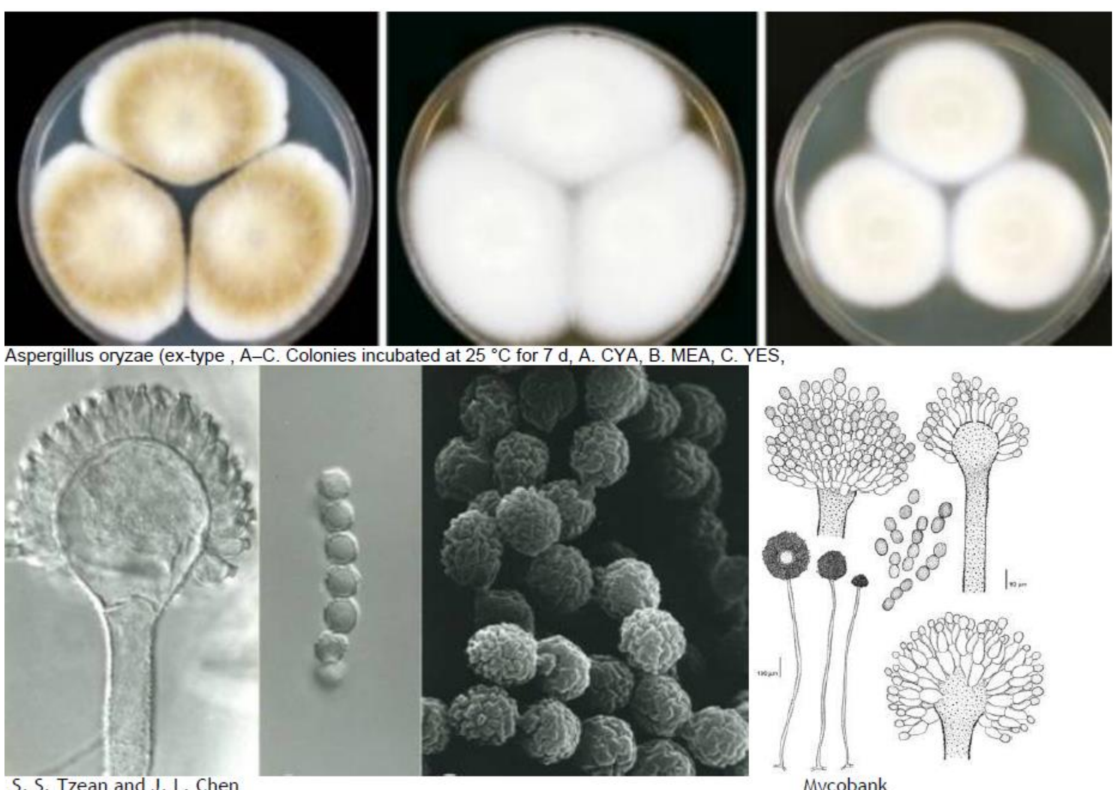
15. Penicillium camemberti
16. Penicillium chrzaszczii
17. Penicillium citrinum
18. Penicillium decaturense;
19. Penicillium expansum
20. Penicillium gorlenkoanum
21. Penicillium hetheringtonii;
22. Penicillium manginii;
23. Penicillium miczynskii
24. Penicillium. odoratum;
25. Penicillium radicicola;
26. Penicillium verrucosum
27. Penicillium westlingii
28. Pythium ultimum
29. Clavariopsis acquatica

Description of some Citrinin producing fungi

1. *Aspergillus oryzae* (Ahlb.) Cohn, 1884

Synonyms:
Eurotium oryzae Ahlb., 1878 *Aspergillus flavus var. oryzae* (Ahlb.) Kurtzman, M.J. Smiley, Robnett & Wicklow1986

Colony diameters on Czapek's Agar 4.5-5.5 cm in 10 days at 25°C, floccose; conidial heads radiate, or splitting into several loose columns, Kronberg's green to citron green; mycelium white; reverse cream color to mustard yellow and pale isabella color; soluble pigment light yellow; stipes smooth to rough, hyaline, 56-1160 × 6.4-20.6 µm; vesicles globose, subglobose, pyriform to somewhat elongate, 15.8-50.0 µm wide. Aspergilla uniseriate, biseriate, or both coexisting on the same vesicle, metulae covering the entire vesicle, 5.2-36.5 × 2.8-9.5 µm; phialides 4.0-14.3 × 2.8-7.1 µm, hyaline to light yellow; phialides of uniseriate aspergilla covering 1/2 to the entire surface of the vesicle. Conidia subglobose, rarely ellipsoidal or ovoid, 2.8-6.0 µm wide, with walls smooth to irregularly roughened. Colony diameters on Malt Extract Agar larger than 9 cm, floccose, in 10 days at 25°C; conidial heads enmeshed within the loosely aerial mycelium, ivy green to citron green, and olive-ocher to olive-yellow; mycelium white; reverse uncolored to pale buffy olive in center.

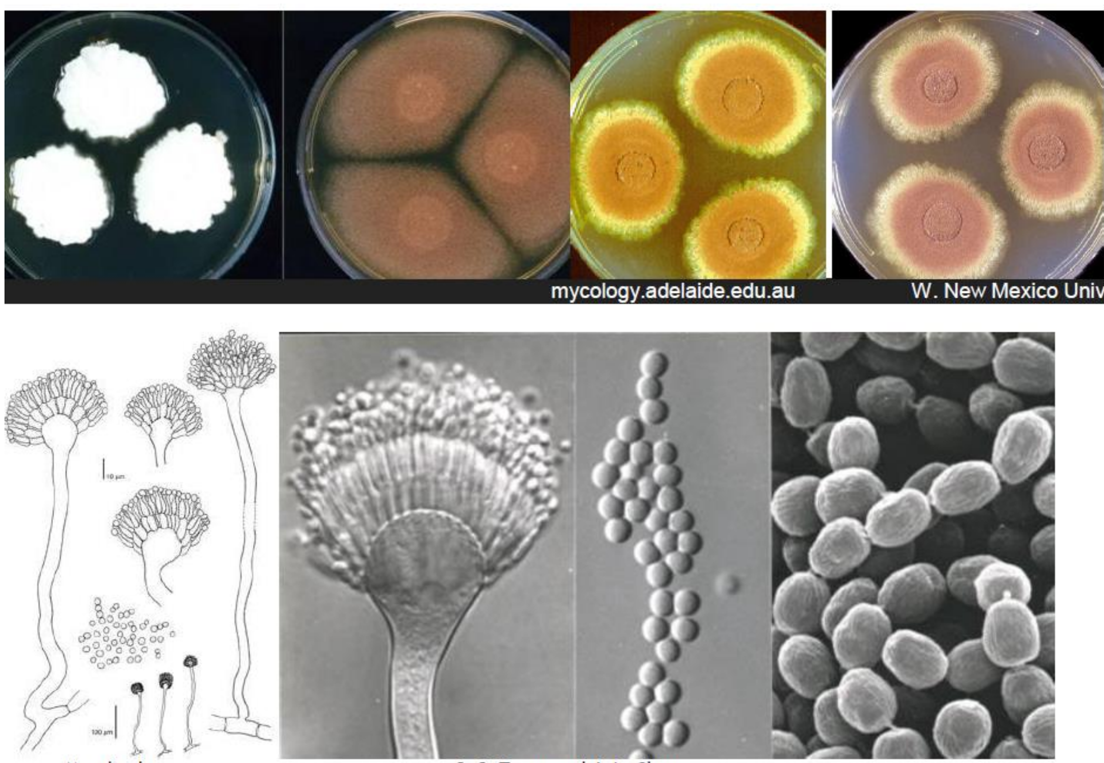

Aspergillus oryzae (ex-type , A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. YES,

S. S. Tzean and J. L. Chen Mycobank

2. *Aspergillus terreus* Thom, (1918)

Synonym:
Aspergillus terrestris

Colonies on potato dextrose agar at 25°C are beige to buff to cinnamon. Reverse is yellow and yellow soluble pigments are frequently present. Moderate to rapid growth rate. Colonies become finely granular with conidial production. Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle). Conidiophores are smooth-walled and hyaline, 70 to 300µm long, terminating in mostly globose vesicles. Conidia are small (2-2.5 µm), globose, and smooth. Globose, sessile, hyaline accessory conidia (2-6 µm) frequently produced on submerged hyphae. On Malt-Agar growth medium (MA) (initial pH 5) – Moderately fast growing colonies (reaching 78 cm in 21 days), velvet-like, white at first and then becoming cinnamon to brown-orange. The reverse is cream to slightly orangey. Emission of a yellowish pigment in the medium. The species slightly acidifies the medium (final pH 4).

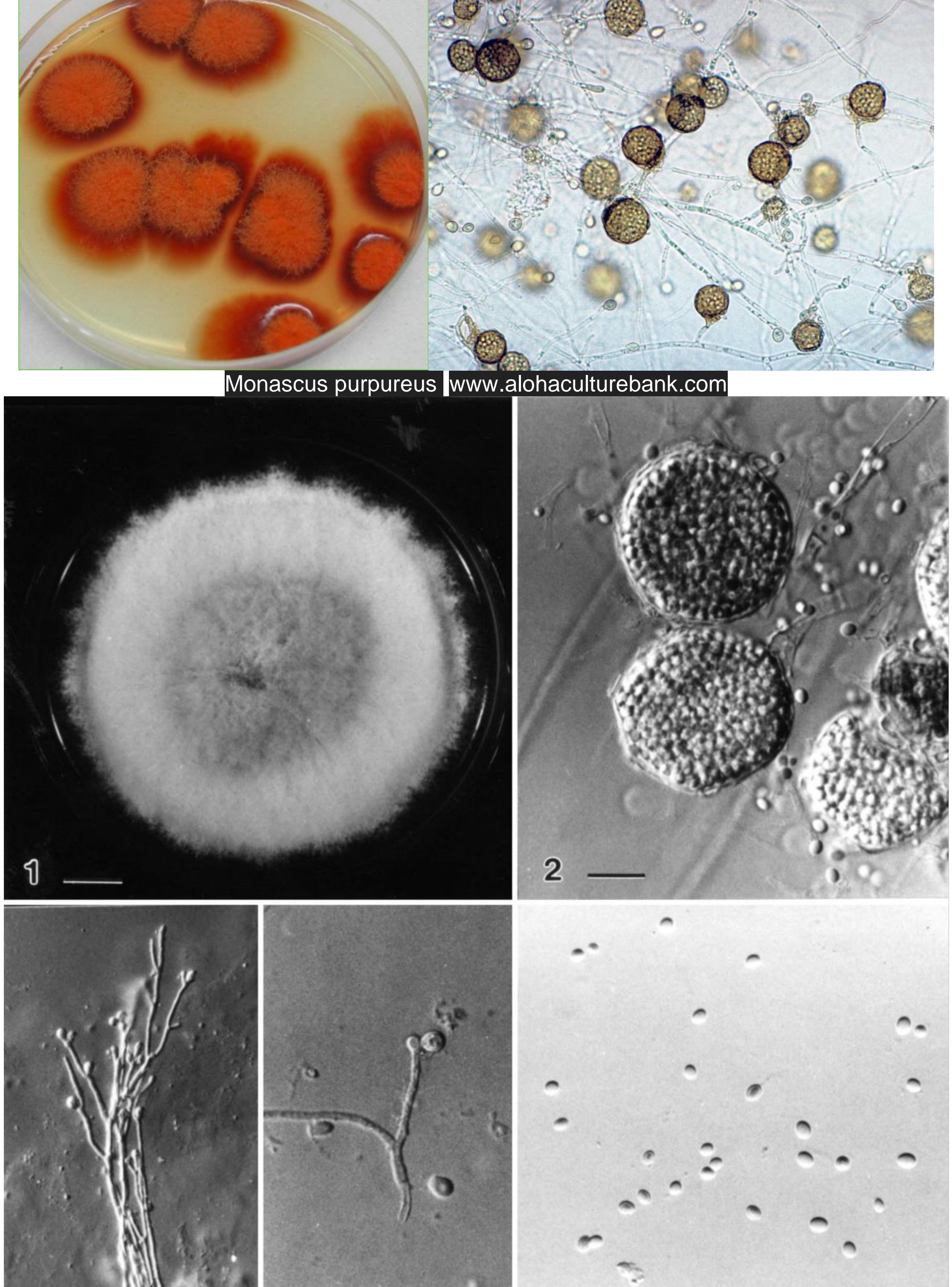


3. *Monascus purpureus* Went, Annales des Sciences Naturelles, Botanique, sér. 8, 1: 13, 1895.

Synonym

= Monascus aurantiacus Li, Acta Microbiol. Sin. 22: 118, 1982.

On MEA at 25°C for 7 d: colonies 18–20 mm diam., white at first, then turned to orange chrome, felted, aerial mycelium sparse and thin, substrate mycelium ivory yellow or pale cinnamon pink, reverse orange red. On CYA at 25°C for 7 d: colonies 25–35 mm diam., low and sparse, white at first, turning to orange yellow, reverse orange. On G25N at 25°C for 7 d: no growth. Hyphae septate, irregularly branched, hyaline initially, becoming pigmented as colonies mature (Fig. 1), in orange shades, guttulate, 3-6 µm wide. Mycelium bearing abundant initials and developing cleistothecia and aleurioconidia. Conidia singular or in short chains, mainly terminal on hyphae but sometimes lateral, obpyriform to globose, hyaline and distinctly truncate at the base, with thick, smooth walls, 6–10 × 5–8 µm (Figs. 3 & 4). Cleistothecia globose, arising on the top of stalk-like hyphae, 30–75 µm diam, hyaline, or appearing orange when pigmented, peridium hyaline, 1.5– 2 µm (Fig. 2). Asci evanescent at an early stage, the ascomata becoming filled by a compact mass of spores. Ascospores oval or broadly ellipsoidal, smooth, hyaline, 4–5.5 × 3– 4 µm



Monascus purpureus (from type culture of Monascus aurantiacus). 1. colony on MEA, 4 weeks. 2. Mature ascomata. 3 & 4. Condiophores and conidia. 5. Ascospores. Bars: 1 = 10 mm; 2 = 20 µm; 3 = 30 µm; 4–5 = 15 µm.

4. *Penicillium citrinum* Thom, U.S.D.A. Bureau of Animal Industry Bulletin 118: 61 (1910)

=Penicillium citrinum var. pseudopaxilli Martínez & Ramírez
=Citromyces subtilis Bainier & Sartory, Bulletin de la Société Mycol de France 28: 46 (1912)
=Penicillium aurifluum Biourge, La Cellule 33: 250 (1923)
=Penicillium phaeojanthinellum Biourge, La Cellule 33: 289 (1923)
=Penicillium sartoryi Thom, The Penicillia: 233 (1930)
=Penicillium sartorii Thom (1930)
=Penicillium botryosum Bat. & H. Maia, Anais da Sociedade de Biologia de

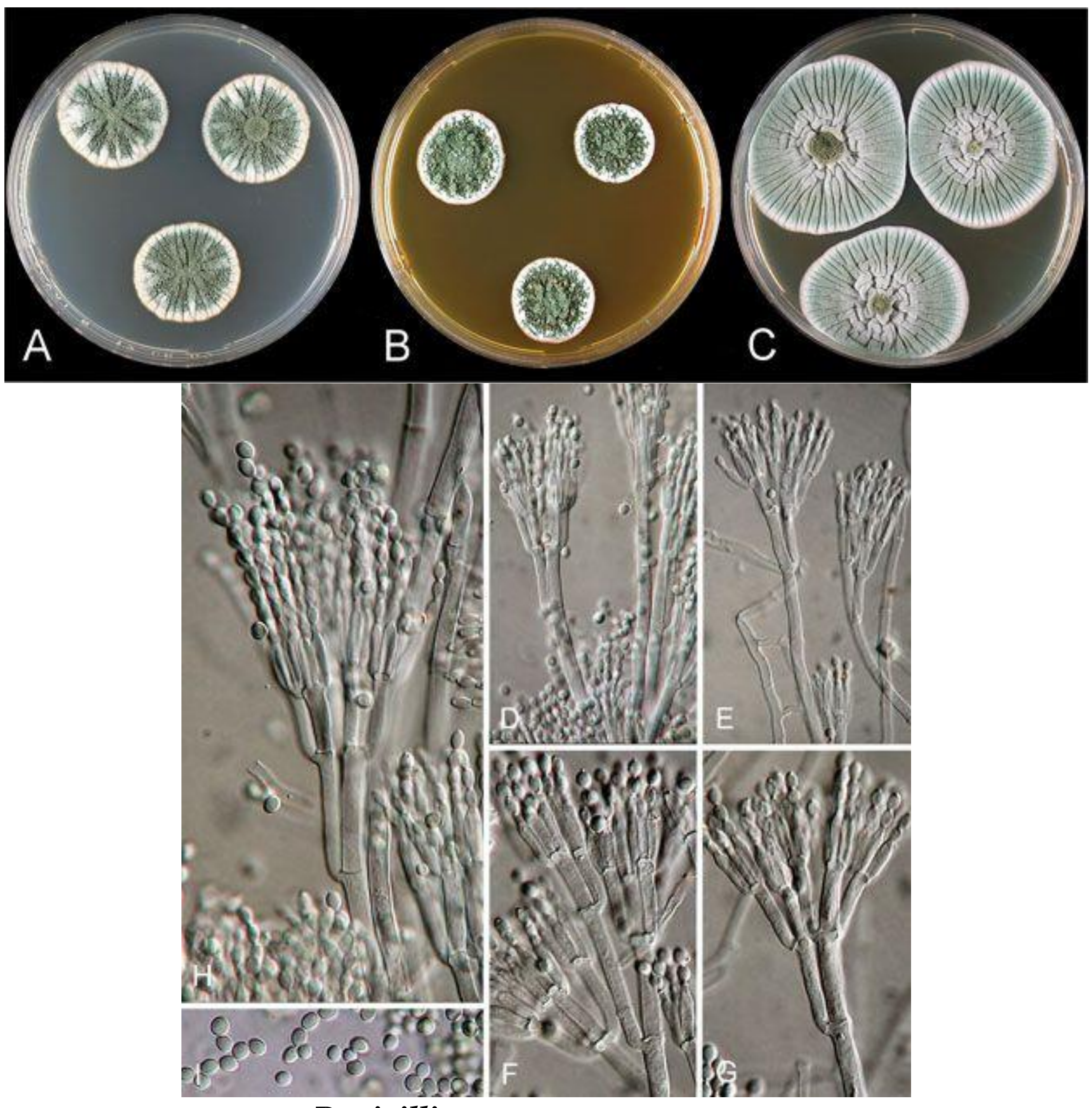
Colony characteristics. Colonies (CzA) with slow to moderate growth, velutinous to floccose; mycelium white to greyish-orange. Conidial masses greyish-turquoise; frequently a pale yellow to reddish-brown soluble pigment is produced. Exudate on MEA greyish-blue.Microscopy. Conidiophore stipes smooth-walled, 100-300 µm long; penicilli biverticillate. Metulae 12-15 µm long, divergent, in whorls of 3-5. Phialides flask-shaped, 7-12 µm long. Conidia spherical to subspherical, smooth-walled or finely roughened, 2.2-3.0 µm diam

P. citrinum thunderhouse4-yuri.blogspot.com

5. *Penicillium expansum* Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3 (1): 17 (1809)

=Coremium leucopus Pers., Mycologia Europaea 1: 42 (1822)
=Penicillium elongatum Dierckx, Annales de la Soci Sci de Bruxelles 25 (1): 87 (1901)
=Penicillium musae Weid., Zentbl. Bakt. ParasitKde, Abt II: 687 (1907)
=Penicillium variabile Wehmer, Mykol. Zentbl.: 195 (1913)
=Penicillium janthogenum Biourge, La Cellule 33: 143 (1923)
=Penicillium aeruginosum Demelius, Verhand Zool –Botan Gesellschaft Wien 72: 76 (1923)
=Penicillium plumiferum Demelius, Verhand Zool –Botan Gesellschaft Wien 72: 76 (1923)
=Penicillium kap-laboratorium Sopp, La Cellule 33: 454 (1925)

Colony characteristics. Colonies (CzA) rapidly growing, fasciculate to synnematal; conidial mass dull green; exudate and soluble pigment brown. Microscopy. Conidiophore stipes smooth-walled, 200-500 µm long; penicilli terverticillate. Metulae 12-18 µm long. Phialides closely packed, flask-shaped, tapering into a short, narrow neck, 8-11 µm long. Conidia ellipsoidal, smooth-walled, 3.0-3.5 µm long.



Penicillium expansum Mycobank

Reports:

Occurrence of citrinin in grains

Scott *et al.* (1972) reported that, 13 of 29 grain samples were found to contain citrinin (0.07 to 80 mg/kg). The contaminated samples included wheat, oats, barley and rye. All samples positive for citrinin were also contaminated with ochratoxin A.

Reddy et al. (1983) found citrinin in six out of 18 parboiled rice samples from Andhra Pradesh, India, with concentrations ranging from 12 to 55 µg/kg. No citrinin was detected in maize (n = 30), sorghum (n = 20), ragi (n = 37) or broken rice (n = 32).

Nishijima (1984) investigated 27 samples of grains for food use taken in the Tokyo metropolitan area and did not detect citrinin.

Dick et al. (1988) found citrinin in two out of four samples of durum wheat with a concentration of 0.3 and 0.7 µg/kg (LOD = 0.1 µg/kg).

Petkova-Bocharova *et al.* (1991) reported a contamination frequency for citrinin in stored maize from endemic and nonendemic areas of 27-44 % and 10-15 %, respectively. The concentrations of citrinin ranged from 50 to 1500 µg/kg for endemic and from 50 to 380 µg/kg for nonendemic areas.

El-Sayed (1996) found citrinin in 56 % of barley samples (n = 27) in Egypt with an average concentration of 64.4 µg/kg, in 8.3 % of yellow maize samples with an average concentration of 62.9 µg/kg, and in 39 % of rice samples with an average concentration of 13.8 µg/kg. Citrinin was not detected in wheat samples.

Scudamore *et al.* (1997) reported that 48 of 141 cereal samples for feed use were found to be contaminated with citrinin with a maximum of 10 µg/kg (LOD = 1µg/kg).

Janardhana *et al.* (1999) collected 197 maize grain samples and, using TLC, found citrinin in only one sample, with a citrinin concentration of 12 µg/kg.

Vrabcheva *et al.* (2000) monitored incidence of ochratoxin A and citrinin in cereal samples intended for use as food and feed from villages in Bulgaria where BEN had occurred. Samples were analysed for citrinin using an ELISA with an LOD of 5 µg/kg. Two of the 3 citrinin-positive wheat samples (37 samples) were also positive for ochratoxin A, and citrinin concentrations were two to 200 times higher than those of ochratoxin A. A maximum citrinin concentration (420 µg/kg) was found in a wheat sample intended for human consumption having also the highest ochratoxin A content (39 µg/kg). Citrinin and ochratoxin A were not detected in barley (6 samples) intended for feed use. Also oats (n = 9) intended for feed use did not contain citrinin in detectable amounts, although ochratoxin A was detected at concentrations up to 140 µg/kg. Maize (n = 23) was found to be free of ochratoxin A and citrinin.

Odhav and Naicker (2002) reported that none of the grain samples (n = 30) for beer production in South Africa contained citrinin

Abd-Allah and Ezzat (2005) detected citrinin in 10 out of 30 samples of rice in Egypt in concentrations between 2.74 and 28.54 µg/kg

Aziz *et al.* (2006) found citrinin in 5 out of 70 samples of grains at concentrations from 100 to 300 µg/kg.

Nguyen *et al.* (2007) investigated the occurrence of 3 mycotoxins (aflatoxin B1, citrinin and ochratoxin A) in rice samples (n = 100) collected from 5 provinces of the central region of Vietnam, using HPLC with fluorescence detection (LOD = 0.11 and LOQ = 0.35 µg/kg). Citrinin was detected in 13 % of the samples at concentrations up to Citrinin in food and feed EFSA Journal 2012;10(3):2605 15 0.42 µg/kg. These samples were collected during the rainy season. No citrinin was found in rice in the dry season.

Kononenko and Burkin (2008) detected citrinin in grains for feed use (LOD = 10 µg/kg). Wheat (n = 43), barley (n = 138) and maize (n = 157) contained citrinin in 5 %, 4 % and 2 % of the samples, with maximum values of 144, 998, and 953 µg/kg respectively. Samples of wheat (n = 25) and maize (n = 30) for animal consumption, that were collected in 1997 from Western Romania were analysed for mycotoxin contamination by Curtui et al. (1998). Citrinin was only found in one maize sample at a concentration of 580 µg/kg.

Tabata *et al.* (2008) investigated citrinin in grains for food use with LC-MS/MS. Citrinin was detected in one wheat sample at a concentration of 0.19 µg/kg, together with ochratoxin A, and in two buckwheat samples at concentrations of 0.55 and 0.62 µg/kg, also with ochratoxin A.

Polisenska *et al.* (2010) analysed 11 wheat samples (for food use) from the Czech Republic shortly after harvest. There was only one sample positive for citrinin, which had a low content not exceeding the LOQ (1.5 µg/kg). The same sample had an ochratoxin A content of 4.7 µg/kg. The authors also analysed three barley samples destined for malt production. One of the samples was offered to a malt house but not accepted due to a higher content of admixtures and impurities and a mouldy smell. This sample contained the highest citrinin content (93.6 µg/kg) and also contained ochratoxin A (31.4 µg/kg). Barley (n = 6) and wheat (n = 11) for feed use were also analysed by these authors and citrinin was found in only 3 barley samples (up to a concentration of 13.2 µg/kg).

Occurrence of citrinin in feed other than grains

Scudamore et al. (1997) analysed animal feed from the UK in 1992. Rice bran, maize products, cottonseed meal, rapeseed, sunflower, olive pulp, palm products, soya, peas and beans, manioc and 9 Mean ± standard error of the mean. Citrinin in food and feed EFSA Journal 2012;10(3):2605 20 citrus pulp (LOD = 5-20 µg/kg) were analysed. Citrinin was detected in one sample of palm kernel meal at a concentration of 7 µg/kg, together with ochratoxin A and aflatoxins, and in one sample of peas/beans at a concentration of 9 µg/kg together with ochratoxin A.

Vrabcheva *et al.* (2000) analysed 24 Bulgarian wheat bran samples (LOD = 5 µg/kg) of which 5 contained citrinin (5 to 230 µg/kg) and ochratoxin A.

Kononenko and Burkin (2008) detected citrinin in feed samples and ingredients (LOD = 10 µg/kg). Out of 829 compounded feeds 8.8 % were positive for citrinin with concentrations in the range of 12 to 182 µg/kg. The highest incidence (28.9 %) was found for sunflower oil-seed meal and cakes with concentrations in the range of 14-397 µg/kg. Also soy-bean samples (2 %), maize gluten samples (16 %) and one wheat bran sample (3 %) contained citrinin at concentrations in the range 14-62 µg/kg.

Talmaciu *et al.* (2008) studied the occurrence of citrinin in feed samples originating from industrial and family-owned farms from Romania in 2007. All samples contained citrinin in the range 17 to > 405 µg/kg with 25 % of the samples containing more than 405 µg/kg.

Stoev *et al.* (2010) analyzed feed samples from pig and chicken farms in Bulgaria that had reported incidences of nephropathy in the livestock (enlarged and mottled or pale appearance of kidneys at slaughter time) were analysed for their mycotoxin

content in 2006 and 2007. Citrinin was found in 92 % and 96 % of the samples, respectively with mean concentrations of 54.7 ± 27.59 µg/kg and 120.5 ± 43.3 µg/kg. Besides citrinin, also other mycotoxins were observed, including ochratoxin A (in 2006: 188.8 ± 27.3 µg/kg and in 2007: 376.4 ± 63.9 µg/kg), penicillic acid (in 2006: 838.6 ± 223.9 µg/kg and in 2007: 904.9 ± 86.5 µg/kg), fumonisin B1 (in 2006: 5564.1 ± 584.4 µg/kg and in 2007: 3254.5 ± 480.6 µg/kg), deoxynivalenol (in 2006: 72.7 ± 18.8 µg/kg and in 2007: 51.4 ± 8.5 µg/kg), penitrem A (in 2006: 1840.4 ± 243.8 µg/kg and in 2007: 713.9 ± 88.2 µg/kg) and zearalenone (in 2006: 133.2 ± 15.5 µg/kg and in 2007: 108.2 ± 9.9 µg/kg)

In France three studies were carried out on the occurrence of mycotoxins in maize silage for dairy cattle. It was observed that the highest citrinin concentrations occurred at the bottom of the silage (average concentration up to 36.6 ± 2.3 µg/kg dry matter). In addition to citrinin, deoxynivalenol, aflatoxin B1, gliotoxin and zearalenone were also detected (Garon et al., 2006; Richard et al., 2007; 2009).

Experimental feeding of citrinin

Ames *et al*. (1976) fed the mature laying hens by CTN @ 0, 50 and 250 mg kg diet for about 3 weeks They found no effect on body weight; feed consumption; egg weight and quality of eggshell. But when diet comprised of 0, 62.5, 125, 250 and 500 mg CTN kg feed was given to them from hatching to 3 weeks, it resulted a significant decrease in body weight at 500 mg CTN kg, while all dose levels also resulted in enlarged kidney and a slight dose resulted an increase in liver weight. In another set of the same experiment, they stated that CTN @ 250 and 500 mg kg feed resulted in a dose dependant increase in water consumption followed by acute diarrhea.

Roberts and Mora [1978] administered various amounts of CTN in per kg feed to broiler chicks for about 4-6 week**.** Diarrhoea was observed at the two highest CTN levels viz., 130 and 260 mg kg feed. At necropsy these experimental chicks had haemorrhages in the jejunum as well as enlarged livers and kidneys. They also interpreted their qualitative observations of anaplastic areas of the kidneys and pancreas observed at the highest concentration of CTN as of being suggestive that CTN could be a carcinogen in chicks.

Wyatt [1979] reported that nephrotoxicity and hepatotoxicity occurs in chickens at dietary levels of 250 g g of CTN with liver and kidney enlargements of 11 and 22%, respectively. Serum sodium levels are also changed. He/She further stated tha necroscopy of affected birds revealed the presence of pale and swollen kidneys. However, toxic effects were not observed in broiler chicks fed with a diet containing 65 mg CTN kg feed.

Mehdi *et al.* (1981) administered citrinin to chickens by crop gavage in dimethylsulphoxide-70% ethanol (3:1, v/v) or mixed with the diet (commercial starter mash) in four separate trials. The single-dose oral LD_{50} value in 7-day-old male broiler chicks was found to be 95 mg/kg. The administration of seven daily doses of citrinin equal to a half and three-quarters of the single-dose LD_{50} produced no

additive toxic effects. The development of tissue lesions was studied in chicks killed sequentially after administration of the LD_{50} in a single dose. Watery diarrhoea and increased water consumption were the most consistent signs of citrinin mycotoxicosis. Histopathological alterations were most prominent in the kidneys and included degeneration and necrosis of tubular epithelial cells of both the proximal and distal convoluted tubules. Hepatic lesions were multiple foci of necrosis and haemorrhage and the severity was correlated with citrinin dose. Lymphoid necrosis and depletion were found in the bursa of Fabricius, thymus, caecal tonsil and spleen in birds given a single and multiple oral doses of citrinin. When chickens were given 100, 250 or 500 ppm citrinin in the diet for 3 wk they showed the clinical signs of citrinin mycotoxicosis and their kidneys were enlarged but no histopathological alterations were found.

Vesela *et al.* (1983) studied the embryotoxic potential of ochratoxin A and citrinin after administering, either subgerminally or intraamniotically, single mounting doses of the mycotoxins to chicken embryos on days 2, 3, and 4. The beginning of the embryotoxicity dose range was found to be between 0.01 to 0.05 microgram for ochratoxin A and 1 to 10 micrograms for citrinin. The maximum response to both mycotoxins occurred after administration on day 3. In addition to significant growth retardation of fetuses, exencephaly, microphthalmia, cleft beak, reduction deformities of the limbs, and abdominal wall and ventricular septal defects were encountered on day 8 of incubation. When 4 micrograms of citrinin was constantly added to ochratoxin A administered in the dose range of 0.03 to 0.5 microgram, a strictly additive effect was seen. It may be supposed that citrinin produced together with ochratoxin A in some strains of Penicillium viridicatum Westling does not potentiate the clear-cut embryotoxic action of the latter mycotoxin.

Manning *et al.* (1985) fed citrinin (CTN) and ochratoxin A (OA) alone and in combination to broilers from day of hatch until 3 weeks of age. Dietary concentrations of 300 micrograms CTN/g and 3.0 micrograms OA/g were used. Birds fed CTN had significantly (P less than or equal to 0.05) lower body weights than controls on days 14 and 21 and increased water consumption on days 7, 14, and 21. Birds fed OA had significantly lower body weights than controls on days 7, 14, and 21 and increased water consumption on day 14. Birds fed CTN and OA in combination had lower body weights than controls and increased water consumption during the experiment, but the alterations were intermediate in severity when compared with those in birds fed CTN or OA alone. Birds fed OA alone or combined with CTN had higher liver and kidney weights than controls, but birds fed CTN alone had only higher kidney weights. Birds fed both CTN and OA had concentrations of serum constituents similar to those in birds fed OA alone, except the levels of cholesterol and triglycerides were not significantly different from those in the controls. Histological evaluation of the kidney indicated no lesions in birds fed CTN alone, but birds fed OA, alone or in combination with CTN, had increased tubular casts and tubular hyperplasia compared with controls. These data suggest that there were no additive or synergistic toxic interactions when 300 micrograms CTN/g and 3.0 micrograms OA/g were fed simultaneously to broiler chicks for 3 weeks. However, the severe growth depression resulting from OA and the increased water consumption associated with CTN toxicosis were ameliorated when CTN and OA were fed in combination. These data may be useful in diagnosing field cases of mycotoxicosis where both CTN and OA are involved.

Brown *et al.* (1986) fed layer chicks 3.0 micrograms of ochratoxin A (OA) and 300 micrograms of citrinin (CTN) per gram of feed, alone and in combination, were evaluated for changes in renal ultrastructure. Feeding OA from 0 to 21 days of age was associated with proximal tubular intranuclear membrane-bound inclusions, elongated tortuous and ring-shaped mitochondria, enlarged mitochondrial matrix granules with hyaline centers, and an increase in number and size of peroxisomes and secondary lysosomes. Birds fed OA from 0 to 7 days and then given untreated ration had similar changes but to a lesser degree. Feeding CTN from 0 to 21 days of age was associated with similar proximal tubular nuclear inclusions, elongated tortuous and ring-shaped mitochondria, and an increase in size and number of peroxisomes and secondary lysosomes. Hyalinized mitochondrial matrix granules were not present, and some proximal tubular cells had cytoplasmic aggregates of smooth endoplasmic reticulum. Birds fed CTN from 0 to 7 days had similar but milder changes. Birds fed CTN + OA for 21 days had changes similar to those fed OA alone and also had cytoplasmic aggregates of smooth endoplasmic reticulum similar to those of CTN-fed birds. Again, changes in birds fed CTN + OA for 7 days were similar but milder.

Hanika *et al.* (1986) gave citrinin to rabbits as a single oral dose of 120 or 67 mg/kg. Rabbits were killed at 4, 6, 8, 10, and 12 hours post dosing, and the kidneys were fixed by intravascular perfusion. Ultrastructural alterations were evident by 4 hours after treatment. In the proximal tubule, alterations were brush border disruption, cytoplasmic rarefaction, and swelling of interdigitating processes. At higher doses, mitochondria were condensed and distorted. Medullary and straight cortical distal tubules had marked distention of the intercellular spaces and disorganization of interdigitating processes. Changes in cortical and outer medullary collecting ducts were similar but less severe. Renal alterations were suggestive of damage to membrane structure and/or transport functions and interference with cellular bioenergetics. Leukocytic infiltration was associated with damaged tubules indicating a contribution of inflammation to the development of the lesions

Glahn *et al.* (1988) designed a study to evaluate the acute effects of ochratoxin A (OA) on pullet renal function, and to determine if the diuretic effects of citrinin are altered by acute ochratoxicosis. Birds were injected intramuscularly with a 1-mg/mL solution of OA at a dose of 5 mg/kg body weight for 2 consecutive days. Control birds received an equal volume injection of the OA carrier vehicle (100% ethanol). On the 3rd day, birds were anesthetized and prepared for renal function studies. Following 30 min of control urine and plasma collection, an intravenous infusion containing 400 ppm citrinin was initiated, and urine and plasma were collected for an additional 70 min. The OA alone caused an increase in manure moisture and increased hematocrits (hemoconcentration), but direct effects on glomerular filtration rate (GFR), urine flow rate/GFR, para-aminohippuric acid clearance (CPAH), free water clearance (FWC), and electrolye excretion (Na, K, Ca, P) were not consistently demonstrated. The OA may cause hemoconcentration by causing a subtle increase in urine flow (diuresis), or by increasing intestinal water loss (diarrhea). Citrinin infusion caused increased urine flow rate, increased urine flow rate/GFR, increased FWC, increased Na excretion, and decreased urine osmolality. Pretreatment with OA attenuated these effects of citrinin, presumably due to renal compensation for the OA-induced hemoconcentration. Citrinin and OA do not appear to have additive diuretic effects during the first 48 h of toxin exposure.

Abdelhamid ***et al.*** **(1990)** offered four groups (each of 8 laying hens plus one cock) commercial laying mash contaminated with 100 ppb of aflatoxins, citrinin, patulin or uncontaminated (control) for 6 weeks. The mycotoxin-contaminated diets led to some significant changes in egg characteristics and composition such as ash and calcium contents of the egg shell. The noticeable changes including also the relative weights of adrenal glands. Blood profile reflected too alterations (P greater than 0.05) in urea content and activity of both glutamic oxaloacetic transaminase and alkaline phosphatase as well. The mycotoxins affected significantly moisture and fat contents of the red muscle and protein content, texture and percentage of lean meat in both types of muscles (red and white). Patulin toxicosis was responsible for the strongest alterations in moisture, fat and vitamin A contents of the laying hen's liver and for the lowest calcium content of egg shell besides the shape alteration of the eggs. Laying hens fed on aflatoxin-contaminated diet produced hatched chicks with higher weight (P less than or equal to 0.05) than those from the controls. Citrinin residues were 10 ppb in the fresh muscles and egg yolk and 6 ppb in egg white.

Stoev ***et al.*** **(2004)** studied the combined toxic effect of ochratoxin A (OTA) and penicillic acid (PA) on the body mass, the weight and pathomorphology of some internal organs in 85 broiler chickens fed a mouldy diet containing 130, 300 or 800 ppb OTA and 1000-2000 ppb PA. The main pathomorphological changes were cloudy swelling and granular degeneration in the epithelium and mononuclear cell proliferation and activation of capillary endothelium in the kidney and liver; degenerative changes and depletion of lymphoid cells in lymphoid organs (bursa of Fabricius, thymus and spleen) were also seen. Protective effects of 5% total water extract of artichoke and a new natural phytosubstance Rosallsat against these pathomorphological changes were observed. A significant decrease in body mass and relative weight of lymphoid organs was found after 6 weeks of exposure and a greater decrease after 10 weeks of exposure to OTA and PA, and a protective effect of artichoke extract and a slight effect of Rosallsat against that decrease was observed. A significant increase in relative weight of liver and kidneys was also observed as well as a protective effect of artichoke extract against that increase. The quantity of OTA and the percentage of positive samples were significantly lower in tissues of chickens treated with artichoke extract or Rosallsat in addition to OTA than in those treated with only OTA.

AHAMAD ***et al.*** **(2006)** studied tha clinico-pathological effects of citrinin (Cln a nephrotoxin and aflatoxin B1 (AFB1), a hepatotoxin in broiler chicken by giving mycotoxins free feed to group A, 150.0 ppm of CIT to group B, 0.5 ppm of AFB1 to group C and combination of these two mycotoxins to group D. The body weight was very poorly pronounced in CIT fed group birds compared with other group birds. A significant decreased feed intake was recorded in CIT (group B) and CIT with AFB1 combined toxins (group D) fed birds compared with other groups. AFB1 alone (group C) and combined toxins (group D) fed birds showed a significant decrease in haematological and serum biochemical values. Significant enlargement of kidneys in group B and hepatomegaly in group C and D was recorded when compared with that in controls. However, in combined toxins fed birds (group D), additive or synergisticeffect of these two toxins was not much appreciated.

Kumar ***et al.*** **(2007)** fed ochratoxin A (OTA) (0.75 mg/kg feed) and citrinin (CIT) (15 mg/kg feed) alone and in combination to young growing New Zealand White rabbits for 60 days to evaluate renal ultrastructural alterations. The severity and

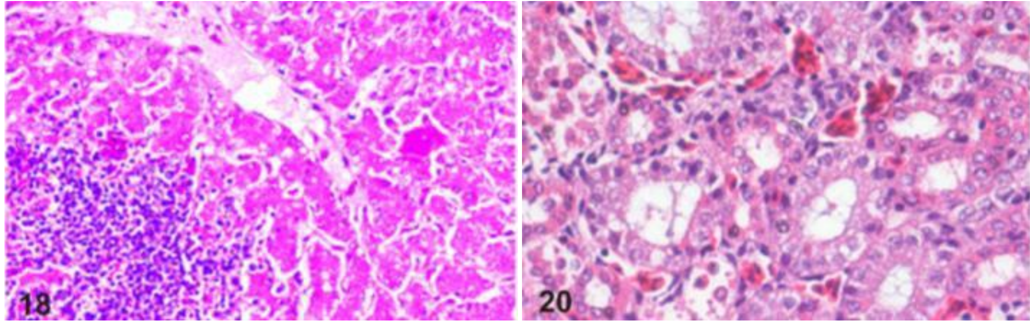
intensity of renal ultrastructural changes varied with the type of the treatment, and predominant and consistent lesions were recorded in the proximal convoluted tubule (PCT) lining cells. The significant changes in mitochondria, the most affected cell organelle in all the treatment groups, included mitochondrial disintegration and distortion, pleomorphism, cluster formation and misshapen appearance such as signet ring, dumbbell, cup and U shapes. Intra-cisternal sequestrations of involuting mitochondria, and thickening of basal layer of PCT epithelial cells with partial detachment, were the characteristic features observed in OTA and combination treatments. CIT treatment revealed crenated nucleus, loss of nucleolus, depletion of cytoplasmic organelles, mitochondrial pleomorphism, nuclear fragmentation, uniform folding of cell membrane and cytoplasmic vacuolations in the PCTs. Focal thickening of the glomerular basement membrane and degeneration of endothelial cells were the prominent alterations in the glomeruli in OTA and combination treatments. Distal convoluted tubules were unaffected in CIT treatment, however, mild to moderate lesions were observed in OTA and combination treated rabbits. It may be concluded that on simultaneous exposure, CIT potentiated the toxic effects of OTA on renal ultrastructure.

Manafi and Bagheri (2011) carried out a study to investigate the combination effect of two mycotoxins in blood parameters of commercial broiler chicks (0-5 weeks) at 7, 14, 21, 28 and 35 days of post intoxication. The broiler chickens were divided into four groups of 40 birds each. Control (group I), OA (1ppm, group II), CTN (12.5ppm, group III) and combination (1ppm OA plus 12.5ppm CTN, group IV) were given in feed up to 35 days of the trial and the control (group I) was fed standard toxin free feed. The levels of blood urea nitrogen (BUN), serum creatinine, uric acid, ALP, AST, ALT and serum triglyceride increased significantly in all the toxin treated groups. However, biochemical alternations were maximum in the combination group than the individual toxin treated group. The interaction of both the toxins was found to be additive.

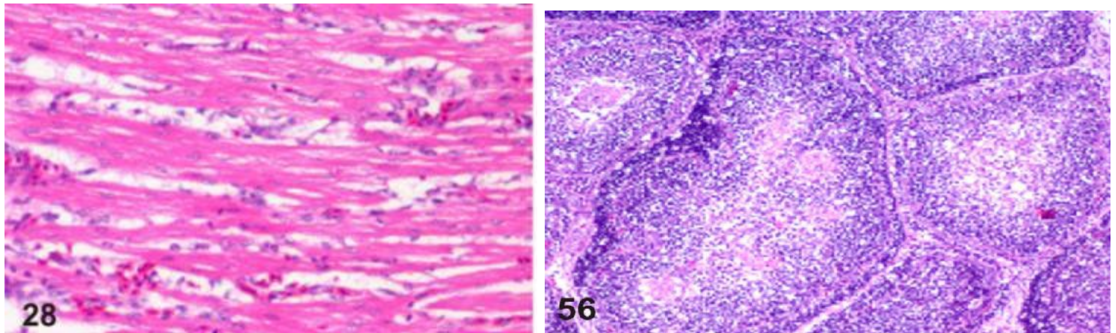
EFSA (2012) reported that Citrinin is a mycotoxin produced by several species of the genera Aspergillus, Penicillium and Monascus and occurs mainly in stored grains. The available occurrence data were not adequate to carry out a dietary exposure assessment. Citrinin is nephrotoxic and a no-observedadverse-effect level (NOAEL) of 20 µg/kg body weight (b.w.) per day was identified from a 90-day study in rats. Due to the limitations and uncertainties in the database, the derivation of a health-based guidance value was not considered appropriate but a level of no concern for nephrotoxicity of 0.2 µg/kg b.w. per day was determined. Based on the available data a concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity. In the absence of adequate exposure data, characterisation of the risk of citrinin as a food contaminant was based on the estimate of the citrinin concentrations in grains and grainbased products that would result in an exposure equal to the level of no concern for nephrotoxicity. For high consuming toddlers, other children and adults this citrinin concentration is between 9 and 53 µg citrinin/kg and between 19 and 100 µg citrinin/kg for average consumers, respectively. For animals, risk characterisation was based on the estimate of the citrinin concentration in grains that would result in exceedance of the NOAEL of 20

µg/kg b.w. per day for pigs, which ranged between 640 and 1 173 µg/kg. The CONTAM Panel concluded that the impact of uncertainties on the risk assessment is large, and more data regarding the toxicity and the occurrence of citrinin in food and feed in Europe are needed to enable refinement of the risk assessment. © European Food Safety Authority, 2012

Jayaramu *et al.* (2013) conducted a study to evaluate the effect of feeding ochratoxin A and citrinin either alone or in combination in broiler chicken. Two hundred broiler chicks were divided into four groups of 50 chicks each with the following treatment viz. Control diet, (group I), OA 1 ppm, (group II), CTN 12.5 ppm (group III) and combination 1 ppm OA plus 12.5 ppm CTN (group IV) up to 35 days of the trial. The experimental and the control birds were sequentially sacrificed and examined at 7, 14, 21, 28 and 35th day of the experiment. On post-mortem examination grossly, the toxin fed birds showed congestion, enlargement, pallor or yellowish discoloration of liver with distended gall bladder, swollen and congested kidneys. In addition, congestion of heart with prominent vasculature, pale, dehydrated and shrunken skeletal muscles, presence of small quantity of semisolid ingesta with slight mucous in crop and proventriculous, dry and shrunken gizzard, congested appearance of intestine with small quantity of mucous and congested pancreas was observed in all the toxin fed groups throughout the period of experimentation. Microscopically degenerative changes in hepatocytes, periportal fibrosis, periductular mononuclear cell infiltration, fatty degeneration, focal necrosis in the liver, degeneration and necrotic changes in the tubular epithelial cells in kidneys, myocardial degeneration, hyaline degeneration of muscle, mucosal hyperplasia of crop, proventriculitis, ventriculitis, catarrhal enteritis, pancreatitis, lymphoid depletion in the spleen, bursa of Fabricius and thymus were the prominent lesions observed when both the toxins were fed to birds from second to fifth week of age. Severity of these lesions was found to be enhanced and suggested the additive or synergistic effect of these toxins in the broiler chicken.



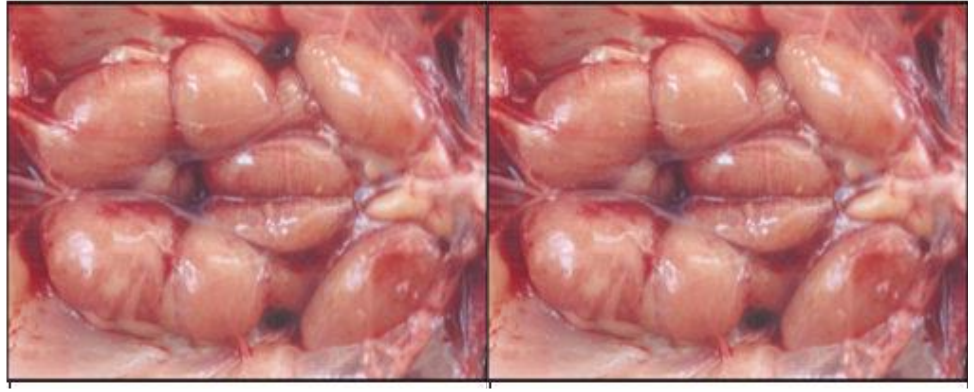
1. Section of Liver from OA and CTN fed bird at 28 days of age showing focal areas of hydrophic degeneration, fatty change and necrosis with infiltration of lymphoid cells. **2**. Section of Kidney from OA fed bird at 28 days showing congestion, haemorrhages, swollen and vacuolated tubular epithelialium, loss of brush border, desquamation of epithelial cells into the tubular lumen and presence of proteinaecious casts in the lumen. **Jayaramu *et al.* (2013)**



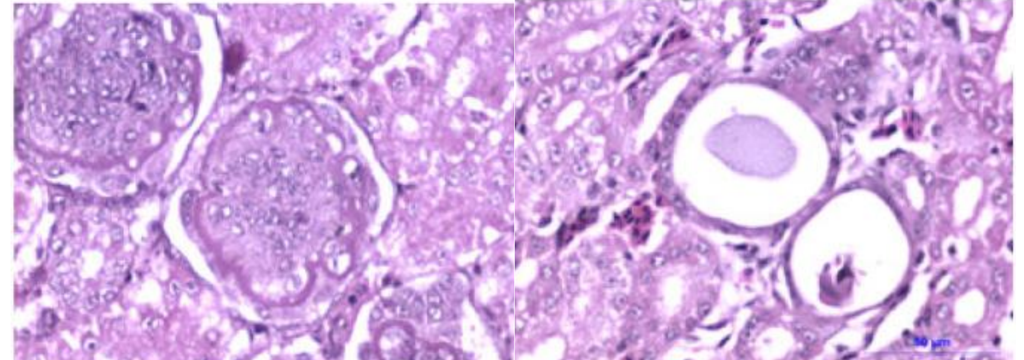
3. Section of heart from OA fed bird at 28 days of age showing edema, haemorrhage, separation and disruption of cardiac fibres with loss of cross striation. 4. Section of bursa of Fabricius from CTN fed bird at 28 days of age showing severe lymphocytolysis with histiocytosis giving starry sky appearance.
Jayaramu *et al.* (2013)

Klarić *et al.* (2014) mentioned that only a limited number of surveys showed that OTA co-occurs in food with mycotoxins (citrinin-CIT, penicilic acid, fumonisin B_1-FB_1, aflatoxins-AF) which exert nephrotoxic, carcinogenic or carcinogen-promoting activity. This review summarises the findings on OTA and its co-occurrence with the mentioned mycotoxins in food as well as experimental data on their combined toxicity. Most of the tested mycotoxin mixtures involving OTA produced additive or synergistic effects in experimental models suggesting that these combinations represent a significant health hazard. Special attention should be given to mixtures that include carcinogenic and cancer-promoting mycotoxins.

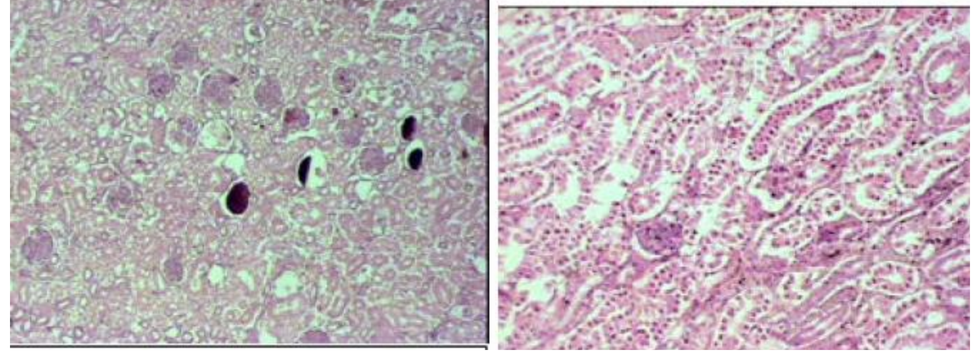
KUMAR *et al.* (2014) studied the individual and combined pathological effects of citrinin (CTN) at 5 ppm and aflatoxin (AF) at 0.5 ppm in broiler chicken by feeding the mycotoxins from 0 to 6 weeks of age. In the entire toxin fed groups, inappetance and brownish diarrhoea were observed from first week onwards. The AF and CTN+AF fed groups showed ruffled feathers, lethargy, and stunted growth from the third week. There was no mortality in the control and mycotoxin fed groups. There was a significant (Pincrease in the relative weight of the liver and spleen and decrease in the bursa of Fabricius in the entire mycotoxin treated groups when compared to the control group. In birds fed with CTN, the liver showed congestion, enlargement, pallor or yellowish discolouration and distended gall bladder. Kidneys revealed swelling, congestion and a few petechiae. Splenomegaly, atrophy of the bursa of Fabricius and catarrhal enteritis was also observed. In the AF group, the lesions were severe, affecting all birds. The gross lesions were pronounced in the sixth week. Microscopically, glomerular basement membrane thickening, degeneration and necrotic changes in the tubular epithelial cells in kidneys, degenerative changes in hepatocytes, microgranuloma, periportal fibrosis, periductular mononuclear cell infiltration, fatty degeneration, focal necrosis and fibrosis in the liver, mucosal hyperplasia of crop, proventriculitis, ventriculitis, catarrhal enteritis, pancreatitis, myocardial degeneration, mycocarditis, hyaline degeneration of muscle, lymphoid depletion and atrophic changes in the bursa of Fabricius, lymphoid depletion and reticulum cell hyperplasia in spleen, lymphoid depletion in caecal tonsils and plasma cell depletion in the Harderian gland were observed in the mycotoxin fed birds. Combined toxicity was more severe when compared to the individual mycotoxin fed groups. However, the effect was less than additive.



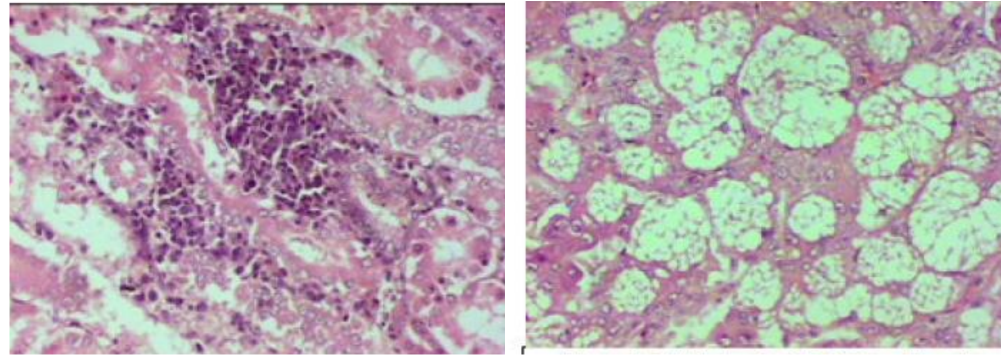
Chick, 3 wks, CIN+AF marked enlargement and pale kidney.
Chick, 6 wks, CIN+AF marked enlargement and pale liver **KUMAR *et al.* (2014)**



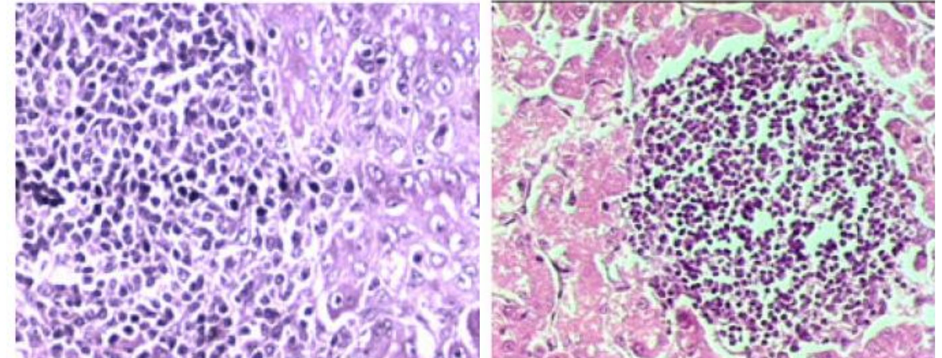
Chick, 3 wks, CIN-kidneys glomerular basement thickening,
Chick, 3 wks, CIN-kidneys, basoeosinophilic fluid in tubular lumen **KUMAR *et al.* (2014)**



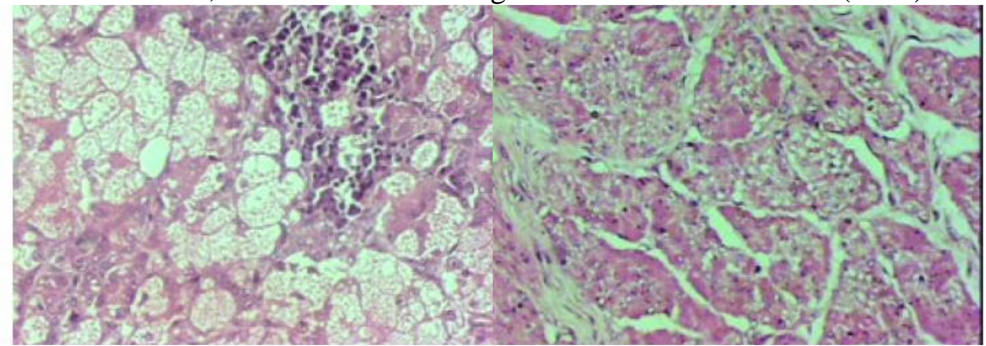
Chick 3 wks, CIN+AF] kidneys glomerular atrophy and calcification,.
Chick, 6 wks, CIN+AF kidneys epithelial cell degeneration and necrosis
KUMAR *et al.* (2014)



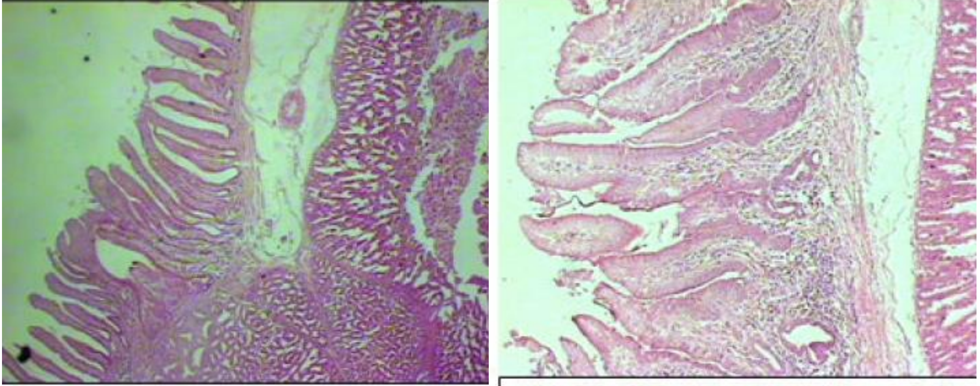
Chick, 6 wks, CIN+AF kidneys interstitial mononuclear cell infiltration,
Chick, 3 wks, CIN-liver vascular degeneration of hepatocytes **KUMAR *et al.* (2014)**



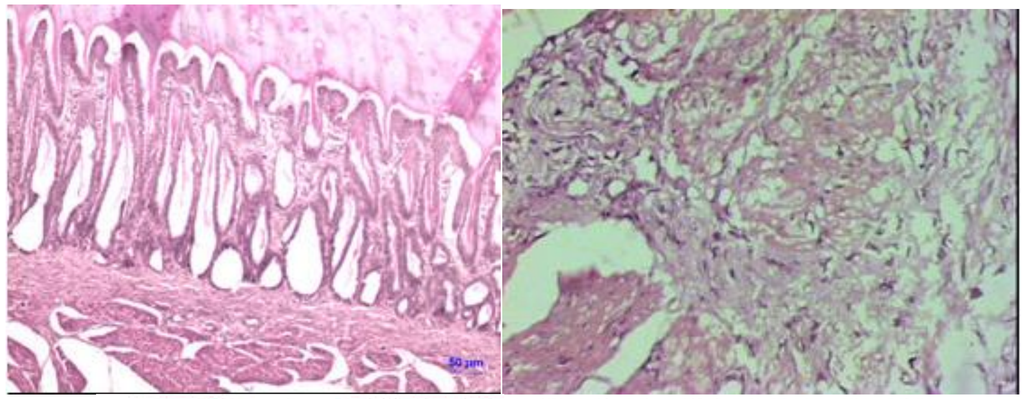
Chick 3 wks, CIN-liver acinar formation and microgranuloma,
Chick 3 wks, CIN+AF-liver microgranuloma **KUMAR** ***et al.*** **(2014)**



Chick 3 wks, CIN+AF-liver mononuclear infiltration in the parenchyma,
Chick 3 wks, CIN+AF-crop muscle degeneration **KUMAR** ***et al.*** **(2014)**



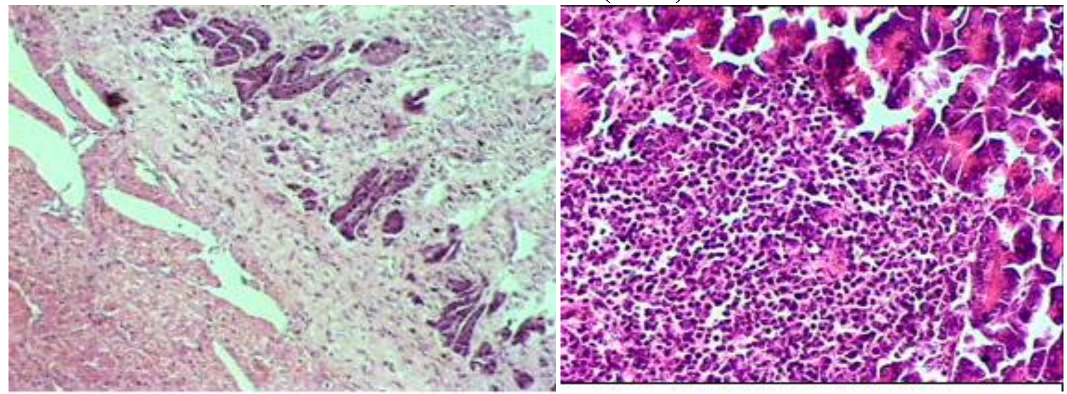
Chick 3 wks, CIN+AF Proventriculus shortning in the villi and ordema in the lamina propria, Chick 6 wks, CIN+AF Proventriculus mononuclear cell infiltration in the mucosa and oedema **KUMAR** ***et al.*** **(2014)**



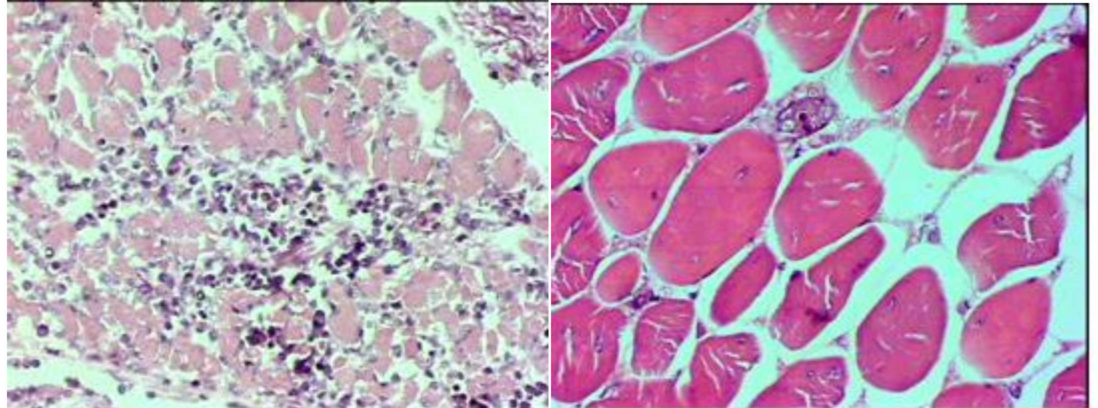
Chick- 3 wks- AF- Gizzard: Dilatation of the glands H&E x 200

Chick- 3 wks-CTN+AF- Gizzard: Muscle degeneration H&E x 200

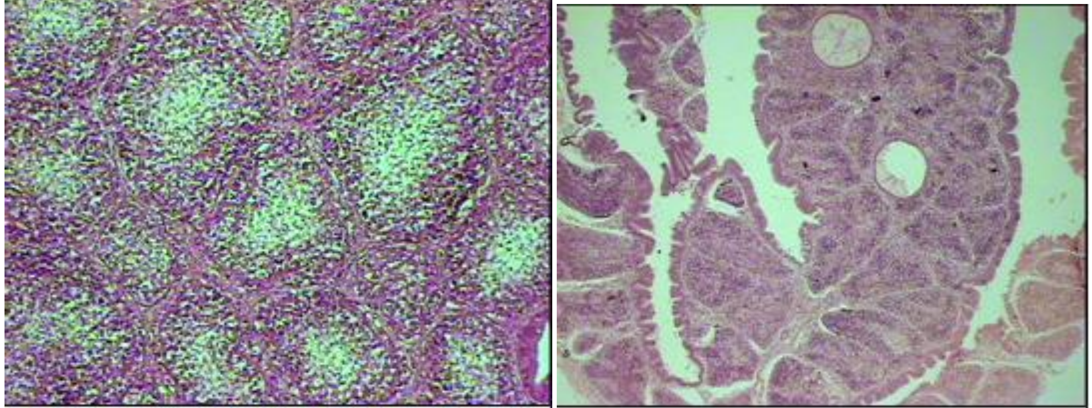
KUMAR *et al.* (2014)



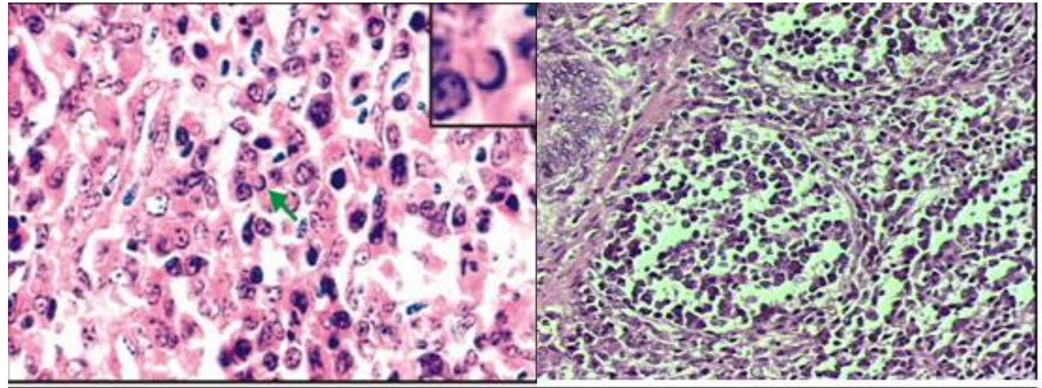
Chick-6 wks CIN+AF Gizzard fibrosis and glandular atrophy,
Chick-3 wks CIN+AF Pancreas mononuclear cell infiltration **KUMAR *et al.* (2014)**



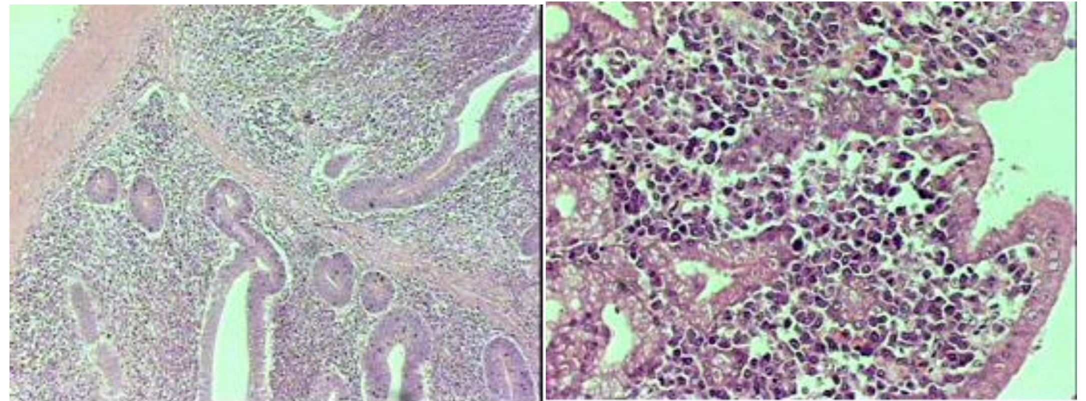
Chick-3 wks CIN+AF Myocarditis,
Chick-3 wks CIN+AF Pectoral muscle hyaline degeneration **KUMAR *et al.* (2014)**



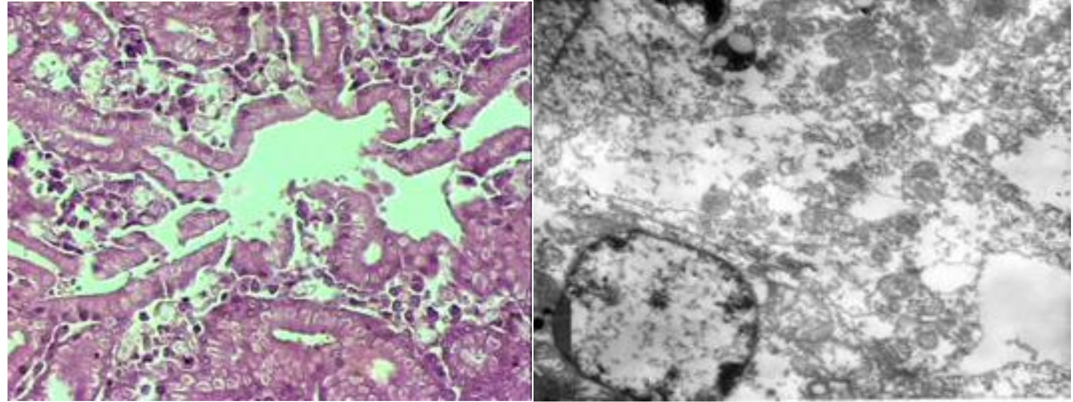
Chick-6 wks CIN+AF Bursa lymphocytosis in the medulla, Chick-6 wks CIN+AF Bursa Atrophy and multiple gollicular cysts **KUMAR *et al.* (2014)**



Chick-3 wks CIN-spleen apoptotic body (arrow),
Chick-3 wks CIN+AF tonsil severe diploid depletion **KUMAR *et al.* (2014)**



Chick-6 wks CIN+AF Chick Caecal tonsil severe lymphoid depletion, Chick -3 wks CIN **Harderious gland plasma cells with Russel bodies KUMAR *et al.* (2014)**



Chick -3 wks CIN+AF **Harderious gland severe plasma cell depletion,**
Chick-6 wks CIN **Transmission electromicrograph Kidney vaculation and swelling mitochondria (arrow) with disruption of cristae-urasyl acetate- lead citrate stain**
KUMAR *et al.* (2014)

Achakzai (2015) mentioned in his review that, Citrinin (CTN) is a fungal secondary metabolite and polyketide nephrotoxic mycotoxin commonly present as a natural hazardous contaminant both in food and feed world wide. Its chemical formula is C H O 13 14 5 with exact mass 250.0841g. It occurs mainly in stored grains. It was first isolated from filamentous fungi i.e. Penicillium citrinum, but now produced by more than 5 fungal genera i.e., Aspergillus, Clavariopsis, Monascus, Penicillium, Pythium and also more than 29 fungal species in grains, foods, feedstuffs as well as in

biological fluids. There are also many forms of CTN degradation products (derivatives), but the main 3 forms are CTN H (non-cytotoxic), CTN H (cytotoxic) and dicitrinin A (cytotoxic). The key ecological determinants for the synthesis of CTN during pre and post-harvest are temperature (°C) and water availability (a_w). Studies revealed that CTN production occurs at an optimum temperature of 20 – 30°C and 0.75 – 0.85a depending upon w fungal species. However, based on the available limited knowledge few researchers stated that CTN is acutely nephrotoxic at relatively high doses in poultry feeds (500 mg CTN kg feed), causing interferences in the 1 function and size of kidneys, liver, heart, pancreas, spleen and gall bladder of chickens. It also reduces body weight, egg weight and feed consumption, but increases water consumption. Therefore, on the basis of available limited data either submitted to EFSA (European Food Safety Authority) or collected from literature were not adequate to carry out poultry dietary exposure assessments for the general or specific groups of any country poults. At this stage it is difficult to establish wide acceptable limits for CTN concentration. Presently, there is no any specific legislation for CTN worldwide in general and Pakistan in particular. The main reason was either lack of suitable analytical routine techniques or its instability in various food and feedstuffs.

References:

1. Abd-Allah EF and Ezzat SM, 2005. Natural occurrence of citrinin in rice grains and its biocontrol by Trichoderma hamatum. Phytoparasitica, 33, 73-84.
2. Abdelhamid AM, Dorra TM. Study on effects of feeding laying hens on separate mycotoxins (aflatoxins, patulin, or citrinin)-contaminated diets on the egg quality and tissue constituents. Arch Tierernahr. 1990 Apr;40(4):305-16.
3. Achakzai, Abdul Kabir Khan. Introduction to Citrinin (CTN) and its Toxicity Status in Poultry Feeds Botany Research International 8 (4): 90-95, 2015
4. Ahamad DB, Vairamuthu S, Balasubramaniam GA, Manohar BM and Balachandran C, 2006. Individual and combined effects of citrinin and aflatoxin B1 in broiler chickens: a clinicopathological study. Indian Journal of Veterinary Pathology, 30, 32-35.
5. Ames DD, Wyatt RD, Marks HL and Washburn KW, 1976. Effect of citrinin, a mycotoxin produced by Penicillium Citrinum, on laying hens and youngbroiler chicks. Poultry Science, 55, 1294-1301.
6. Aziz NH, Mattar ZA and Mahrous SR, 2006. Contamination of grains by mycotoxin-producing molds and mycotoxins and control by gamma irradiation. Journal of Food Safety, 26, 184-201.
7. BASHEER AHAMAD. D., S. VAIRAMUTHU2 G.A. BALASUBRAMANIAM, B. MURAL I MANOHAR3 AND C. BALACHANDRAN. INDIVIDUAL AND COMBINED EFFECTS OF CITRININ AND AFLATOXIN Bl IN BROILER CHICKENS: A CLINICO-PATHOLOGICAL STUDY. Indian Journal of Veterinary Pathology 30(1): 32-35,2006

8. Brown, TP, Manning, RO, Fletcher, OJ, Wyatt, RD 1986.The individual and combined effects of citrinin and ochratoxin A on renal ultrastructure in layer chicks. Avian Dis. 30191198
9. Carlton W 1980. Penicillic acid, citrinin and xanthomegnin quinone metabolites, a review. Conference on mycotoxins in animal feeds and grains related to animal health, FDA, Rockville, Maryland, US, 13 June 1980. 345 pp.
10. Dick R, Baumann U and Zimmerli B, 1988. Zum Vorkommen von Citrinin in Cerealien. Mitteilungen Gebiete Lebensmitte Hygiene, 79, 159-164.
11. EFSA (2012) SCIENTIFIC OPINION Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed1 EFSA Panel on Contaminants in the Food Chain (CONTAM)2, 3 European Food Safety Authority (EFSA), Parma, Italy
12. El-Sayed AMAA, 1996. Natural occurrence of ochratoxin A and citrinin in food stuffs in Egypt. Mycotoxin Research, 12, 41-44. Endo A and Kuroda M, 1976. Citrinin, an inhibitor of cholesterol synthesis. The Journal of Antibiotics, 29, 841-843.
13. Glahn RP and Wideman RF, Jr., 1987. Avian diuretic response to renal portal infusions of the mycotoxin citrinin. Poultry Science, 66, 1316-1325.
14. Glahn R.P., Wiederman R.F., Evangelisti J.W. Effects of ochratoxin A alone and in combination with citrinin on kidney function of single comb white leghorn pullets. Poult. Sci. 1988;67:1034–1042. doi: 10.3382/ps.0671034.
15. Glahn RP, Shapiro RS, Vena VE, Wideman RF, Jr. and Huff WE, 1989. Effects of chronic ochratoxin A and citrinin toxicosis on kidney function of single comb White Leghorn pullets. Poultry Science, 68, 1205-1212.
16. Hanika, C, Carlton, WW, Hinsman, EJ, Tuite, J 1986. Citrinin mycotoxicosis in the rabbit: Ultrastructural alterationsVet Pathol2324525
17. Janardhana GR, Raveesha KA and Shetty HS, 1999. Mycotoxin contamination of maize grains grown in Karnataka (India). Food and Chemical Toxicology, 37, 863-868. Jarvis B, 1983.
18. Jayaramu G.M., Satyanarayana, M.L., Ravikumar, P., Jagadeesh Sanganal, Paniraj, K.L. & Suguna Rao. EFFECT OF FEEDING OCHRATOXIN A AND CITRININ TOXINS ON CERTAIN BIOCHEMICAL PARAMETER IN BROILER CHICKEN. G.J.B.B., VOL.1 (2) 2012: 186-190
19. Klarić, M. Š., Rašić, D., & Peraica, M. (2013). Deleterious Effects of Mycotoxin Combinations Involving Ochratoxin A. *Toxins*, *5*(11), 1965–1987. http://doi.org/10.3390/toxins5111965
20. Kononenko GP and Burkin AA, 2008. A survey on the occurrence of citrinin in feeds and their ingredients in Russia. Mycotoxin Research, 24, 3-6.
21. Kumari CK and Nusrath M, 1987. Natural occurrence of citrinin and ochratoxin A in coconut products. National Academy Science Letters-India, 10, 303-305.
22. Kumar M., Dwivedi P., Sharma A.K., Singh N.S., Patil Ochratoxin A and citrinin nephrotoxicity in New Zealand White rabbits: An ultrastructural assessment. Mycopathologia. 2007;163:21–30
23. Kumar M, Dwivedi P, Sharma AK, Telang AG, Patil RD and Singh ND, 2008. Immunotoxicity of ochratoxin A and citrinin in New Zealand White rabbits. World Rabbit Science, 16, 7-12.
24. KUMAR, T. A. AND C. BALACHANDRAN, PATHOLOGICAL EFFECT OF CITRININ AND AFLATOXIN IN BROILER CHICKEN .Int J life Sci Pharma Res. VOL 4/ ISSUE 4/OCT-DEC 2014

25. Manafi M and H Bagheri, 2011. Effect of combination of Aspergillus ochraceus (NRRL-3174) and Penicillium citrinum (NRRL 1841) on blood parameters of commercial chicks. Res. Opin. . Anim. Vet. Sci., 1(S), S23-S29
26. Manning R.O., Brown T.P., Wyatt R.D., Fletcher O.J. The individual and combined effects of citrinin and ochratoxin A in broiler chicks. Avian Dis. 1985;29:986–997.
27. Mehdi, NAQ, Carlton, WW 1981Citrinin mycotoxicosis in broiler chickensFood Cosmet Toxicol19723733
28. Monbaliu S, Van Poucke C, Detavernier Cl, Dumoulin F, Van De Velde M, Schoeters E, Van Dyck S, Averkieva O, Van Peteghem C and De Saeger S, 2010. Occurrence of Mycotoxins in Feed as Analyzed by a Multi-Mycotoxin LC-MS/MS Method. Journal of Agricultural and Food Chemistry, 58, 66-71.
29. Nguyen MT, Tozovanu M, Tran TL and Pfohl-Leszkowicz A, 2007. Occurrence of aflatoxin B1, citrinin and ochratoxin A in rice in five provinces of the central region of Vietnam. Food Chemistry, 105, 42-47. Citrinin in food and feed EFSA Journal 2012;10(3):2605 57
30. Nishijima M, 1984. Survey for mycotoxins in commercial rations. In: Toxigenic Fungi: Their Toxins and Health Hazards. Eds Kurata H and Ueno Y. Elsevier. Developments in Food Science 7, Amsterdam, 172-189.
31. Odhav B and Naicker V, 2002. Mycotoxins in South African traditionally brewed beers. Food Additives and Contaminants, A, 19, 55-61. Osborne BG, 1980. The occurrence of ochratoxin A in mouldy bread and flour. Food Cosmetic Toxicology, 18, 615-617.
32. Petkova-Bocharova T, Castegnaro M, Michelon J and Maru V, 1991. Ochratoxin A and other mycotoxins in cereals from an area of Balkan endemic nephropathy and urinary tract tumours in Bulgaria. In: Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours. Eds Castegnaro M, Plestina R, Dirheimer G, Chernozemsky IN and Bartsch H. IARC Scientific Publications, 83-87.
33. Polisenska I, Pfohl-Leszkowicz A, Hadjeba K, Dohnal V, Jirsa O, Denesova O, Jezkova A and Macharackova P, 2010. Occurrence of ochratoxin A and citrinin in Czech cereals and comparison of two HPLC methods for ochratoxin A detection. Food Additives and Contaminants, A, 27, 1545- 1557.
34. Reddy RV, Taylor MJ and Sharma RP, 1988. Evaluation of citrinin toxicity on the immune functions of mice. Journal of Food Protection, 51, 32-36. Citrinin in food and feed EFSA Journal 2012;10(3):2605 58
35. Roberts WT and Mora EC, 1978. Toxicity of Penicillium citrinum AUA-532 contaminated corn and citrinin in broiler chicks. Poultry Science, 57, 1221-1226.
36. Scott PM, Van Walbeek W, Kennedy B and Anyeti D, 1972. Mycotoxins (ochratoxin A, citrinin, and sterigmatocystin) and toxigenic fungi in grains and other agricultural products. Journal of Agricultural and Food Chemistry, 20, 1103-1109.
37. Scudamore KA and Hetmanski MT, 1995. Natural occurrence of mycotoxins and mycotoxigenic fungi in cereals in the United Kingdom. Food Additives and Contaminants, A, 12, 377-382.
38. Scudamore KA, Hetmanski MT, Chan HK and Collins S, 1997. Occurrence of mycotoxins in raw ingredients used for animal feeding stuffs in the United Kingdom in 1992. Food Additives and Contaminants, A, 14, 157-173.
39. Šegvić Klarić M, Želježić D, Rumora L, Peraica M, Pepeljnjak S and Domijan A-M, 2012. A potential role of calcium in apoptosis and aberrant chromatin forms in porcine kidney PK15 cells induced by individual and combined ochratoxin A and citrinin. Archives of Toxicology, 86, 97-107.

40. Stoev S.D., Stefanov M., Denev S., Radić B., Domijan A., Peraica M. Experimental mycotoxicosis in chickens induced by ochratoxin A and penicillic acid and intervention by natural plant extracts. Vet. Res. Commun. 2004;28:727–746.
41. Tabata S, Iida K, Kimura K, Iwasaki Y, Nakazato M, Kamata K and Hirokado M, 2008. Investigation of ochratoxin A, B and citrinin contamination in various commercial foods. Journal of the Food Hygienic Society of Japan, 49, 111-115.
42. Talmaciu E, Sandu I and Banu T, 2008. Researches regarding the fungal contamination and the presence of citrinin in feed. Fungi & Mycotoxins, 2, 212-217.
43. Vesela D., Vesely D., Jelinek R. Toxic effect of ochratoxin A and citrinin, alone and in combination, on chicken embryos. Appl. Environ. Microbiol. 1983;45:91–93
44. Vrabcheva T, Usleber E, Dietrich R and Märtlbauer E, 2000. Co-occurrence of ochratoxin A and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy. Journal of Agricultural and Food Chemistry, 48, 2483-2488.

4.6. Penicillic Acid Mycotoxicosis

Penicillic acid is a mycotoxin isolated from various strains of *Penicillium* and *Aspergillus fungi* that demonstrates mutagenic and cytotoxic effects. This compound is demonstrated to induce single-strand DNA breaks and to inhibit DNA synthesis in CHO cells. Penicillic acid is also reported to irreversibly inactivate GMD (GDP-mannose dehydrogenase), interrupting the committed step in alginate biosynthesis. Penicillic acid is an inhibitor of ADH/AKR1A1 and LDH.

Penicillic acid is a tetraketide derivative, and exhibits hepatotoxic, antibacterial, antiviral, cytotoxic, carcinogenic and phytotoxic properties (**Keromnes and Thouvenot 1985)**.

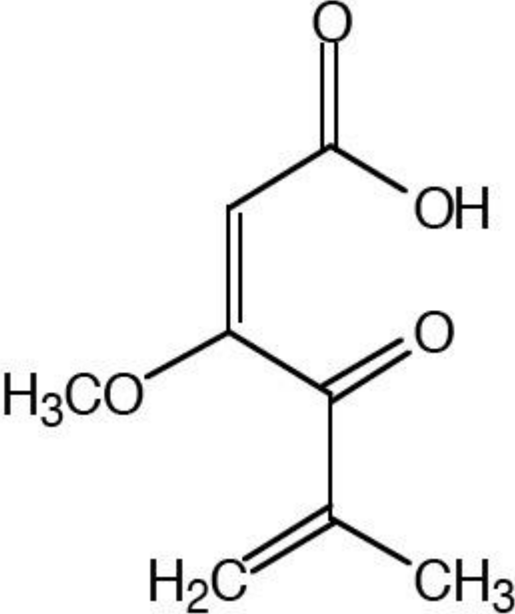
Physical and chemical properties

Physical State: Solid

Solubility: Soluble in water (50 mg/ml), DMSO (>10 mg/ml), chloroform (10 mg/ml), ethyl acetate (>10 mg/ml), and acetonitrile (>10 mg/ml).

Melting Point: 88° C

Boiling Point: ~285.7° C at 760 mmHg (Predicted)



Chemical structure

Fungi producing penicillic acid

- Penicillic acid was first identified in P. puberulum and P. cyclopium (**Birkinshaw *et al.* 1936**).
- Penicillic acid was found in several Penicillium (e.g., P. aurantiogriseum, P. carneum, P freii, P. melanoconidium, P. neoechinulatum, P. polonicum, P. pulvillorum, P. radicicola, P. tulipae, P. viridicatum; **(Ciegler and Kurtzman 1970; Frisvad et al. 2004)**
- Penicillic acid was also found in several Aspergillus species (A. ochraceus, A. ostianus, A. melleus, A. sulphureus, A. westerdijkiae, A. westlandense, A. steynii, A. sclerotiorum, A. roseoglobulosus, A. pseudoelegans, A. persii, A. muricatus, A. flocculosus, A. auricomus, A. bridgeri, A. cretensis (**Ciegler 1972; Samson et al. 2004; Visagie et al. 2014).**

Penicillic Acid **producing Penicillium species**

1. *Penicillium aurantiogriseum,*
2. *Penicillium aurantiocandidum,*
3. *Penicillium brasilianum,*
4. *Penicillium carneum,*
5. *Penicillium cyclopium,*
6. *Penicillium freii,*
7. *Penicillium melanoconidium,*
8. *Penicillium neoechinulatum,*
9. *Penicillium polonicum,*

10. *Penicillium radicicola,*
11. *Penicillium tulipae*
12. *Penicillium viridicatum*

Penicillic Acid producing Aspergillus species

1. *Aspergillus affinis*
2. *Aspergillus auricomus*
3. *Aspergillus bridgeri*
4. *Aspergillus cretensis*
5. *Aspergillus elegans*
6. *Aspergillus insulicola*
7. *Aspergillus melleus*
8. *Aspergillus muricatus*
9. *Aspergillus neobridgeri*
10. *Aspergillus occultus*
11. *Aspergillus ochraceopetaliformis (= A. flocculosus)*
12. *Aspergillus ochraceus*
13. *Aspergillus ostianus*
14. *Aspergillus pallidofulvus*
15. *Aspergillus persii*
16. *Aspergillus pseudoelegans*
17. *Aspergillus roseoglobulosus*
18. *Aspergillus salwaensis*
19. *Aspergillus sclerotiorum*
20. *Aspergillus subramanianii*
21. *Aspergillus westerdijkiae*
22. *Aspergillus westlandense*

Natural occurrence:

- Natural occurrence of penicillic acid has been detected in the poultry feed, corn, dried beans, cheese, salami and tobacco products.
- Penicillic acid was isolated from blue-eye diseased corn, poultry feed, commercial corn, dried beans, cheese and tobacco products **(LeBars J. 1980).**

Effects on poultry

- Graded levels of PA fed up to 400 mg/kg of diet to broiler chicks produced no significant effects on growth or efficiency of feed utilization, suggesting that PA alone has little toxicity in chickens (**Huff *et al.* 1980).**
- The residue of penicillic acid in tissue and organs of the broiler chickens within 60 days (**HE *et al.*, 2002**) showed:
    - major pathological changes was fatty and vacuolar degeneration of hepatocytes,cloudy swelling of renal epithelial cells and myocardial cells;

- contents of MCV and MCHC in poisoned groups were less than that in control group,
- activities of SGPT, LDH and AKP in the formers were higher than those in the latter;
- the amount of penicillic acid, was the highest in liver, and the residue in kidney outweighed that in heart.
- there was a relation between the residue of penicillic acid and the degree of lesion.
- The target organ of penicillic acid poisoning wasthe liver.

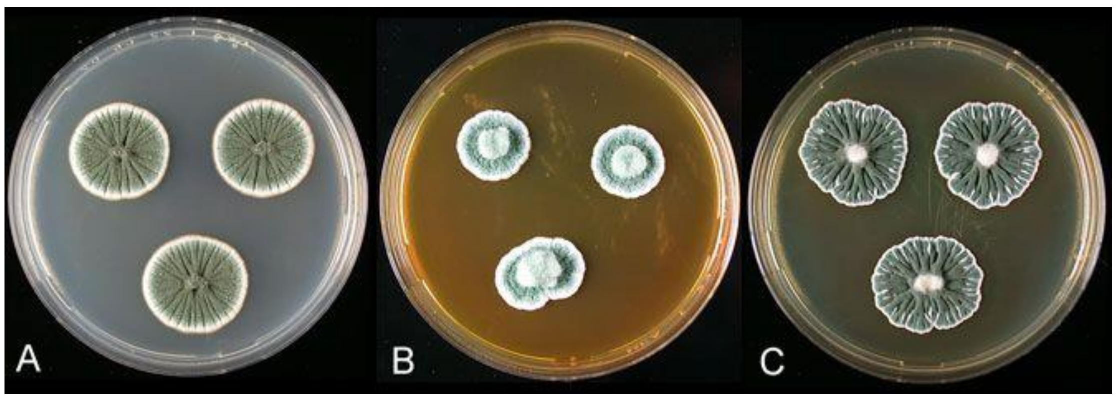
- Broiler chickens fed a mouldy diet containing 130, 300 or 800 ppb OTA and 1000–2000 ppb PA **Stoev *et al*. (2004)** showed:
  - cloudy swelling and granular degeneration in the epithelium and mononuclear cell proliferation and activation of capillary endothelium in the kidney and liver;
  - degenerative changes and depletion of lymphoid cells in lymphoid organs (bursa of Fabricius, thymus and spleen)
  - A significant decrease in body mass and relative weight of lymphoid organs was found after 6 weeks of exposure
  - a greater decrease after 10 weeks of exposure to OTA and PA,
- The peak induction of apoptosis was observed at 24 h treatment of penicillic acid (15 ppm) **PAZHANIVE *et al.* (2014)**.

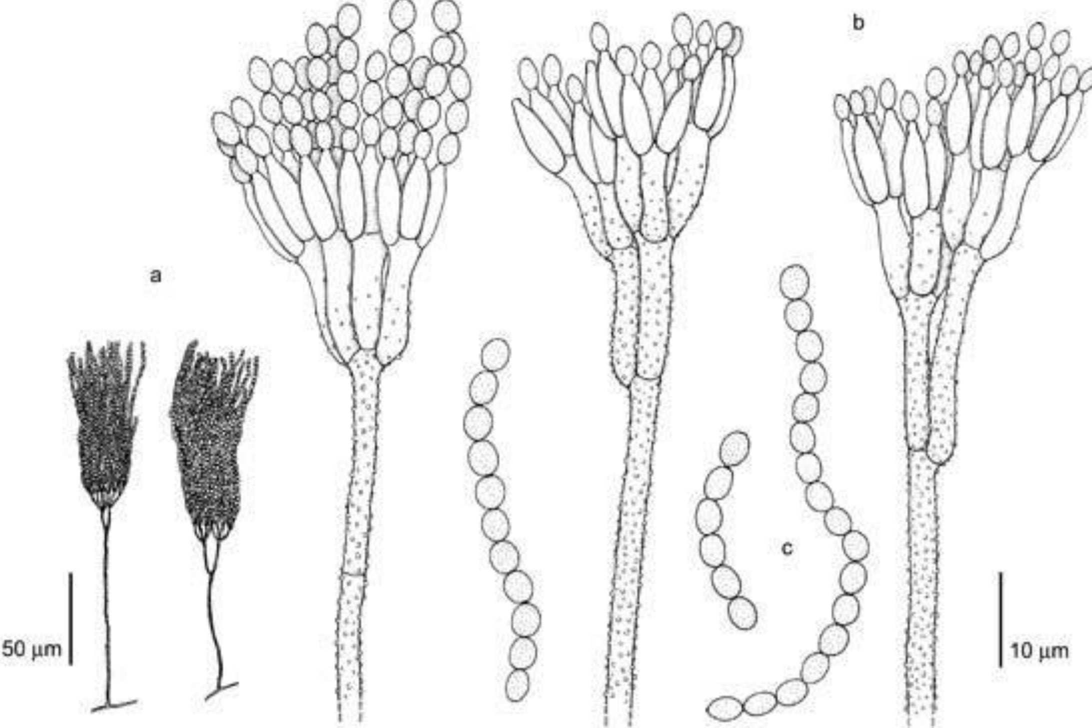
Description of some Penicillium and Aspergillus species producing penicillic acid

1. Penicillium aurantiogriseum Dierckx, Annales de la Société Scientifique de Bruxelles 25 (1): 88 (1901)

=Penicillium aurantioalbidum Biourge, La Cellule 33: 197 (1923) [MB#257873]
=Penicillium ochraceum Bainier, The Penicillia: 309 (1930)

Colonies (CzA) growing moderately rapidly, granular, somewhat floccose, bright greyish-green, sometimes exuding a reddish-brown pigment into the medium; reverse orange to brown. Microscopy. Conidiophore stipes mostly roughened, 200-400 µm long; penicilli usually terverticillate. Metulae 10-12 µm long. Phialides flask-shaped, 9-10 µm long. Conidia smooth-walled, subspherical to ellipsoidal, 3.5-4.0 µm long, bluish-grey.





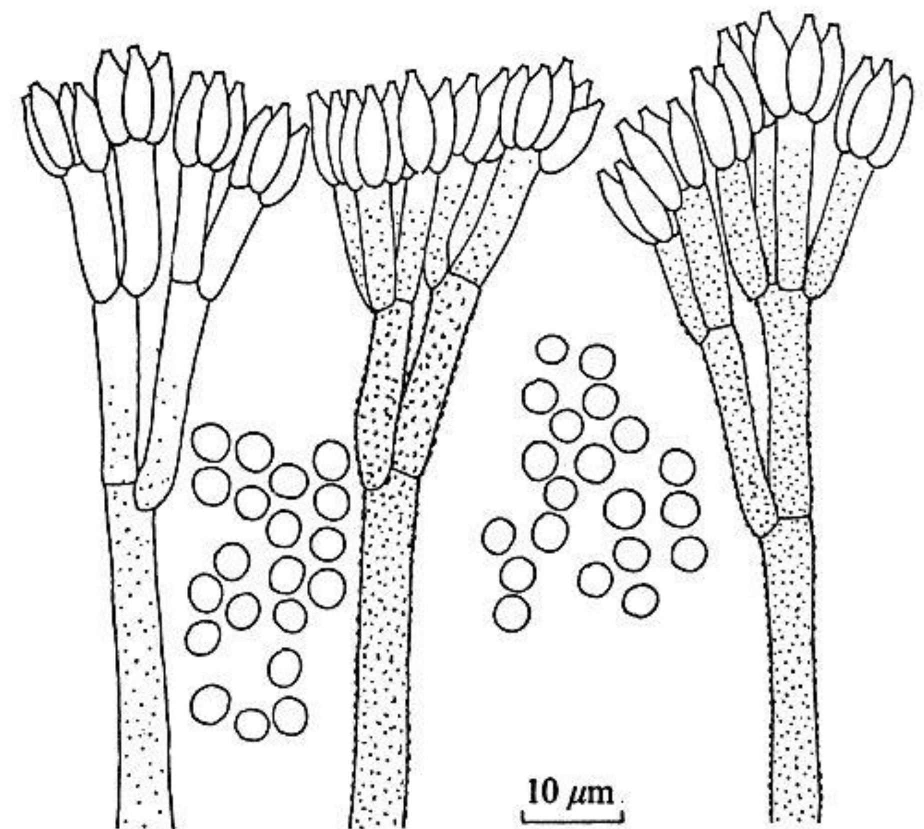
Penicillium aurantiogriseum, Mycobank

2. Penicillium cyclopium Westling, Arkiv før Botanik 11 (1): 90 (1911)

≡Penicillium verrucosum var. cyclopium (Westling) Samson, Stolk & Hadlok, Studies

Colonies on Czapek agar and CYA at 25°C growing restrictedly producing grey green conidia with a granular to fasciculate colony surface, often with exudate droplets. The colony reverse is orange to red or pinkish brown with the colour often diffusing into the agar medium or more rarely creamish yellow. On MEA the conidia are blue green with a strong blue element and colonies have a distinct yellow reverse, often with the yellow colour diffusing into the medium. On YES agar there is no sporulation and the colony mycelium if often strongly yellow, reverse colour distinct yellow. On CREA weak growth but strong acid production. Conidiophores two-stage branched

(terverticillate) with all elements adpressed, stipes rough-walled. Conidia smooth-walled, globose to subglobose, 3-3.5 µm in diam.

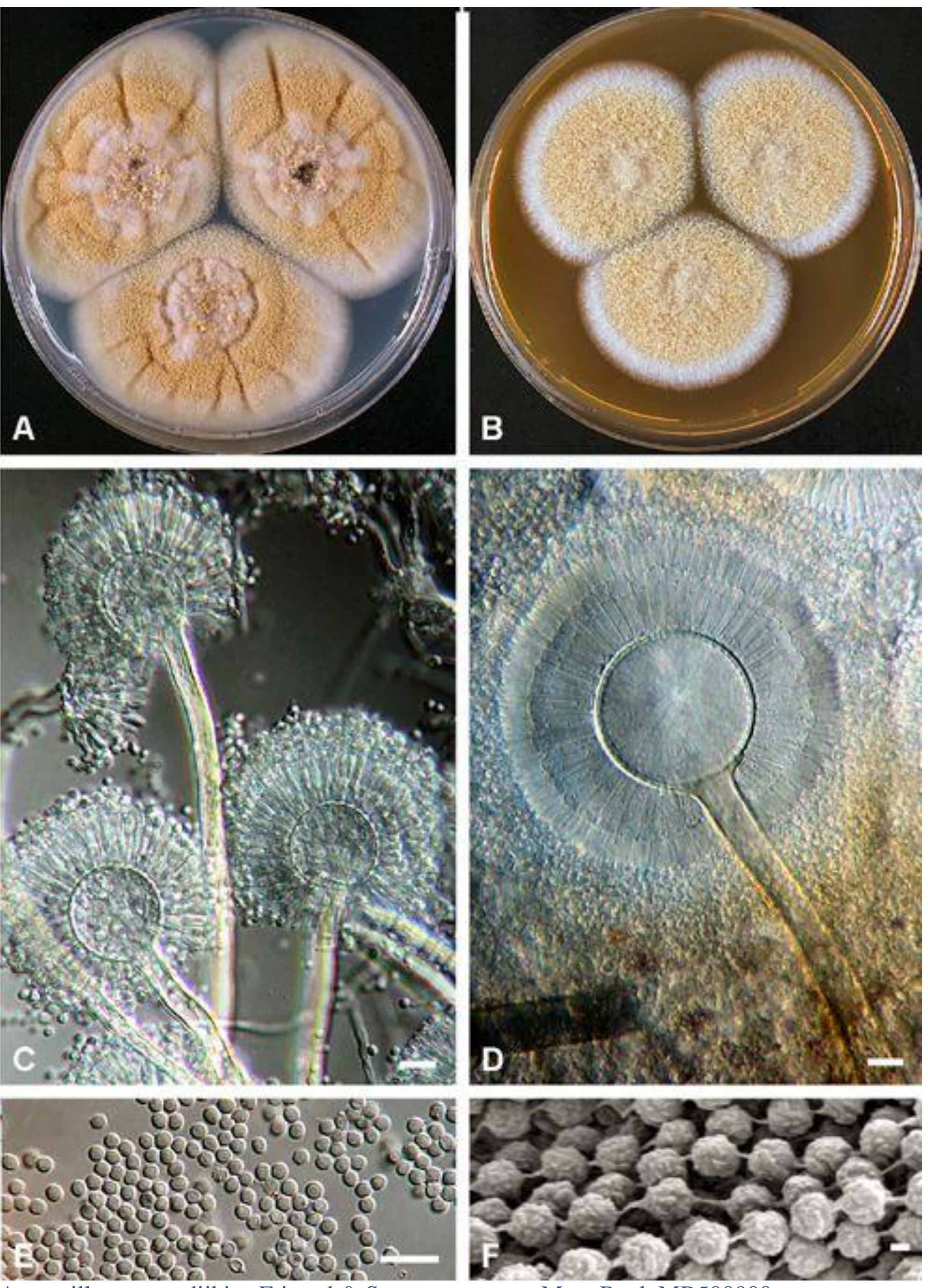


Penicillium cyclopium, Mycobank

3. Aspergillus westerdijkiae Frisvad & Samson, Studies in Mycology 50 (1): 30 (2004)

Colony diameters after 7 d at 25 °C in mm: CYA25 49-57; MEA 42-47; no growth on CYA37. Colony colours and textures. Moderate to good conidia production on CYA25, pale to light to dull yellow (3B3-3A3); mycelium white, inconspicuous; sclerotia sparsely produced; pale yellow after 7 d, becoming dull orange at age. Reverse crème brown, no soluble pigment present. Good sporulation on MEA, velvety, pale to light or dull yellow (3A3-3B3) after 7 d; mycelium white, sclerotia sparsely formed, overgrown by conidial state and in shades of orange, reverse brown

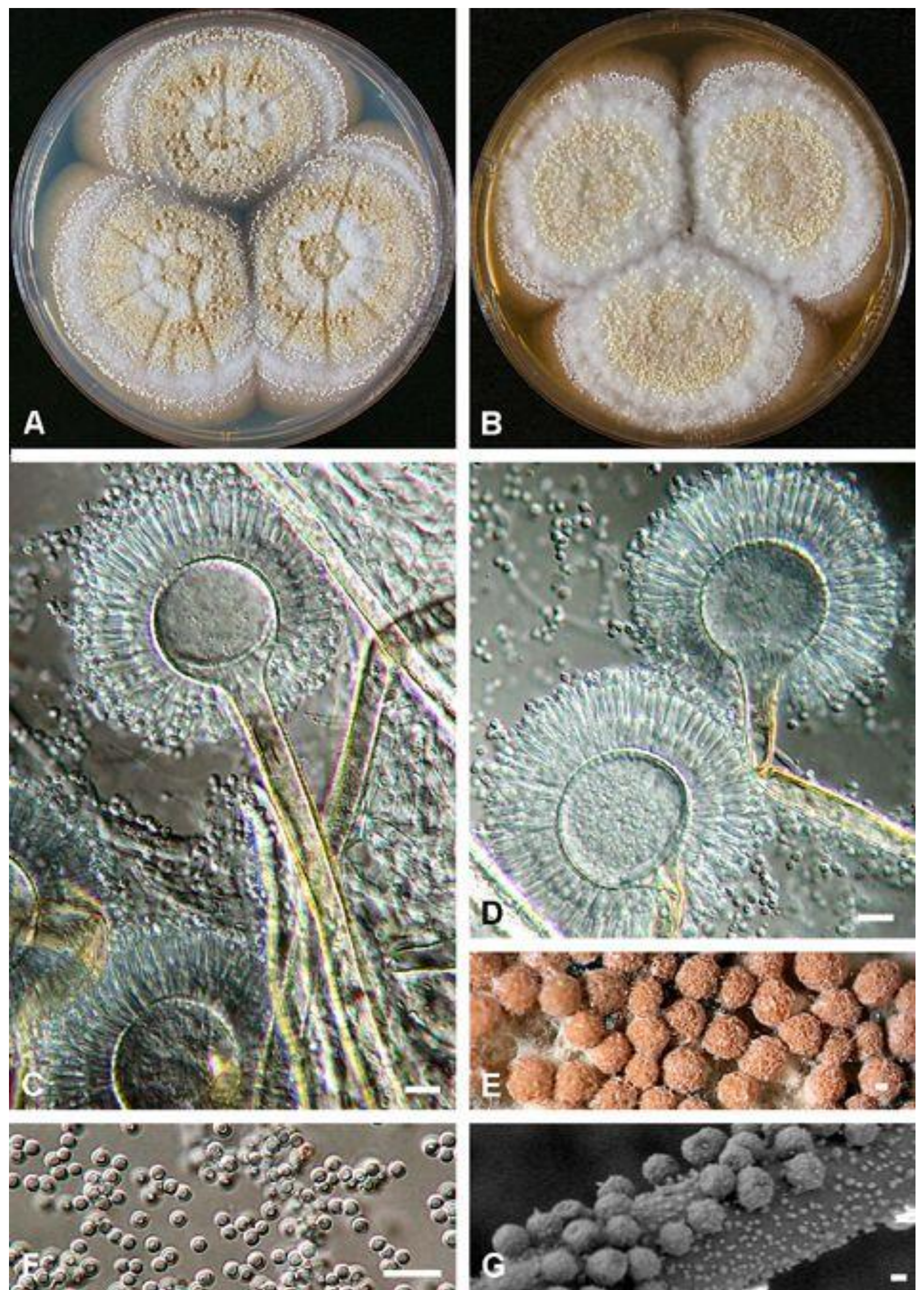
centre with yellow to medium-coloured edge. No growth on CYA37. Conidial heads radiate, splitting into columns; stipes up to 1800 µm in length, walls rough, uncoloured to yellow pigmented; vesicles globose to spathulate, (16-)20-35(-42) — (3-)3.5-5.7(-7.1)µm; biseriate; metulae covering the entire vesicle, measuring (10.5-)11 ´ 19(-23) µm; phialides (6.8-)7.3-9.7(-10.5) — (2-)2.1-3(-3.5) µm; conidia predominantly globose, finely roughened, (2.3-)2.5-3(-3.1) ´ (2.2-)2.3-2.8(-3.1) µm; sclerotia sparsely produced, white to cream, globose to subglobose, (460-)480-760(-840) — (430-)480-660(-720) µm on CYA and (440-)450-720(-750) — (430-)430-650(-700) µm on OA.



Aspergillus westerdijkiae Frisvad & Samson, sp. nov. MycoBank MB500000.

4. Aspergillus pseudoelegans Frisvad & Samson, Studies in Mycology 50 (1): 35 (2004)

Colony diameters after 7 d of incubation, in mm: CYA25 39-48; MEA 38-47; CYA37 0-8. Colony colours and textures. No conidia are produced on CYA25 after 7 d of incubation, light brown to yellowish brown (5D5-6) conidia are formed after prolonged incubation; mycelium white, inconspicuous; sclerotia abundantly present; white after 7 d, becoming brownish grey, (4C2-4D2) after 30 d of incubation. Reverse (light) red brown, light brown soluble pigment present. On MEA conidial production absent, prolonged incubation showed sparse production of conidiophores; sclerotia covered by mycelium, greyish beige (4C2) after 30 d of incubation, reverse light brown centre with medium-yellow edge. No or weak growth on CYA37, 0-8 mm. Conidial heads radiate, splitting into columns; stipes up to 1000-1200 µm in length, walls distinct rough, yellow to light brown; vesicles globose to spathulate, (26-)28-34(-36) µm; biseriate; metulae covering the entire vesicle, measuring (8-)9.5-17(-18) — (3.7-)4.1-6.1(-7) µm; phialides (6.6-)7-9.5(-10) — (1.7-)2.1-3(-3.6) µm in length; conidia globose to subglobose, smooth, (2-) 2.1-2.5 (-2.6); sclerotia abundant, globose to subglobose, (285-) 300-430(-500) µm on CYA and somewhat larger on MEA (360-)400-590(-650) µm.



Aspergillus pseudoelegans Frisvad & Samson, sp. nov. MycoBank

Reports:

Bacon *et al.* (1973) proved that a strain of *Aspergillus ochraceus* Wilhelm, isolated from poultry feed, produced both penicillic acid and ochratoxin A. Studies demonstrating the ability of this fungus to colonize poultry feed and produce these two mycotoxins under various temperatures and moistures indicated that the interaction was complex. The optimal temperature for conidial development did not vary with moisture, but accumulation of both toxins did. A combination of low temperature, 15 or 22 C, and low moisture favored the production of penicillic acid, whereas high temperature, 30 C, and high moisture favored the production of ochratoxin A.

Huff *et al.* (1980) evaluated penicillic acid for its toxicity in broiler chickens by feeding graded concentrations (0, 100, 200, and 400 microgram/g of diet) to 4 groups of 10 birds per treatment. No significant (P greater than .05) effects were measured on growth rate, feed conversion, relative size of pancreas, spleen, liver, heart, bursa, or kidney or on hemoglobin, packed cell volume, liver lipid, plasma protein, or glucose. The only significant effects were a slight reduction in the size of the proventriculus and gizzard at dose levels of 200 and 400 microgram/g. Neither the salt nor lactone forms of penicillic acid had any detectable effect. The acute oral LD50 for the sodium salt form was 92 +/- 9 mg/kg. These data suggest that penicillic acid by itself has little toxicity (less than 1% of that of aflatoxin) in chickens.

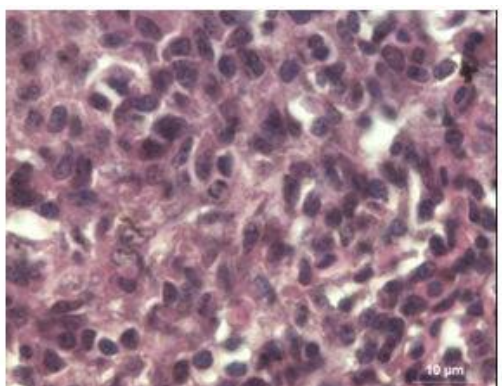
Chan and Haye (1981) reported that the hepatotoxicity of penicillic acid (PA), a carcinogenic mycotoxin, was substantiated by a variety of hepatic functional tests. Involvement of an active metabolite as the toxic species was proposed. The toxicity of PA was dependent on the route of administration with intraperitoneal (ip) being the most toxic followed by intravenous (iv) and oral. This difference in toxicity was explained by the kinetic data for PA if liver were assumed to be the site of activation. One-, 2- and 3-compartment open models were proposed to fit the plasma parent compound concentration after oral, ip, and iv administration of PA. Liver, kidneys, heart, lungs and spleen contained more radioactivity than brain, fat and muscle after [^{14}C] - PA administration. Only a fraction of the radioactivity in the blood was detected as the parent compound. Most of the recovered radioactivity in the kidneys and liver was in the cytosol fraction. [^{14}C]PA was readily metabolized in the liver. The metabolites were excreted in the bile and effectively cleared by the kidneys. Fecal and respiratory CO_2 were minor excretory routes. Over 90% of the urinary and 99% of the biliary metabolites were not extracted with polar organic solvents. Three water-soluble metabolites (derived from GSH or cysteine) were resolved by HPLC in urine and bile. About 10% of the urinary metabolites were detected as glucuronide conjugates. These data supported the hypothesis that an active metabolite which can be detoxified by GSH is involved in the toxicity of PA.

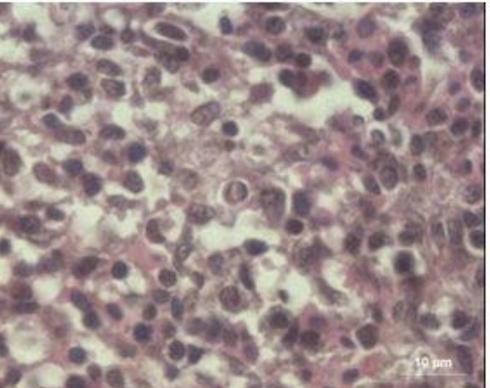
HE *et al.* (2002) investigated the toxicity of penicillic acid in broiler chickens. The major pathological changes was fatty and vacuolar degeneration of hepatocytes, cloudy swelling of renal epithelial cells and myocardial cells; the contents of MCV and MCHC in poisoned groups were less than that in control group, whereas the activities of SGPT, LDH and AKP in the formers were higher than those in the latter. As for the distribution of penicillic acid, the amount was the highest in liver, and the residue in kidney outweighed that in heart. It was understandable that there was a relation between the residue of penicillic acid and the degree of lesion. The target organ of penicillic acid poisoning was the liver.

Stoev *et al*. (2004) studied the combined toxic effect of ochratoxin A (OTA) and penicillic acid (PA) on the body mass, the weight and pathomorphology of some internal organs in 85 broiler chickens fed a mouldy diet containing 130, 300 or 800 ppb OTA and 1000–2000 ppb PA. The main pathomorphological changes were cloudy swelling and granular degeneration in the epithelium and mononuclear cell proliferation and activation of capillary endothelium in the kidney and liver; degenerative changes and depletion of lymphoid cells in lymphoid organs (bursa of Fabricius, thymus and spleen) were also seen. Protective effects of 5% total water extract of artichoke and a new natural phytosubstance Rosallsat against these

pathomorphological changes were observed. A significant decrease in body mass and relative weight of lymphoid organs was found after 6 weeks of exposure and a greater decrease after 10 weeks of exposure to OTA and PA, and a protective effect of artichoke extract and a slight effect of Rosallsat against that decrease was observed. A significant increase in relative weight of liver and kidneys was also observed as well as a protective effect of artichoke extract against that increase. The quantity of OTA and the percentage of positive samples were significantly lower in tissues of chickens treated with artichoke extract or Rosallsat in addition to OTA than in those treated with only OTA.

PAZHANIVE *et al.* (2014) studied the apoptosis in the sublethal dose of penicillic acid mycotoxicosis in broiler chicken. Eighteen day-old broiler chicks were fed with control diet for the period of three weeks. Subsequently, the birds were randomly distributed to two groups of nine birds each and fed with control and 15 ppm of penicillic toxin diets. Three birds from control and treated groups were sacrificed at 24, 48 and 72 h after treatment in the acute toxicity trial. Similarly, six birds (3 control and 3 penicillic acid–15 ppm) were used for subacute toxicity trial (21 days) and sacrificed to study the apoptosis in the spleen and thymus by using flow cytometric analysis with the Annexin V kit to assess apoptosis and necrosis in splenocytes and thymocytes. This research indicated that peak induction of apoptosis was observed at 24 h treatment of penicillic acid (15 ppm).





Spleen apoptotic cell chromatin margination, Spleen apoptotic cell shrinkage Nd chromatin margination, **PAZHANIVE *et al.* (2014)**

References

1. Bacon CW, Sweeney JG, Robbins JD, Burdick D. Production of Penicillic Acid and Ochratoxin A on Poultry Feed by *Aspergillus ochraceus*: Temperature and Moisture Requirements. *Applied Microbiology*. 1973;26(2):155-160.
2. Birkinshaw JH, Oxford AE, Raistrick H (1936) Studies in the biochemistry of micro-organisms: Penicillic acid, a metabolic product of Penicillium puberulum Bainier and P. cylopium Westling. Biochem J 30:394-411.
3. **Chan, P. K. and A. W. Haye Hepatotoxicity of the mycotoxin penicillic acid: A pharmacokinetics consideration**J American. Oil Chemists' Society
4. December 1981, 58:A1017
5. Ciegler A (1972) Bioproduction of ochratoxin A and penicillic acid by members of the Aspergillus ochraceus group. Can J Microbiol 18:631-663.

6. Frisvad JC, Frank JM, Houbrakren JAMP, Kuijpers AFA, Samson RA (2004) New ochratoxin A producing species of Aspergillus section Circumdati. Stud Mycol 50:23- 43
7. Frisvad JC (2015) Taxonomy, chemodiversity, and chemoconsistency of Aspergillus, Penicillium and Talaromyces species. Front Microbiol 5:1-7
8. HE Zu-ping[1];YUAN Hui[2];FENG Mei-**Toxicity of Penicillic Acid in Broiler Chickens**. ZOOLOGICAL RESEARCH 2002 23 (3): 261-265
9. Huff WE, Hamilton PB, Ciegler A. **Evaluation of penicillic acid for toxicity in broiler chickens.** Poult Sci. 1980 Jun;59(6):1203-7.
10. János Varga1 *, Nikolett Baranyi1 , Muthusamy Chandrasekaran2 , Csaba Vágvölgyi1,2, Sándor Kocsubé**.** Mycotoxin producers in the Aspergillus genus: an update**.** Volume 59(2):151-167, 2015 Acta Biologica Szegediensis http://www2.sci.u-szeged.hu/ABS
11. Keromnes J, Thouvenot D (1985) Role of penicillic acid in the phytotoxicity of Penicillium cyclopium and Penicillium canescens to the germination of corn seeds. Appl Environ Microbiol 49:660-663.
12. Kubena, L. F. T. D. Phillips, D. A. Witzel and N. D. Heidelbaugh Environmental Contamination |and Toxicology Toxicity of Ochratoxin A and Penicillic Acid to Chicks. Bull. Environ. Contam. Toxicol. (1984)32:711-716
13. PAZHANIVEL, N C. BALACHANDRAN, 3 B.MURALI MANOHAR, 4 G.DHINAKAR RAJ, 5 V.BALAKRISHNAN AND 6 A.RAJA. STUDY OF APOPTOSIS IN THE SUBLETHAL DOSE OF PENICILLIC ACID TOXICITY IN BROILER CHICKENS. Int. J. Life Sci Pharma Res. VOL 4/ ISSUE 3/JULY-SEPTEMBER 2014

14. Stoev, S,.D. , St. Denev, M. Stefanov, B. Radic, A M. Domijan, M. Peraica. **Experimental Mycotoxicosis in Chickens Induced by Ochratoxin A and Penicillic Acid and Intervention with Natural Plant Extracts**. Veterinary Research Communications November 2004, Volume 28, Issue 8, pp 727–746
15. Visagie CM, Varga J, Houbraken J, Meijer M, Kocsubé S, Yilmaz N, Fotedar R, Seifert KA, Frisvad JC, Samson RA (2014) Ochratoxin production and taxonomy of the yellow aspergilli (Aspergillus section Circumdati). Stud Mycol 78:1-61.

4.7. Avian fusariotoxicosis

Fusarium fungi are field fungi that produce mycotoxins on the crops in the field, *Fusarium* fungi have traditionally been associated with temperate climatic conditions, since they require somewhat lower temperature for growth and mycotoxin production than, for example, the *Aspergillus* species.

Fusarium mycotoxins

Extensive data now exist to indicate the global scale of contamination of cereal grains and animal feed with *Fusarium* mycotoxins **(D'Mello and Macdonald, 1998).**

- Of particular importance are the trichothecenes, zearalenone (ZEN) and the fumonisins.
    - The trichothecenes are subdivided into four basic groups, with types A and B being the most important.
    - Type A trichothecenes include T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol (DAS).
    - Type B trichothecenes include deoxynivalenol (DON, also known as vomitoxin), nivalenol and fusarenon-X.
    - The production of the two types of trichothecenes is characteristic for a particular *Fusarium* species. However,
    - a common feature of the secondary metabolism of these fungi is their ability to synthesize ZEN which, consequently, occurs as a co-contaminant with certain trichothecenes.
- The fumonisins are synthesized by another distinct group of *Fusarium* species. Three members of this group (fumonisins B_1, B_2 and B_3) often occur together in maize.
- Virtually all the toxigenic species of *Fusarium* listed are also major pathogens of cereal plants, causing diseases such as head blight in wheat and barley and ear rot in maize.
- Harvested grain from diseased crops is therefore likely to be contaminated with the appropriate mycotoxins, and this is supported by ample evidence.
- Surveillance of grain and animal feed for the occurrence of *Fusarium* mycotoxins has been the subject of many investigations over recent years.
- The global distribution of these mycotoxins is a salient feature, but striking regional differences should also be noted.
- Another aspect worthy of comment is consistent evidence of the co-occurrence of various *Fusarium* mycotoxins in the same sample. These issues have been considered at greater length by **Placinta, D'Mello and Macdonald (1999)** who, for example, referred to
    - a German study in which 94 percent of wheat samples analysed were contaminated by between two and six *Fusarium* mycotoxins and 20 percent of the samples were co-contaminated with DON and ZEN
    - The most frequent combination included DON, 3-ADON and ZEN. T-2 and HT-2 toxins were detected at levels ranging from 0.003 to 0.250 mg/kg and 0.003 to 0.020 mg/kg, respectively, but these mycotoxins only occurred in combination with DON, NIV and ZEN.

- Animal feeds are routinely subject to contamination from diverse sources, including environmental pollution and activities of insects and microbes.
- Animal feeds may also contain endogenous toxins arising principally from specific primary and secondary substances produced by fodder plants. Thus, feed toxins include compounds of both plant and microbial origin.
- Although these toxins are often considered separately, because of their different origins, they share several common underlying features. Thus, particular compounds within both plant and microbial toxins may exert antinutritional effects or reduce reproductive performance in farm animals. Furthermore,
- the combined effects may be the result of additive or synergistic interactions between the two groups of compounds. The extent and impact of these interactions in practical livestock feeding remain to be quantified.
- Feed contaminants and toxins occur on a global scale but there are distinct geographical differences in the relative impact of individual compounds. article is limited to a review of those contaminants and toxins that represent significant risks to farm livestock.

Toxigenic Fusarium species

From the available data, almost 80 Fusarium species have been confirmed to be toxin producers. Almost all toxigenic Fusarium species produce more than one toxin reaching in some species up to 9 different toxins. Moreover, the same toxin was found to be produced by several Fusarium species, reaching up to 22 in case of fumonisins and 18 species in case of moniliformin.

1. Fusarium acaceae-mearnsii: nivalenol, 3A- Deoxynivalenol
2. Fusarium acuminatum: trichothecenes, enniatin B, moniliformin
3. Fusarium acutatum: beauvericin, fumonisin
4. Fusarium aethiopicum : 15A- Deoxynivalenol
5. Fusarium anantum: fumonisins
6. Fusarium andiyazi: moniliformin, fumonisins
7. Fusarium anthophilum: moniliformin, fumonisins
8. Fusarium armeniacum: T-2. HT-2, neosolaniol
9. Fusarium asiaticum: trichothecines
10. Fusarium astroamericanum: nivalenol, 3A- Deoxynivalenol
11. Fusarium avenaceum: beauvericin, fusarin C, moniliformin, enniatins A,B,C
12. Fusarium begonia: moniliformin, fumonisin B1
13. Fusarium beomiforme: moniliformin, beauvericin
14. Fusarium boothii : 15A- Deoxynivalenol
15. Fusarium brasilicum: nivalenol, 3A- Deoxynivalenol
16. Fusarium brevicatenulatum: fumonisin B1
17. Fusarium chlamydosporum: moniliformin
18. Fusarium circinatum: beauvericin, fusaric acid
19. Fusarium compactum trichothecenes
20. Fusarium concentricum: fumonisins
21. Fusarium crookwellense: nivalenol, zearalenone, fusaric acid, fusarin C

22. Fusarium culmorum: moniliformin, deoxynivalenol, fusarin C, zearalenone, trichothecines
23. Fusarium dactylidis: nivalenol , zearalenone
24. Fusarium delphinoides: indole-3-acetic acid
25. Fusarium denticulatum: moniliformin
26. Fusarium dlaminii: beauvericin, moniliformin, fumonisins
27. Fusarium equiseti: butenolidem, beauvericin, trichothecenes, nivalenol, T-2 toxin, fusarochromanon, zearalenone, equisetin,
28. Fusarium fujikuroi: moniliformin, beauvericin, fusaric acid
29. Fusarium gerlachii: nivalenol
30. Fusarium globosum: fumonisin, beauvericin, fusaproliferin
31. Fusarium graminearum: zearalenon, nivalenol, 3ADeoxynivalenol, 15A-Deoxynivalenol
32. Fusarium guttiforme: beauvericin, fusaproliferin
33. Fusarium heterosporum: fusaric acid
34. Fusarium konzum: fumonisins, beauvericin, fusaproliferin
35. Fusarium kyushuense: trichothecenes
36. Fusarium lactis: moniliformin
37. Fusarium lateritium: enniatins, lateropyrone
38. Fusarium longipes: beauvericin
39. Fusarium langsethiae: diacetoxyscirpenol, T-2 toxin , HT- 2 toxin, neosolaniol culmorins, chrysogine, aurofusarin, and enniatins
40. Fusarium louisianense: nivalenol
41. Fusarium mangiferae: azepinostatin
42. Fusarium meridionale: nivalenol
43. Fusarium mesoamerricanum: nivalenol, 3A- Deoxynivalenol
44. Fusarium musae: moniliformin
45. Fusarium musarum: trichothecenes
46. Fusarium napiforme: moniliformin, fusaric acid, fumonisins
47. Fusarium nepalense: 15A- Deoxynivalenol
48. Fusarium nisikadoi: moniliformin
49. Fusarium nygamai: beauvericin, fusaric acid, fumonisins, moniliformin
50. Fusarium oxysporum: beauvericin, bikaverin, enniatins, fusaric acid, fusarin C, isoverrucanol, moniliformin, sambutoxin, wortmannin, fumonisins
51. Fusarium phyllophilum: fumonisins, moniliformin, beauvericin, fusaproliferin
52. Fusarium poae: beauvericin, fusarin C, trichothecenes
53. Fusarium polyphialidicum: fuminosins
54. Fusarium proliferatum: gibberellic acid, beauvericin, fusaproliferin, fusaric acid, fusarins,,moniliformin
55. Fusarium pseudoanthophilum: beauvericin
56. Fusarium pseudocircinatum: moniliformin, fusaproliferin, fumonisins
57. Fusarium pseudograminearum: deoxynivalenol, 3-acetyl deoxynivalenol, zearalenone
58. Fusarium pseudonygamai: moniliformin, fusaproliferin, fumonisins
59. Fusarium ramigenum: moniliformin, fusaproliferin, beauvericin, fumonisin B1, fumonisin B2
60. Fusarium redolens: fusaric acid, fumonisins
61. Fusarium sacchari: fusaric acid, fumonisins
62. Fusarium sambucinum: enniatins, beauvericin, fusaric acid, fusarin C, sambutoxin, wortmannin

63. Fusarium semitectum: apicidins, beauvericin, equisetin, fusapyrone, moniliformin, sambutoxin, trichothecenes, zearalenone
64. Fusarium sibiricum : trichothecenes
65. Fusarium solani: deoxynivalenol, T-2 toxin, zearalenone
66. Fusarium sporotrichioides: butenolide, fusarin C, monilformin, scirpentriol, zearalenone, T-2 toxin
67. Fusarium sterilihyphosum: monilformin
68. Fusarium subglutinans: moniliformin, beauvericin, fusaric acid, fusaproliferin, fumonisins
69. F. succisae fusaproliferin
70. Fusarium temperatum: moniliformin, beauvericin, enniatins, fumonisin B1
71. Fusarium thapsinum: moniliformin, fusaric acid, fumonisins
72. Fusarium torulosum: enniatin B, wortmannin
73. Fusarium tricinctum :fusarin C, enniatins, moniliformin
74. Fusarium tumidum: neosolaniol
75. Fusarium udum: fusaric acid
76. Fusarium ussurianum : trichothecenes 3A- Deoxynivalenol
77. Fusarium venenatum: trichothecenes
78. Fusarium virguliforme: toxin FvTox1
79. Fusarium verticillioides : fumonisins, fusaric acid, fusarin C, beauvericin
80. F. vorosi: trichothecenes 3A-Deoxynivalenol

The major Fusarium mycotoxins occurring in poultry feeds are:

- Deoxynivalenol (DON)
- Fumonisins B_1 (FB_1)
- Fusaric acid (FA)
- Moniliformin (M)
- T-2 toxin
- Zearalenone (ZEN)

Fusarium toxins occurrence in feed and feed raw materials worldwide (Placinta *et al.,* 1999).

- From a global perspective, three classes of *Fusarium* mycotoxins may be considered to be of particular importance in animal health and productivity deoxynivalenol (DON) is widely associated with feed rejection in pigs, The surveillance of grain and animal feed for the occurrence of *Fusarium* mycotoxins continues to attract worldwide attention and has been the subject of extensive investigations over recent years high incidence rates of contamination with DON and another trichothecene, nivalenol (NIV), have been reported in maize samples in New Zealand.
- In Poland, unacceptably high values (up to 927 mg/kg) for DON were recorded for maize grain and cobs.

- Potentially harmful levels of DON (up to 40 mg/kg) were also observed in wheat produced in Germany, Poland, Japan, New Zealand, USA, Canada and Argentina.
- Samples of barley grain in Norway, Japan and USA were found with DON levels of up to 71 mg/kg.
- In the Norwegian study oat samples were also contaminated with DON at levels ranging from 7 to 62 mg/kg grain.
- Abnormally high concentrations of both NIV and ZEN have been observed in some Japanese barley samples (up to 26 and 15 mg/kg, respectively), and in maize produced in New Zealand (up to 7 and 10.5 mg/kg, respectively).
- Other trichothecenes such as 3-acetyl DON, diacetyoxyscirpenol (DAS), T-2 toxin and HT-2 toxin have also been found in cereals and animal feed in both temperate and tropical countries.
- In Uruguay all samples of maize-based animal feeds tested were positive for fumonisin B_1 (FB_1).
- The highest FB_1 values were observed in South Africa for compound feed (11 000 µg/kg), and in Thailand and China for maize (18 800 and 25 970 µg/kg, respectively). In a study of Argentinian maize, FB_2 was the major fumonisin at values of up to 11 300 µg/kg.

It is concluded that, although sample size has been small in a number of surveys, there is nevertheless unequivocal evidence of global contamination of cereal grains and animal feed with several trichothecenes, ZEN and fumonisins. Furthermore, it is clear that legislation for the control of these mycotoxins in animal feed is now overdue and that further work is required to exploit cereal genotypes that are resistant to diseases caused by toxigenic *Fusarium* phytopathogens

- During an 8-year period, 17 316 samples of feed and feed raw materials from all over the world were analysed for contamination with aflatoxins, ochratoxin A, zearalenone, deoxynivalenol and fumonisins. (**Streit et al., 2013).**

    - Overall, 72% of the samples tested positive for at least one mycotoxin and 38% were found to be co-contaminated.
    - Mycotoxin concentrations were generally low and the majority of the samples were compliant with the most stringent EU guidance values or maximum levels for mycotoxins in feed.
    - In their present state these regulations do not address co-contamination and associated risks.
    - Long-term trends are difficult to establish as strong yearly variations were observed regarding mycotoxin prevalence and contamination levels. In some cases unusual weather conditions can be linked with high observed mycotoxin loads.
    - An exception to this rule is South-East Asia, where a steady increase of aflatoxin prevalence has been observed.

Global implications of Fusarium toxins for animal health, welfare and productivity (D'mello *et al.,* 1999)

- Trichothecenes, zearalenone (ZEN) and fumonisins are the major Fusarium mycotoxins occurring on a worldwide basis in cereal grains, animal feeds and forages. Other important Fusarium mycotoxins include moniliformin and fusaric acid.
- Spontaneous outbreaks of Fusarium mycotoxicoses have been recorded in Europe, Asia, New Zealand and South America and, in addition, chronic exposure occurs on a regular and more widespread scale.
- The metabolism and adverse effects of the Fusarium mycotoxins are considered in this review with particular reference to recent data on specific and proposed syndromes and to interactions among co-occurring mycotoxins.
    - Within the trichothecene group, deoxynivalenol (DON) is associated with emesis, feed refusal and depressed feed intake in pigs,
    - T-2 toxin and diacetoxyscirpenol (DAS) are now clearly linked with oral lesions in poultry.
    - The gut microflora of farm livestock are able to transform DON to a de-epoxy derivative.
    - The ovine metabolism of ZEN results in the production of five metabolites and relatively high levels of these forms may be excreted in the urine as glucuronides.
    - Fumonisins are positively linked with pulmonary edema in pigs, leukoencephalomalacia in equines and with deranged sphingolipid metabolism in these animals.
    - Fusarium mycotoxins have also been provisionally implicated in ovine ill-thrift, acute mortality of poultry and in duodenitis/proximal jejunitis of horses. Several Fusarium mycotoxins may co-occur in a particular feed ingredient or in compound feedingstuffs.

- In general, combinations of Fusarium mycotoxins result in additive effects, but synergistic and/or potentiating interactions have been observed and are of greater concern in livestock health and productivity.
    - Synergistic effects have been reported between DON and fusaric acid; DON and fumonisin B1 (FB1); and DAS and the Aspergillus-derived aflatoxins.
    - Limited evidence of potentiation between FB1 and DON or T-2 toxin has also emerged recently.
    - Additive and synergistic effects between known and unidentified mycotoxins may account for enhanced adverse effects observed on feeding Fusarium-contaminated diets.

Impact of *Fusarium* mycotoxins on animal host susceptibility to infectious diseases (Antonissen *et al*., 2014)

Fusarium mycotoxins are capable of inducing both acute and chronic toxic effects. These effects are dependent on the mycotoxin type, the level and duration of exposure, the animal species that is exposed and the age of the animal.

- Oral intake of low to moderate amounts of *Fusarium* mycotoxins are capable of inducing both acute and chronic toxic effects:
    - **The gastro-intestinal epithelial cell layer** will be exposed first
    - The intestinal mucosa acts as a barrier, preventing the entry of foreign antigens including food proteins, xenobiotics (such as drugs and toxins), commensal microbiota and pathogens into the underlying tissues
    - **The mucosal immunity**, which consists of an innate and adaptive immune system, can be affected by *Fusarium* mycotoxins
        - DON and FB1 are able to increase the permeability of the intestinal epithelial layer avian origin
        - the viability and proliferation of intestinal epithelial cells can be negatively affected by *Fusarium* mycotoxins
        - Their effect on mucus production is variable:
            - Co-exposure of low doses of DON, T-2 and ZEN reduces the number of goblet cells
            - ZEN given alone at higher doses increases the activity of goblet cells
            - Several mycotoxins are also able to modulate the production of cytokines *in vitro*and *in vivo* ,
            - DON increases the expression of TGF-β and IFN-γ in mice and fumonisins decrease the expression of IL-8 in an intestinal porcine epithelial cell line (IPEC-1)

- ***Fusarium* mycotoxins can cross the intestinal epithelium and reach the systemic compartment affecting the immune system**.
    - Exposure to these toxins can either result in immunostimulatory or immunosuppressive effects depending on the age of the host and exposure dose and duration
    - Mycotoxin-induced immunomodulation may affect innate and adaptive immunity by an impaired function of macrophages and neutrophils, a decreased T- and B-lymphocyte activity and antibody production

.

- Low amounts may impair intestinal health, immune function and/or pathogen fitness, resulting in altered host pathogen interactions and thus a different outcome of infection.
    - exposure to deoxynivalenol and other *Fusarium* mycotoxins generally exacerbates infections with parasites, bacteria and viruses across a wide range of animal host species. Well-known examples include

coccidiosis, salmonellosis, colibacillosis necrotic enteritis, aspergillosis etc.

  - On the other hand, T-2 toxin has been shown to markedly decrease the colonization capacity of *Salmonella* in the intestine.

- Published data papers clearly indicate a negative influence of *Fusarium* mycotoxins on the intestinal function and immune system. Since the intestinal tract is also a major portal of entry to many enteric pathogens and their toxins, mycotoxin exposure could increase the animal susceptibility to these pathogens. This is illustrated in the following 2 examples

1. **The potential for Fusarium mycotoxins to modulate immunity was studied in chickens raised to 10 weeks of age using an enteric coccidial infection model**. Experimental diets included: control, diets containing grains naturally contaminated with Fusarium mycotoxins, and diets containing contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GMA). Contaminated diets contained up to 3.8 microg/g deoxynivalenol (DON), 0.3 microg/g 15-acetyl DON and 0.2 microg/g zearalenone**.(Girgis *et al.,* 2008)**

- Total serum immunoglobulin (Ig) A and IgG concentrations in challenged birds fed the contaminated diet were higher than controls at the end of the challenge period.
- Serum concentration of IgA, but not IgG, was significantly decreased at the end of the recovery period in birds fed the contaminated diet.
- The percentage of CD4+ and CD8+ cell populations in blood mononuclear cells decreased significantly at the end of the challenge period in birds fed the control or the contaminated diet compared to their percentages prior to challenge.
- The pre-challenge percentage of CD8+ population was restored at the end of the recovery period only in birds fed the control diet.
- Interferon-gamma (IFN-gamma) gene expression in caecal tonsils was up-regulated in challenged birds fed the contaminated diet at the end of the challenge period.
- No significant effect of diet was observed on oocyst counts despite the changes in the studied immune parameters.
- It was concluded that Fusarium mycotoxins modulate the avian immune system. This modulation involves alteration of gene expression but apparently does not enhance susceptibility or resistance to a primary coccidial challenge.

- To conclude, *Fusarium* mycotoxins negatively affect the innate and adaptive cellular immune response against *Eimeria*, though without changing the oocyst yield. Further data of clinical coccidiosis lesion scoring is still needed in order to evaluate the effect of *Fusarium* mycotoxins on the severity of the disease.

2. The intake of DON-contaminated feed is a predisposing factor for the development of necrotic enteritis in broiler chickens (Antonissen *et al*.,2013)

- An experimental Clostridium perfringens infection study revealed that DON, at a contamination level of 3,000 to 4,000 mg/kg feed, increased the

percentage of birds with subclinical necrotic enteritis from 2062.6% to 4763.0% (P<0.001).

- DON significantly reduced the transepithelial electrical resistance in duodenal segments (P<0.001) and decreased duodenal villus height (P = 0.014) indicating intestinal barrier disruption and intestinal epithelial damage, respectively. This may lead to an increased permeability of the intestinal epithelium and decreased absorption of dietary proteins.

- DON had no effect on in vitro growth, alpha toxin production and netB toxin transcription of Clostridium perfringens.

- In conclusion, feed contamination with DON at concentrations below the European maximum guidance level of 5,000 mg/kg feed, is a predisposing factor for the development of necrotic enteritis in broilers. These results are associated with a negative effect of DON on the intestinal barrier function and increased intestinal protein availability, which may stimulate growth and toxin production of Clostridium perfringens

Toxicokinetics (Guerre, 2015)

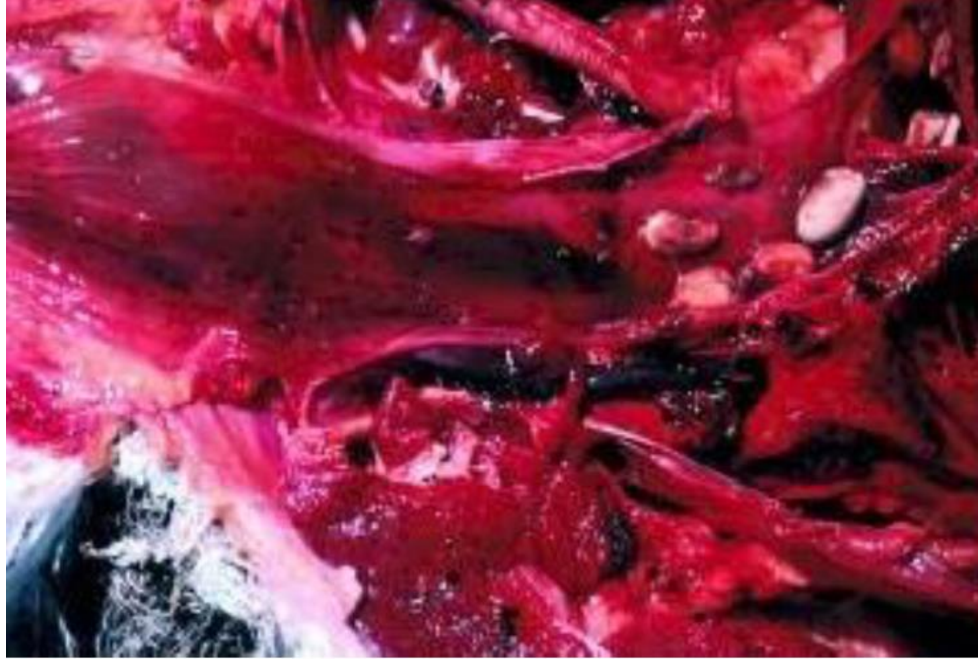
Toxinokinetic studies have focused on the main fusariotoxins deoxynivalenol, T-2 and HT-2 toxins, zearalenone and fumonisin B1 and B2. The key parameters used in the toxicokinetic studies are presented along with the factors responsible for their variations. Then, each toxin was analyzed separately. Results of studies conducted with radiolabelled toxins were compared with the more recent data obtained with HPLC/MS-MS detection.

- Recent studies demonstrated the important role of metabolism in avian species. Even if the metabolic pathways are the same as those in mammals, different metabolites can be formed.
    - Deep oxidation of DON, which is the main detoxification mechanism in mammals, appears to play a less important role in avian species, whereas in these species, sulfation is a key protective mechanism.
    - The metabolism schedule also varies with the toxin.
        - Sulfation and glucuronidation are important steps in DON and zearalenone metabolism.
        - Hydroxylation and deacetylation are important in T-2 toxin metabolism,
        - No sulfate or glucuronide of T-2 toxin have been reported.
- The metabolites formed from a toxin are the same among the avian species tested, the ratio of the metabolites appears to vary with the species.
    - This has been demonstrated for the DON-3α-sulfate:DON ratio in broilers and turkey poults and in the α:β ratio of zearalenol in different avian species.
    - The metabolism of fumonisins in avian species indicated that their oral bioavailability, clearance and persistence in tissues appear to vary between broilers, ducks and turkeys.

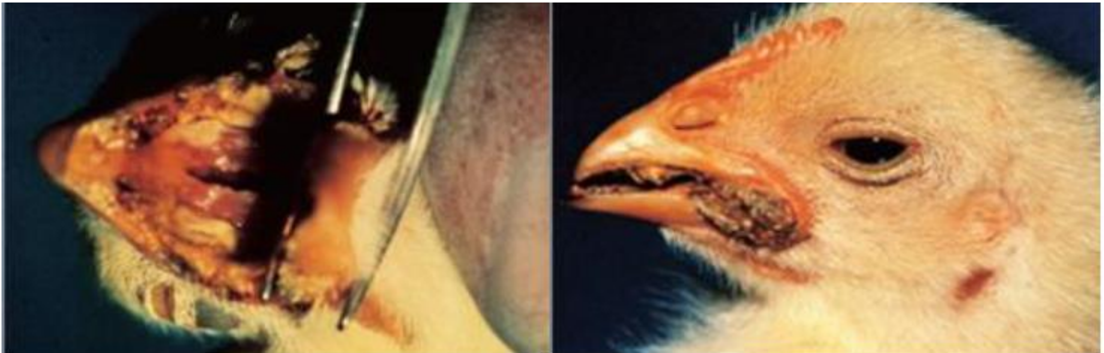
- Taken together, these results suggest major differences in the toxicokinetics of fusariotoxins in avian species, and marked high variation between species in the level of some key metabolites
- The use of radiolabelled DON, T2-toxin, and zearalenone revealed high biliary excretion of these toxins whereas the amount of the parent compound in plasma was low.
- This observation and the low level of radioactivity found in tissues led to the conclusion that fusariotoxins are weakly absorbed and rapidly eliminated.

Pathology of fusariotoxicosis in birds

A sandhill crane suffering from fusariotoxicosis, Fluid beneath the skin of the head and neck of a sandhill showing wing and head droop, **Ronald M. Windingstad** crane with fusariotoxicosis. **J. Christian Franso**



Inflammation and ulceration of the mucosal surface of the oesophagus in a sandhill crane with fusariotoxicosis. James Runningen



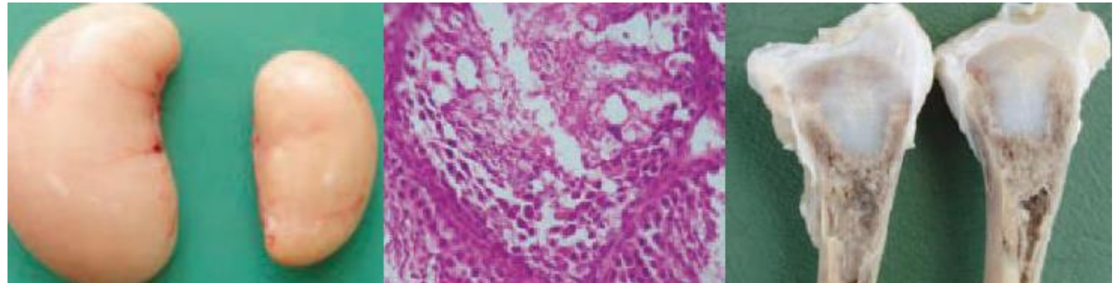
Stomatitis following consumption of T2 fusariotoxin. Chick showing stomatitis attributed to T2.fusariotoxicosis, **Dr.Mohamed Abdel - Moniem Amer**

Ivan Dinev, Diseases of Poultry

erosions and ulcers in gizzard cuticulum , thickened wall of the proventriculus hyperaemic and haemorrhagic mucous coat of the gizzard **Ivan Dinev, Diseases of Poultry**

reddening and hemorrhage of intestinal muscosa. **Ivan Dinev, Diseases of Poultry**

Frequent findings in fusariotoxicoses are the massive subcapsular liver haematomas, causing sudden death in broilers. **Ivan Dinev, Diseases of Poultry**



The fusariotoxin zearalenone has an effect, identical to that of oestrogenic hormones and results in reduction of testes in cocks. Left - normal; right - atrophied testis in a cock, in whose diet high zearalenone concentrations have been determined. Microscopically, the testes of cocks with zearalenone fusario-toxicosis, show a fatty infiltration and atrophy of the germinative epithelium with the exception of the basal layer as well as interruption of the spermatogenesis., Fusarochromanone causes tibial dyschondroplasia in broiler chickens, manifested with long bone deformation. **Ivan Dinev, Diseases of Poultry**

Human risk from avian fusariotoxicosis

- The potential for transmission of DON into eggs and of ZEN into porcine kidney and liver has been demonstrated. .
- It is concluded that livestock health, welfare and productivity may be severely compromised by consumption of DON, T-2 toxin, DAS, ZEN and fumonisins and by interactions among these mycotoxins.
- Safety of some animal products may also be at risk. Furthermore, in view of the limited options available for remediation, it is concluded that exploitation of crops resistant to Fusarium infection offers the most viable strategy for reducing mycotoxin contamination of grain and animal feed

- It is generally accepted that absorption of fusariotoxins by avian species is limited and that their elimination is rapid, thereby reducing the risk of toxicity and persistence in tissues. Consequently, human exposure to fusariotoxins through consumption of poultry meat and eggs is considered to be negligible compared with exposure through the consumption of cereals.

Identification of Fusarium species

Morphological identification

Fusarium cultures are examined for macromophological features typical for Fusarium species namely, woolly to cottony, flat, spreading colonies, white, cream, tan, salmon, cinnamon, yellow, red, violet, pink or purple; and on the reverse, it may be colourless, tan, red, dark purple, or brown, and the micromorphological features namely: curved, transversely septate conidia macroconidia, produced from sporodochia or pionnotes, smaller conidia of various shapes and septation (“microconidia” and/or “mesoconidia”) produced from unbranched or branched mycelial conidiophores, producing conidiogenous cells with monophialidic polyphialidic openings, and chlamydospores which are thick-walled, generally globose thallospores, produced in or on hyphae or conidia, singly or in chains or bunches, in addition to sexual spores, when observed, which are produced in flask-shaped fruiting bodies (perithecia) that are usually in shades of red, orange, blue or purple, with little or no stromatal tissue.

Asci produced from distinct hymenia, single-walled (unitunicate) containing eight ascospores, which usually possess one or more septa, but can be aseptate.

Molecular Methods for Identification of Fusarium

Molecular biology has offered a number of insights into the detection and enumeration of fungal pathogens and information on identifying unknown species from their DNA sequences. In recent years, there has been vast progress in the development of molecular biological tools and technologies. Each technique can be used as a tool to study variation amongst fungal isolates, and hence provide important information on genetic relationships, taxonomy, population structure and epidemiology associated with fungi.

Molecular markers used for identification of Fusarium

- sequence characterized amplified regions (SCAR),
- single strand conformational polymorphism (SSCP),
- randomly amplified polymorphic DNA (RAPD),
- amplified fragment length polymorphism (AFLP),
- restriction fragment length polymorphism (RFLP),
- sequence related amplified polymorphism (SRAP),
- single nucleotide polymorphism (SNP),
- variable number of tandem repeat (VNTR)
- SNP-based multilocus genotyping assay

Identification by comparison with databases

- The FUSARIUM-ID server at http://fusarium.cbio.psu.edu
- BLAST search tool that allows users to query unknown sequences against the database.
- GenBank database is publicly available for identification purposes, and can be accessed via the Entrez website at the US National Center for Biotechnology Information (NCBI): http://www.ncbi.nlm.nih.gov/Entrez/.
- It is strongly recommend to use FUSARIUM-ID because it contains vouchered and well-characterized sequences that correspond to publicly available cultures that can be used for confirmation.
- FUSARIUM-ID can be used in conjunction with GenBank.

References

1. Antonissen G., Van Immerseel F., Pasmans F., Ducatelle R., Haesebrouck F., Timbermont L., Verlinden M., Janssens G.P.J., Eeckhout M., de Saeger S., et al. Deoxynivalenol predisposes for necrotic enteritis by affecting the intestinal barrier in broilers; Proceedings of the International Poultry Scientific Forum; Atlanta, Georgia, USA. 28–29 January 2013; pp. 9–10.
2. Antonissen, G., Martel, A., Pasmans, F., Ducatelle, R., Verbrugghe, E., Vandenbroucke, V., Croubels, S. (2014). The Impact of *Fusarium*Mycotoxins on Human and Animal Host Susceptibility to Infectious Diseases.*Toxins*, *6*(2), 430–452. http://doi.org/10.3390/toxins6020430

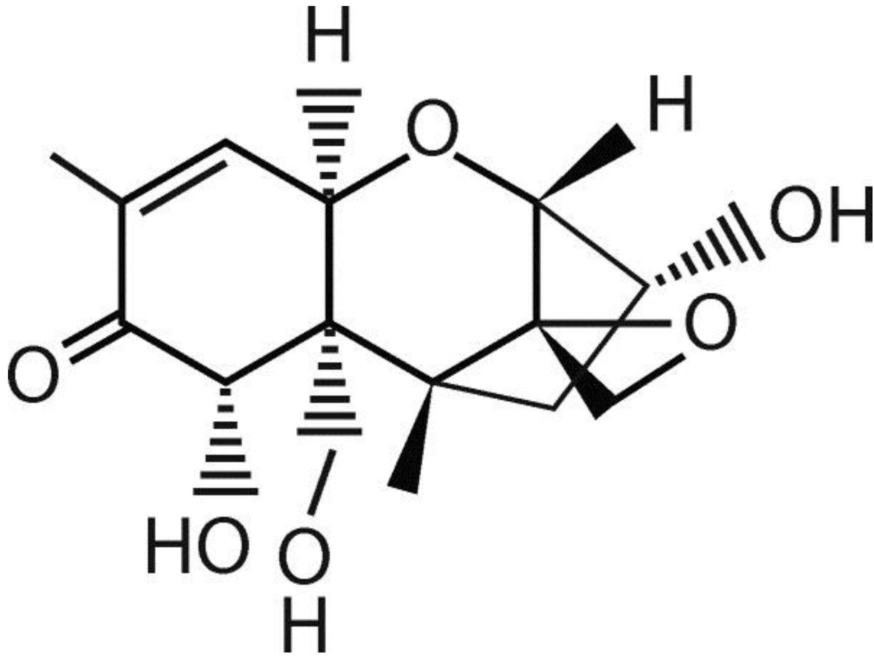
3. Devreese M., de Backer P., Croubels S. Overview of the most important mycotoxins for the pig and poultry husbandry. Vlaams Diergeneeskundig Tijdschrift. 2013;82:171–180.
4. D'mello J., Placinta C., Macdonald A. *Fusarium* mycotoxins: A review of global implications for animal health, welfare and productivity. Anim. Feed Sci. Tech. 1999;80:183–205
5. Girgis G.N., Sharif S., Barta J.R., Boermans H.J., Smith T.K. Immunomodulatory effects of feed-borne*fusarium* mycotoxins in chickens infected with coccidia. Exp. Biol. Med. 2008;233:1411–1420. doi: 10.3181/0805-RM-173. [PubMed] [Cross Ref]\
6. Guerre, P. (2015). Fusariotoxins in Avian Species: Toxicokinetics, Metabolism and Persistence in Tissues. *Toxins*, *7*(6), 2289–2305. http://doi.org/10.3390/toxins7062289
7. Murugesan G.R., Ledoux D.R., Naehrer K., Berthiller F., Applegate T.J., Grenier B., Phillips T.D., Schatzmayr G. Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. [(accessed on 18 June 2015)];Poult. Sci. 2015 94:1298–1315.
8. Placinta C., D'mello J., Macdonald A. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim. Feed Sci. Tech. 1999;78:21–37
9. Streit E., Naehrer K., Rodrigues I., Schatzmayr G. Mycotoxin occurrence in feed and feed raw materials worldwide-long term analysis with special focus on Europe and Asia. J. Sci. Food Agric. 2013;93:2892–2899.

4.7.1. Deoxynivalenol (DON)

Deoxynivalenol (DON) is a natural-occurring mycotoxin mainly produced by *Fusarium graminearum* (**Kushiro, 2008**). It is also known as vomitoxin due to its strong emetic effects after consumption, because it is transported into the brain, where it runs dopaminergic receptors. The emetic effects of this mycotoxin were firstly described in Japanese men consuming mouldy barley containing *Fusarium* fungi in 1972 (**Ueno, 1985; Ueno, 1988**). DON is probably the best known and most common contaminant of grains and their subsequent products. Its occurrence in food and feed represent more than 90% of the total number of samples and it is a potential marker of the occurrence of other mycotoxins.

Chemical properties of DON

Chemically DON is a member of the trichothecenes family of mycotoxins. Structurally, it is a polar organic compound, which belong to the type B trichothecenes and its chemical name is 12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8-on (Nagy *et al*., 2005). In its molecule it contains 3 free hydroxy groups (-OH), which are associated with its toxicity.



Chemical structure of deoxynivalenol (DON).

Physicochemical property of DON

- One of the most important physicochemical property of DON is its ability to withstand high temperatures, which is the risk of its occurrence in food **(Hughes *et al*., 1999).**
- Numerous studies have documented that DON was heat-stable. DON is very stable under temperature within the interval from 170°C to 350°C, with no reduction of DON concentration after 30 min at 170°C.
- DON levels are reduced in cooked pasta and noodles because of leaching into the cooking water (**Manthey *et al*., 2004; Sugita-Konishi *et al*.,2006; Visconti *et al*., 2004**)
- DON is water-soluble, but no reduction of its concentration was observed during frying DON-contaminated food in oil.
- Some evidence indicates that DON levels may be reduced during the processing, mainly boiling in water

Natural occurrence of DON

- DON is the most common contaminant of feedstuffs worldwide. It was found in cereal grains (wheat, maize, barley, oat and rye and less often in rice, sorghum and triticale).
- DON contaminates mainly corn and wheat, while small grains, such as oats, rye and barley, have less DON contamination [**CAST, 2003].**
- The natural occurrence of DON in grains used for poultry is normally between 0 and 5 mg/kg, although concentrations can be higher
- DON contamination can be noticed when corn kernels ripen prematurely and unevenly and have a blanched appearance. At harvest, kernels may show pink

color. The natural occurrence of DON in grains used for poultry is normally between 0 and 5 mg/kg, although concentrations can be higher

- DON production was minimized by improved storage conditions (<14% moisture), while cool temperatures and high humidity are the environmental conditions that favour the fungal development in the field [**Richard, 2007].**

Susceptibility of poultry to DON:

- Chickens are considered to be less sensitive compared to other species, especially the pig. This can be attributed to differences in DON absorption, distribution, metabolism and elimination [**Pestka et al., 2005]**
    - Chickens resist DON due to the low level of absorption into plasma and tissues in addition to rapid clearance (**Prelusky *et al.,* 1986**)
    - A limited oral absorption and rapid plasma clearance of DON was found in turkeys (**Gauvreau, 2000)**
    - The intestinal microflora convert DON to de-epoxy DON (DOM-1) in birds (**He *et al.*, 1992)**
    - The degradation of the epoxide group by reductive cleavage of the toxic 12, 13-epoxy ring is carried out by intestinal microflora in chickens.
    - *Eubacterium* sp. DSM 11798 was capable of completely compensating for the adverse effect of DON in poultry (Awad *et al.*, 2013)

Effects of DON on growth performance:

- DON impact on growth performance in poultry is highly variable, due to differences in strains of poultry and diets used.
- Some studies failed to notice an adverse effect on performance of poultry, including broilers, laying hens, ducklings and turkeys. In broilers, even levels of DON up to 15 mg/kg could not produce an adverse influence on body-weight gain, feed intake or feed efficiency **(Harvey *et al.*, 1997, Swamy *et al.,* 2002. Li et al., 2003, Awad et al., 2004, 2006a and b,** 2011a and b)
- A reduction in body weight, feed intake and body weight gain of broilers fed diets artificially contaminated with 10 mg DON/kg diets was documented **(Awad *et al.***, 2012, **Ghareeb *et al.***2012)
- **In laying hens**, performance traits were adversely affected by chronic feeding of DON (**Dänicke et al., 2002)**
- Performance of **laying hens**, egg production, fertility and hatchability of eggs remained unaffected after feeding of 2–3 mg/kg DON **(Keshavarz, 1993)**
- **Egg production** was negatively affected in **hens** fed a diet containing sorghum that was contaminated with zearalenone (ZON) at a level of 1.1 mg/kg and DON at a level of 0.3 mg/kg (**Branton et al.(1989)**

- **In Peking ducklings**, feed refusal was observed after natural contamination of the diet with 0.3–1.2 mg DON/kg and 0.01 mg of aflatoxin B1/kg feed (**Davis et al.,1994)**
- **In turkeys**, feeding of corn contaminated with DON up to 10 mg/kg reduced poults body weight gain at the third week of life (Xu et al., 2011)
- Only a slight reduction in the body weight gain was found in **turkeys** when fed increasing proportions of *Fusarium* toxin-deoxynivalenol contaminated wheat (0.10, 1.96, 4.66 and 5.42 mg DON/kg diet) **(** Dänicke et al., 2007)
- DON decreased the small intestinal absorption of glucose and amino acids in **broilers and laying hens**, which can displace the nutrient uptake to the intestinal distal parts. (**Keshavarz, 1993, Awad, 2005**)
- DON can be completely transformed to de-epoxy-DON after incubating for 96 h with the content of the large intestine of hens. This may explain why DON did not strongly influence the performance traits in some studies regarding broilers, laying hens, ducklings and turkeys **(He *et al.,* 1992)**

Effects of DON on internal organs

- Feeding of **Peking ducks** with an increasing proportion of DON contaminated wheat (6–7 mg DON/kg and 0.05–0.06 mg ZON/kg) led to a relative decrease of the mass of the bursa of Fabricius, which may reduce the production of antibodies (**Dänicke *et al.,* 2004)**
- In **ducks,** higher heart, liver and pancreas weight were reported after feeding of DON, and in broilers gizzard, heart and bursa of Fabricius were having a higher weight. On the other hand, the liver mass was reduced in broilers fed diets containing (9 or 18 mg DON/kg) (**Kubena et al., 1985, 1997, Cheng et al.,2004.**
- Gizzard mucosa had small erosions in laying hens fed DON in a very high concentration of 82.8 mg/kg for about four weeks in addition to higher absolute and relative gizzard weights. This was considered as an irritant effect of DON on the mucosa as reported (**Lun *et al,1986***)
- In hens, a decrease of the weight of the small intestine was observed after *Fusarium* mycotoxin (0.02 DON and 0.002 mg ZON/kg) intake (**Dänicke *et al.*, 2002)**
- In broiler chickens, villus atrophy and alteration of villus crypts of broilers were found after feeding of either artificial or natural DON contaminated diets, and the structure of duodenal and jejunal mucosa was affected in the form of shorter and thinner villi due to DON exposure . Those results suggest that DON adversely affects the intestinal digestive and absorptive functions. Contrary to that, in ducks and in turkey poults, DON did not affect the intestinal histology (Branton ***et al.,* 1989, Morris *et al.,* 1999, Awad *et al.,* 2004, 2006, Dänicke *et al.,* 2007. Awad *et al*., 2011a and b, Xu *et al.,* 2011,).**

Effects of DON on humoral and cellular immune response

- The feeding of diets containing 50 mg of purified DON/kg of depressed mitogen induced lymphocyte proliferation and the antibody response to the Newcastle disease vaccine in 3-wk-old broiler chickens (**Harvey et al., 1991**).
- The feeding of diets containing 17.6 mg of DON/kg and 1.6 mg of ZEN/kg. decreased antibody titer against the Newcastle disease virus in laying hens (**Dänicke *et al.* (2002)**
- In chickens, humoral immunity can be either stimulated or impaired by DON and other trichothecenes. In poultry, serum antibody titers to common viral vaccines can be useful to evaluate the humoral immunotoxicity of DON (Dänicke et al., 2003)
- In broiler chickens, DON was shown to suppress the vaccination response to infectious bronchitis virus (IBV), Newcastle disease virus (NDV). Recently, DON was shown to suppress the antibody response to infectious bronchitis vaccine (IBV) in broiler chickens **(Harvey *et al.,* 1991, Dänicke *et al*., 2002,** Yegani ***et al.,* 2006, Yunus *et al.,* 2011, Ghareeb *et al.,* 2012)**
- Feeding of contaminated diets with *Fusarium* mycotoxins to chickens did not cause significant changes in serum or bile immunoglobulin concentrations **(Swamy *et al.,* 2004)**
- Furthermore, a higher biliary IgA level was reported in turkey fed DON (**Chowdhury *et al*., 2005)**
- Contrary to this, it was shown that the biliary IgA was reduced by DON **(Li et al., 2003)**
- Feeding a mixture of mycotoxins, including DON to broiler chickens, was shown to reduce IgA, the relative weight of the spleen, the mRNA expression of IFN-γ and the antibody titers against Newcastle disease **(Li et al., 2012)**
- Dietary DON alters immune function in laying hens. An important immunotoxic effect was seen after dietary inclusion of DON in diets for laying hens and broilers, such as the reduction of white blood cell and total lymphocyte numbers **(Chowdhury *et al.,* 2005)**
- Deoxynivalenol produced genotoxic effects on circulating blood lymphocytes **(Awad** *et al.,* 2012)
- Leukocytes, isolated from chicken spleen, had higher DNA fragmentation when animals were exposed to 10 mg DON/kg feed **(** Frankic *et al.,* 2006)
- Chronic feeding of 10 mg DON/kg feed to broilers decreased the plasma concentration of TNF-α **(Awad *et al*., 2012).**
- in broiler chickens, splenic mRNA expression of IFN-γ was downregulated as a result of chronic feeding of naturally contaminated diets with DON and other *Fusarium* mycotoxin contaminated diets **(Li *et al*., 2012)**

- Furthermore, plasma concentration of TNF-α was significantly reduced after chronic exposure to 10 mg DON/kg diet in 5 wk old broiler chickens **(Awad *et al*., 2012).**
- In contrast, interferon-γ (IFN-γ) gene expression was upregulated in the caecal tonsils of chickens fed *Furarium* mycotoxins challenged with coccidia. In this context, it becomes evident that further research is required to investigate the effects of DON on the innate immune response **(Girgis *et al.,* 2008).**

Toxicokinetics and the persistence of DON in tissues:

Toxicokinetics and the persistence of DON in tissues have been investigated using ^{14}C- and ^{3}H-radiolabeled DON.

1. **In early studies, uniformly labeled ^{14}C-DON was solubilized in methanol and administered to hens with 5 g of feed (1.5 µCi/bird/day** equivalent to 2.2 mg/bird/day) following a fasting period of 3 h, after which feed and water were provided *ad libitum* (Prelusky *et al*., 1986)
   - Maximum radioactivity in plasma was measured three hours after administration, and represented less than 1% of the amount administrated.
   - The maximum concentrations in tissues were found in the small intestine, liver and kidney, while the concentrations in muscle and fat were lower.
   - The highest concentrations were measured in the bile, suggesting a strong first pass effect and biliary excretion of the toxin.
   - Elimination via the excreta accounted for 78.6%, and 98.5% of the dose after 24 and 72 h, respectively.
   - Daily administration of a similar level over a period of eight to 12 days revealed minimal accumulation of the toxin
2. **Transmission of ^{14}C-DON and of its metabolites to eggs was studied in White Leghorn hens fed an equivalent of 5.5 mg DON/kg feed for 65 days**
   - Radioactivity in the eggs increased rapidly to reach a maximum of 28 ng equivalent DON/g on day 8 after administration.
   - Subsequently, radioactivity slowly decreased until day 30, and stabilized at 7 ng equivalent DON/g egg, although exposure to ^{14}C-DON remained the same. The reason for this decline was unknown, but the authors suggested that prolonged exposure to DON could change the level of enzymes responsible for its metabolism (Prelusky *et al.,* 1989)**.**

3. **In ducks fed a diet containing up to 7 mg DON/kg,** UV/diode array detection with an LOD of above 5 and 10 ng/mL for DON and its deepoxidized metabolite, respectively, failed to reveal these compounds in plasma (Dänicke ***et al.,* 2004)**

4. **In broilers fed a diet containing 1 and 5 mg DON/kg** with HPLC/MS detection of DON at an LOD of 5 ng/ml (**Awad *et al.,* 2011)**
    - **a.** DON and de-epoxy DON were not found in plasma,
    - **b.** very small amounts of DON were recovered in excreta,
5. the rapid absorption of DON across the intestinal epithelium by passive diffusion has been demonstrated in chicken DOM (**Awad et ail., 2007)**

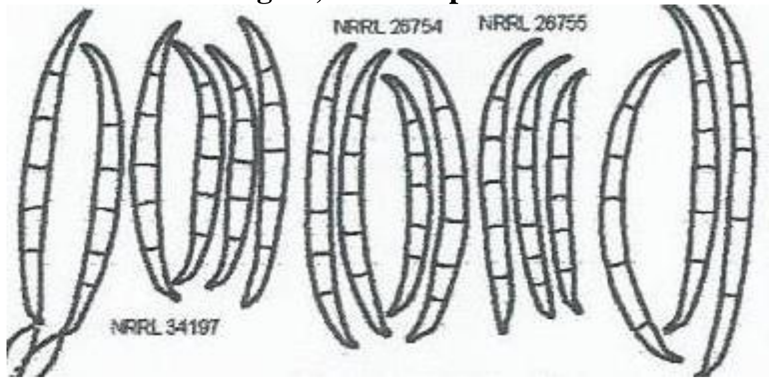
DON producing fungi

1. *Fusarium acaceae-mearnsii*
2. *Fusarium aethiopicum*
3. *Fusarium austroamericanum*
4. *Fusarium boothii*
5. *Fusarium brasilicum*
6. *Fusarium culmorum*
7. *Fusarium graminearum*
8. *Fusarium nepalense*
9. *Fusarium pseudograminearum*
10. *Fusarium solani*
11. *Fusarium ussurianum*
12. *Fusarium vorosi*

Description of Fusarium species

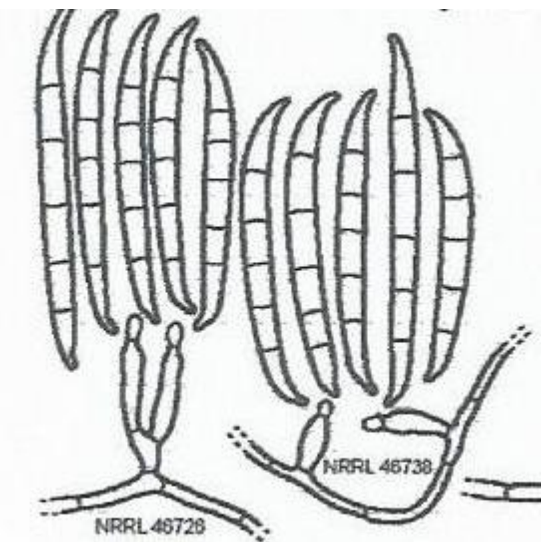
1. Fusarium acacia-mearunsii

Macroconidia 5-septate,gradually curved, asymmetric upper and lower haves, widest above and lower mid-region,narrow apical beak



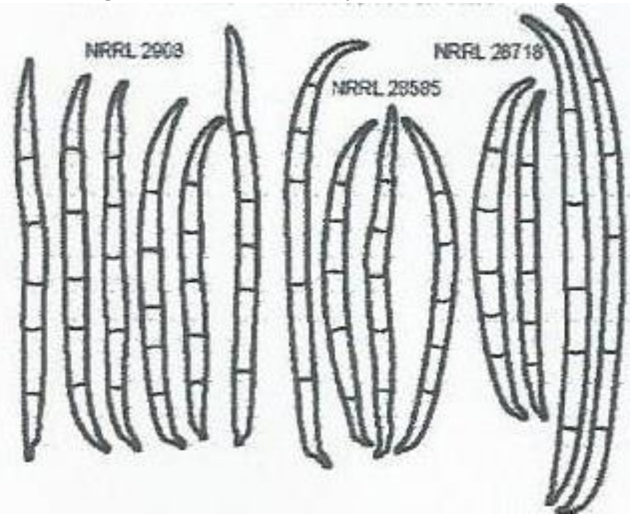
2. Fusarium aethiopicum **O'Donnell, Aberra, Kistler & T. Aoki (2008)**

F. aethiopicum produces mostly straight conidia, which are asymmetrical in that they are typically widest above the mid-region



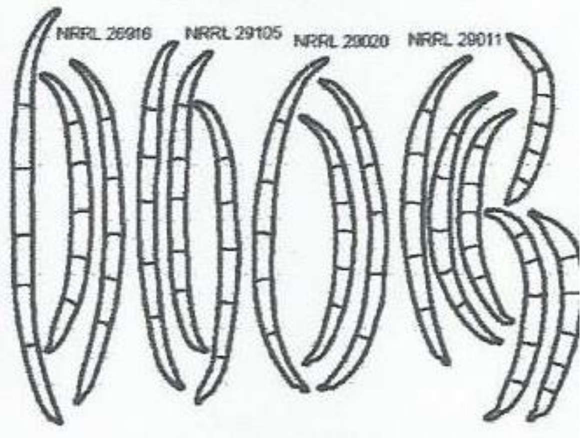
3. **Fusarium austroamericanum.** T. Aoki, Kistler, Geiser & O'Donnell, Fungal Genetics & Biology 41 (6): 617 (2004)

Macroconidia 5-septate, with longitudinal axis typically straight, asymmetric lower and upper halves, widest in mid-region, with narrow apical beak.



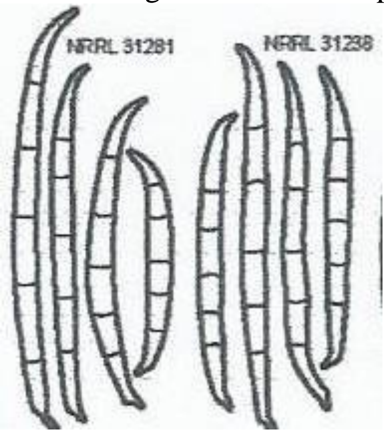
4. **Fusarim boothii** O'Donnell, T. Aoki, Kistler & Geiser, Fungal Genetics & Biology 41 (6): 618 (2004)

Colonies produce white mycelium with light brown coloue in the center, macroconida 5-septate , gradually curved, upper and lower halves are mostly symmetric, widest in the mid-region, with a narrow apical peak,



5. Fusarium brasilicum T. Aoki, Kistler, Geiser & O'Donnell, Fungal Genetics & Biology 41 (6): 620 (2004)

Macroconidia 5-septate , straight or gradually curved, upper and lower halves asymmetrical, widest below the mid- region and narrow apical peak

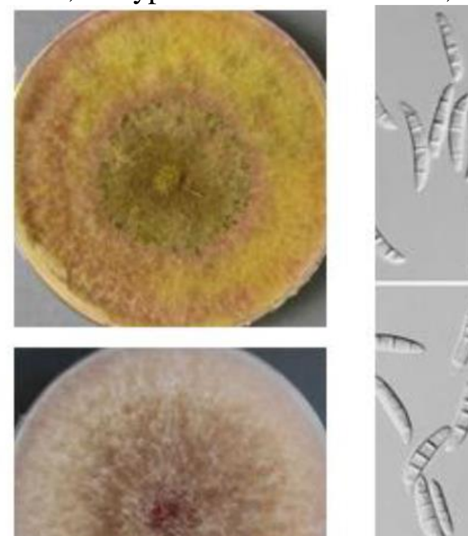


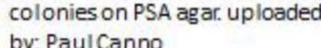
6. Fusarium culmorum (W.G. Sm.) Sacc., Sylloge Fungorum 11: 651 (1895)

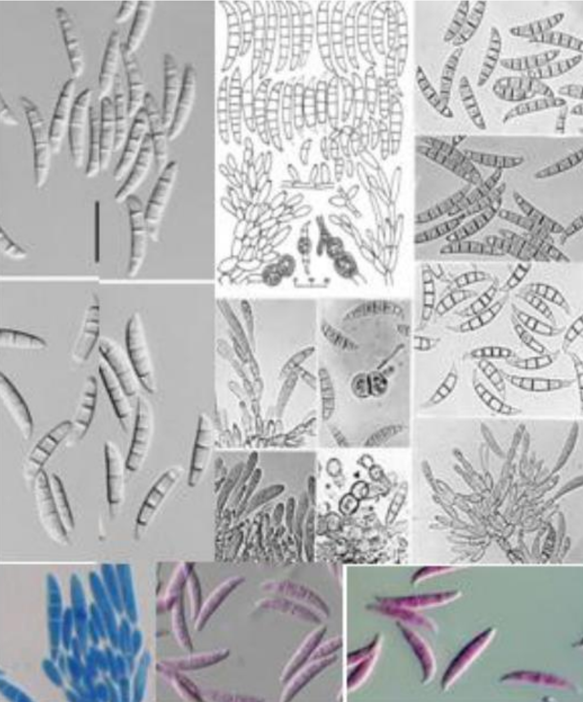
=Fusisporium culmorum Wm.G. Sm., Diseases of field and garden crops, chiefly as are caused by fungi: 209 (1884)

≡Fusarium culmorum (W.G. Sm.) McAlpine, Agricultural Gazette of New South Wales 7: 299-306 (1896)

Macroconidia: abundant, relat. Short, thick-walled, dorsal curvature and straight ventrally, 5 septa, apical cell rounded ant blunt , basal cell notched. Sporodochia: orange –brown, abundant. Microconidia: absent. Chlamydospores : abundant in 3-5 weeks, in hyphae and macroconidia, in chains and clusters



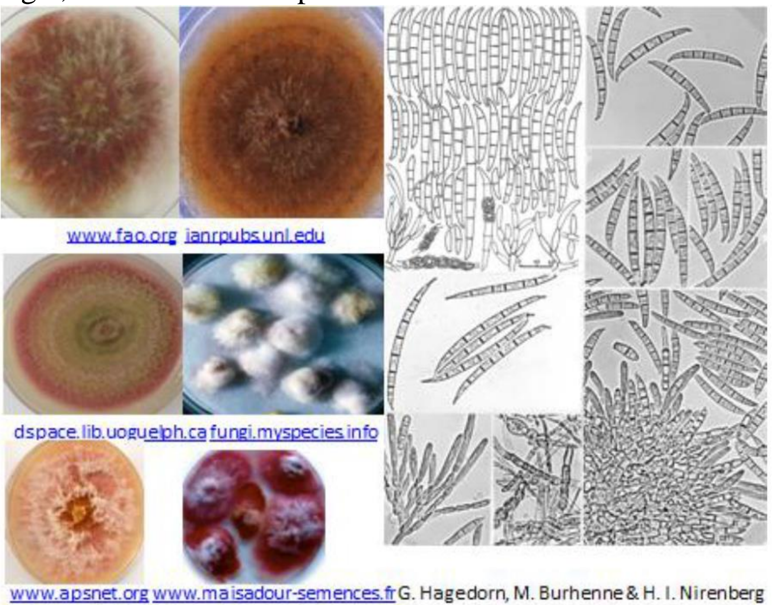

colonies on PSA agar. uploaded by: Paul Canno



John F. Leslie and Brett A. Summerell , G. Hagedorn, M. Burhenne & H. I. Nirenberg, Wikipedia

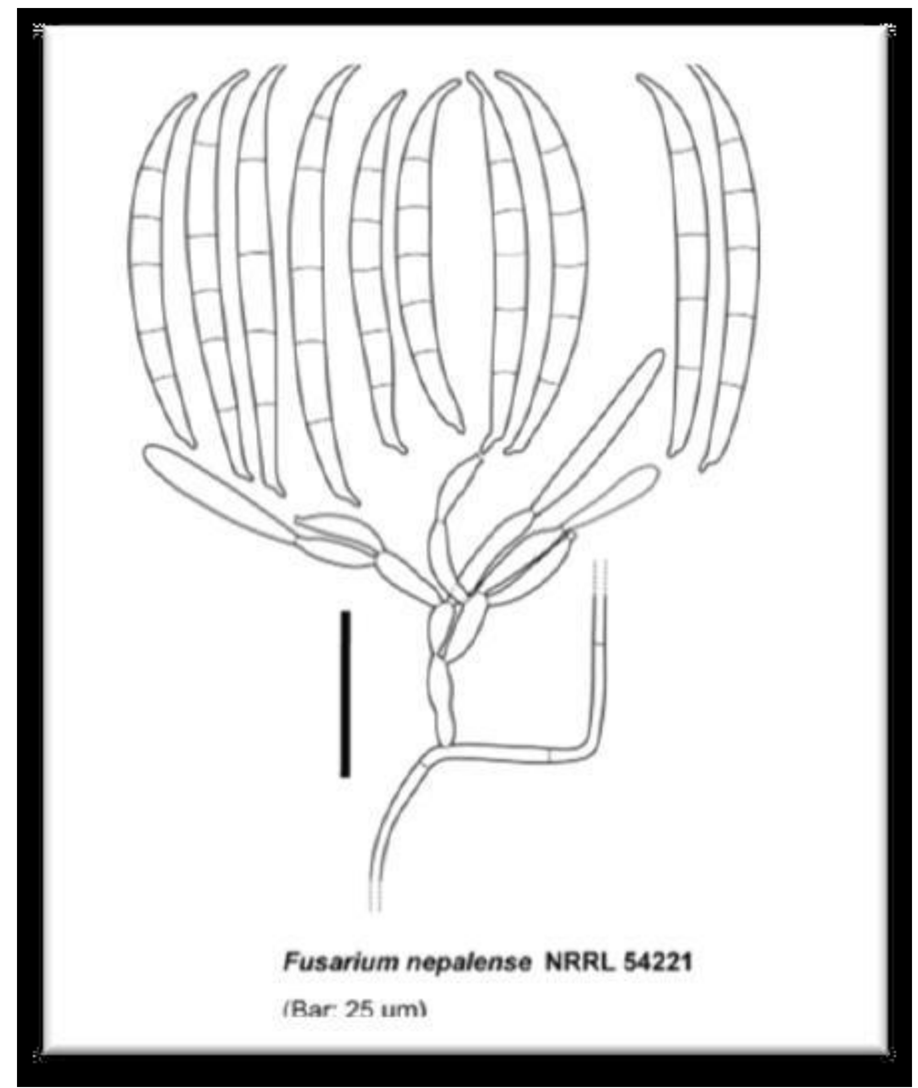
7. Fusarium graminearum **Schwabe, Flora Anhaltina 2: 285 (1839)**

Macroconidia: abundant in sporodochia, slender-slightly curved, thick-walled, 5-6 septa, apical cell tapering, basal cell foot-shaped. Sporodochia: pale orange. Microconidia: absent . Chlamydospores : are formed in the macroconidia, finely roughened, single, in chains or clumps



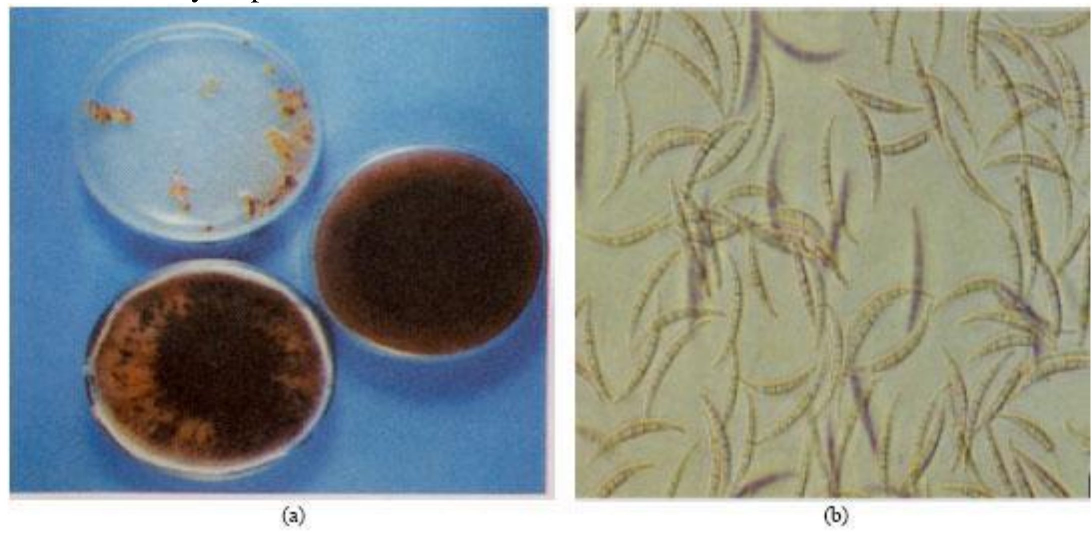
8. Fusarium nepalense **T. Aoki, Carter, Nicholson, Kistler & O'Donnell, Fungal Genetics & Biology 48: 1105**

Colonies abundant, sometimes sparsely developed, loosely to dense floccose, white, reddish-white, pale red to grayish red, grayish-orange aerial mycelium. Colony margin entire to undulate, often forming colony sectors of different growth rates. Sporodochia formed abundantly or sparsely. Conidiophores branched or unbranched, terminating with monophialides on the apices. Phialides simple, subulate, ampulliform to subcylindric, monophialidic. Conidia of a single type, typically falcate and curved, dorsiventral, most frequently widest slightly above the midregion of their length, tapering and gradually curving toward both ends, with an arcuate and beaked apical cell and a distinct basal foot cell, upper and lower halves asymmetric, 3-7-septate. Chlamydospores absent.



9. Fusarium pseudograminearum O'Donnell & T. Aoki, Mycologia 91 (4): 604 (1999)

Macroconidia: slender, almost straight to moderately curved, 1-11 septa, apical cell curved, basal cell foot-shaped. Sporodochia: abundant, pale orange. Microconidia: absent. Chlamydospores: abundant within 4 weeks



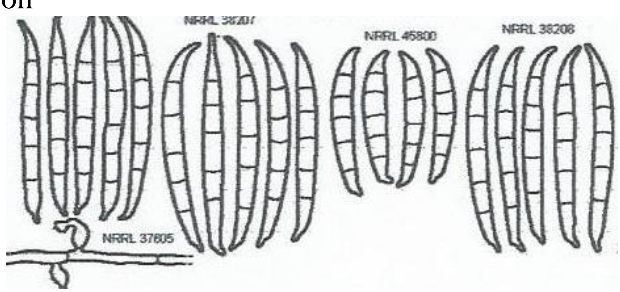
(a) (b)

10. Fusarium ussurianum **T. Aoki, Gagkaeva, Yli-Mattila, Kistler & O'Donnell, Mycologia 101 (6): 841-852 (2009)**

Colonies produce loose to densely floccose, white, reddish-white, brownish-yellow, brownish-orange to grayish-brown mycelium. Conidiophores branched verticillately or unbranched, forming monophialides. Phialides simple, subulate, ampulliform to subcylindrical, sometimes doliiform, monophialidic. Conidia of a single type, typically falcate and curved, dorsiventral, most frequently widest slightly above the mid-region of their length, mostly tapering and curving equally toward both ends, with an arcuate apical cell and a distinct basal foot cell, forming symmetrical upper and lower halves, 1-7-septate. Chlamydospores and sclerotia absent but some globose hyphal swelling sometimes present, intercalary or occasionally terminal.

11. Fusarium vorosii B. Tóth, Varga, Starkey, O'Donnell, H. Suga & T. Aoki, Fungal Genetics & Biology 44 (11): 1191-1204 (2007)

Fusarium vorosii is morphologically similar to F. graminearum including colony characters on PDA, but has slightly different conidial features from it. Macroconidia 5-septate, typically straight but sometimes gradually curved and frequently widest above the mid-region



.

Reports:

Prelusky *et al.* (1986) determined the disposition of [14C]deoxynivalenol ([14C]DON) administered to **hens** as either a single oral dose or consumed in spiked feed over a 6-day period by tracing the specific radioactivity of tissues and excreta. Following a single intubated dose (2.2 mg [14C]DON; 2.4 microCi/bird), the toxin was found to be poorly absorbed; peak plasma levels (2-2.5 hr post-treatment) accounted for less than 1% of the administered dose. Maximum tissue residues were measured at 3 hr in all tissues (liver, kidney, brain, heart, spleen, proventriculus, gizzard, small intestine) except for fat, muscle, and oviduct which occurred at 6 hr postdosing. Among the organs, the highest activities were measured in kidney, liver, and spleen; however, these levels were equal to less than 500 ng DON equivalents/g tissue, and declined quickly. Clearance of radioactivity from tissue had an average

half-life of 16.83 +/- 8.2 hr (range 7.7-33.3 hr, depending on the tissue). Elimination of the labeled toxin in excreta occurred rapidly; recovery of radioactivity accounted for 78.6, 92.1, and 98.5% of the dose by 24, 48, and 72 hr, respectively. In continuously dosed birds fed 2.2 mg unlabeled DON for 6 days followed by 2.2 mg (1.5 microCi) [14C]DON for 6 days, accumulation of radioactivity in tissues did not occur. Maximum residual levels, which occurred in the kidneys, were only 60 ng DON equivalents/g. Estimated level of residues contained in the edible tissues amounted to only 13-16 micrograms DON/1.5 kg hen.

KUBENA *et al.* (1987) fed White Leghorn chickens starter and grower diets containing either a control (non-contaminated) wheat diet or a naturally contaminated deoxynivalenol (DON) wheat diet (18 mg DON/kg) from 1 day of age to the onset of egg production. The hens were then placed on their respective layer diets of control wheat or DON-contaminated wheat (18 mg DON/kg) for six 28-day egg production periods. Feeding the DON-contaminated diet did not significantly influence body weights during the growing or the laying phases. Overall, hen-day egg production and egg weights were significantly higher for hens receiving the DON diet. Feeding DON contaminated wheat caused no significant changes in percent shell, albumen height, percent fertility, percent hatch of fertile eggs, percent hatch of eggs set, or weight of chicks at hatch. There were slight, although significant, changes in shell weight and shell thickness and in some serum chemistry values. There were no significant differences in the hematology parameters measured or in prothrombin times. None of the eggs collected from hens fed the control and the DON-contaminated wheat diet contained detectable quantities of DON. Microscopic examination of sections of the liver, kidney, and proventriculus of control and treated hens revealed no unusual histopathology. The results indicate that feeding DON at relatively high levels beginning at 1 day of age and continuing through six egg production periods had only slight effects on the parameters measured.

Lun *et al.* (1988) intubated **laying domestic chickens** with DON contaminated feed and Chromium (Cr) from sodium dichromate marked soybean meal served as a feed marker. DON Concentration was measured in the digesta as it progressed along the gastrointestinal tract (GIT). Recovery of the Cr marker was over 90%, regardless of time after intubation. A similar recovery of DON only occurred immediately after intubation when most of the original dose was confined to the crop Recovery of DON within the proventriculus-gizzard and all areas following was low, regardless of time after intubation. It was concluded that, the present experimentation has shown that DON as such largely disappeared from the GIT between the crop and Jejunum. This disappearance was presumed to have occurred because of its absorption by the enterocyte and conversion to another form. High radioactivity in the liver and bile in birds given labeled DON suggested that the metabolite was being excreted in association with bile back into the small intestine.

Branton *et al.* (1989) carried out an experiment to determine the effect of corn vs. grain sorghum on performance of laying hens. Egg production decreased significantly in the grain sorghum-fed hens in each of two trials starting 24 weeks after the trials began. Necropsy of chickens fed both diets revealed buccal ulceration at the ventral aspect of the oral cavity and squamous metaplasia of the esophageal glands and submaxillary salivary glands. Lesions were much more severe in the sorghum-fed birds than in the corn-fed birds. Analysis of the grain sorghum and corn revealed the presence of mycotoxins. Zearalenone and deoxynivalenol were present in the grain sorghum, and a lower amount of deoxynivalenol and a trace of aflatoxin B1 were

found in the corn. Although mycotoxin levels were low, interaction between these mycotoxins and others may have decreased egg production.

Prelusky ***et al.*** **(1989)** investigated the transmission of radioactive residues of 14C-labelled deoxynivalenol (DON; vomitoxin) to eggs during prolonged administration of low levels of DON-contaminated feed to White Leghorn chickens. Laying hens were provided with a 5.5 ppm 14C-DON-spiked diet (.55 mg DON; .8.25 microCi bird/day) for a 65-day period, after which they received a clean, unadulterated diet for 21 days. Total residues (based on specific radioactivity) increased daily until the 8th day of 14C-DON exposure, when levels reached a plateau for several days, then decreased slowly thereafter. Maximum radioactivity measured was equivalent to 1.7 micrograms DON or metabolites per 60-g egg; the yolk, albumen, and shell membrane contributed 70, 29, and 1% of the total amount, respectively. By Day 30, levels had declined to 25% of peak levels (.40-micrograms DON equivalents/egg) and remained relatively constant until the spiked feed was removed at Day 65, at which time residues quickly declined to negligible values. These findings indicate that although very low concentrations of DON can be found in eggs under these feeding conditions, levels are so low that a potential health hazard to humans would likely be minimal.

He *et al.* (1992) tested microbial inocula from rumen fluid, soil, and contents of the large intestines of chickens (CLIC) and of swine (SLIC) for their ability to transform deoxynivalenol (vomitoxin) in vitro. Microorganisms in (CLIC) completely transformed pure vomitoxin, and this activity was retained through six serial subcultures. No alteration of the toxin by incubation with SLIC was detected, whereas 35% of the vomitoxin was metabolized in the original culture of rumen fluid and 50% was metabolized by the soil sample, though metabolism was decreased in subsequent subcultures of either sample. A single metabolite was isolated and identified as deepoxy vomitoxin. The increase in concentration of deepoxy vomitoxin in the culture medium corresponded with the decrease in vomitoxin concentration. The vomitoxin transformation rate was not affected by either the ratio of CLIC to vomitoxin (5 to 0.2 g of CLIC per mg of vomitoxin) or the initial concentration of vomitoxin (14 to 1,400 ppm) in the medium. Biotransformation of vomitoxin was completely inhibited when the pH in the medium was lowered to 5.20. Sodium azide at a 0.1% (wt/vol) concentration in the medium blocked the transformation of vomitoxin, suggesting that the deepoxidation of vomitoxin is an energy-dependent process. About 50% of the vomitoxin in moldy corn in culture medium was transformed by microorganisms from CLIC. The vomitoxin transformation rate in moldy corn was not affected when the concentration of CLIC changed from 0.2 to 0.8 g/ml of medium. Vomitoxin in the moldy corn was not transformed when CLIC were added to corn without culture medium.

Keshavarz (1993) conducted an experiment to determine the effects of feeding corn contaminated naturally with deoxynivalenol (DON, vomitoxin) on performance of laying hens and growing chicks. Ten dietary regimens used in the laying hen experiment contained incremental levels of 0-2.1 ppm DON and 0-0.42 ppm zeralenone. Six dietary regimens used in the growing chick experiment contained 0 or 2.1 ppm DON and 0-0.42 ppm zeralenone. The criteria used for evaluating the effect of dietary treatments were body weight, body weight gain, egg production, feed consumption, feed conversion, egg weight, egg grades, shell quality, albumen quality, fertility and hatchability, organ weight, and presence of lesions in the mouth. No

adverse effects were observed in laying hens or growing chicks for any of these parameters even at the highest levels of DON contamination, which provided 2.1 ppm DON and 0.42 ppm zeralenone in the finished feeds. The data indicate that growing chicks and laying hens are relatively insensitive to corn contaminated naturally with 2-3 ppm DON and 0.4-0.6 ppm zeralenone, and having specifications similar to the corn samples used in this study. The results do not support the notion that corn contaminated with more than 0.5 ppm DON should be rejected for use in poultry feeds.

Davis *et al.* (1994) reported high mortality in two flocks of ducklings at rates of 20% and 50% by 4 and 7 days of age, respectively. The feeds were found to contain 300 to 1176 ppb of deoxynivalenol (DON), 4.5 ppm of fumonisin, and 10 ppb of aflatoxin B1. No other mycotoxins were detected. Pathological analysis indicated that the ducklings were dehydrated with no feed in the gastrointestinal tract. Histopathology revealed no significant lesions. A necropsy diagnosis indicated a condition similar to starve-outs and feed refusal. An infectious cause of mortality was not suspected.

Boston *et al.* (1996) fed captive mallards (Anas platyrhynchos) wheat containing 5.8 ppm deoxynivalenol (DON, vomitoxin) from an outbreak of Fusarium gramineariumhead-blight that occurred on grain crops in Manitoba, Canada, during 1993. There was no evidence of taste aversion to this grain during a 10-day palatability trial. No significant differences were detected in serum protein, calcium, glucose, creatinine kinase, aspartate aminotransferase or uric acid levels, blood packed cell volume, or body or organ weight, between ducks fed contaminated wheat and those fed uncontaminated wheat during a 14-day feeding trial. No gross or microscopic lesions were detected in birds fed contaminated wheat for 14 days. Based on these results, ducks will consume grain containing moderate levels of DON and short-term exposure to this grain will not result in obvious adverse effects.

Harvey *et al.* (1997) evaluated the effects of feeding diets containing 100 mg moniliformin (M)/kg of feed from culture material and 16 mg deoxynivalenol (DON)/kg of feed from naturally contaminated wheat in growing broiler chicks from 1 day to 21 days of age. Body weight (BW), body-weight gain, and feed consumption were decreased by feeding M and M plus DON diets. Relative heart weight was increased by the M diet, whereas relative weights of proventriculus, gizzard, and heart were increased by the M plus DON diet. The M diet increased alanine transferase and aspartate transaminase activities and creatinine concentration and decreased mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (MCHC). The M and DON diet decreased glucose, hemoglobin, and MCHC. Histopathological lesions from the M diet were limited to the kidney and consisted of extensive renal tubular epithelial degeneration plus luminalmineralization. A moderation of the severity of lesions was seen in the tissues of the M plus DON-fed chicks, consisting of generally mild tubular epithelial degeneration. None of the parameters measured were affected by the DON diet. Results indicate additive or less-than-additive toxicity for most parameters when chicks were fed diets containing 100 mg M plus 16 mg DON/kg of feed. Although the concentration of M in this study was high compared with that reported for feedstuffs, additional information on the occurrence and toxicity of M will need to be collected in order to assess the importance of M to the poultry industry.

Kubena *et al.* (1997) evaluated the individual and combined effects of feeding diets containing 300 mg fumonisin B1 (FB1), and 5 mg T-2 toxin (T-2)/kg of diet, or 15

mg/kg deoxynivalenol (DON, vomitoxin) from naturally contaminated wheat in two studies in male broiler chicks from day of hatch to 19 or 21 d of age in Experiments 1 and 2, respectively. When compared with controls, body weight gains were reduced 18 to 20% by FB1, 18% by T-2, 2% by DON, 32% by the FB1 and T-2 combination, and 19% by the FB1 and DON combination. The efficiency of feed utilization was adversely affected by FB1 with or without T-2 or DON. Mortality ranged from none for the controls to 15% for the FB1 and T-2 combination. Relative weights of the liver and kidney were significantly increased by FB1 with or without T-2 or DON. Serum concentrations of cholesterol were increased in chicks fed FB1 with or without T-2 or DON. Activities of aspartate aminotransferase, lactate dehydrogenase, and gamma glutamyltransferase were increased in chicks fed FB1 at 300 mg/kg alone and in combination with T-2 or DON, indicating possible tissue damage and leakage of the enzymes into the blood. Results indicate additive toxicity when chicks were fed diets containing 300 mg FB1 and 5 mg T-2/kg of diet and less than additive toxicity when chicks were fed 300 mg FB1 and 15 mg DON/kg of diet. Of importance to the poultry industry is the fact that toxic synergy was not observed for either of these toxin combinations and the likelihood of encountering FB1 at this concentration in finished feed is small. However, under field conditions with additional stress factors, the toxicity of these mycotoxins could be altered to adversely affect the health and performance of poultry.

Morris *et al.* (1999) evaluated the effects of feeding diets containing either 20 mg deoxynivalenol (DON)/kg, 100 mg moniliformin (M)/kg, or a combination of DON and M (20 mg/kg DON and 100 mg M/kg) in growing turkey poults, from 1 to 21 d of age. Feed intake and BW gains were decreased ($P < 0.05$) by dietary treatments containing M. Feed conversion was not affected by any of the dietary treatments, and no interactive effects on performance were evident between M and DON. Absolute weights of hearts and kidneys were increased ($P < 0.05$) in poults fed diets containing M. Mean cell volume was decreased by the M and DON-M treatments; however, the decrease was much smaller in poults fed the combination DON-M treatment resulting in a significant ($P < 0.05$) DON by M interaction. Mean cell hemoglobin and mean cell hemoglobin concentrations were not affected by any of the dietary treatments. No histological lesions were seen in control poults or poults fed DON alone. Lesions associated with dietary treatments were only observed in the heart and kidney. Poults fed diets containing M alone or the DON-M combination exhibited an increased incidence of variable sized cardiomyocyte nuclei, with numerous large giant nuclei, and a generalized loss of cardiomyocyte cross striations. Isolated renal tubules in sections of kidney were noted to have mild diffuse mineralization in poults fed M and the combination DON-M treatments. None of the response variables measured were affected by DON alone. No toxic synergy was observed when these toxins were fed simultaneously to turkey poults for 21 d.

Swamy *et al.* (2002) fed three hundred sixty, 1-d-old male broiler chicks, diets containing grains naturally contaminated with Fusarium mycotoxins for 56 d. The four diets included control (0.14 mg/kg deoxynivalenol, 18 mg/ kg fusaric acid, < 0.1 mg/kg zearalenone), low level of contaminated grains (4.7 mg/kg deoxynivalenol, 20.6 mg/kg fusaric acid, 0.2 mg/kg zearalenone), and high level of contaminated grains without (8.2 mg/kg deoxynivalenol, 20.3 mg/kg fusaric acid, 0.56 mg/kg zearalenone) and with (9.7 mg/kg deoxynivalenol, 21.6 mg/kg fusaric acid, 0.8 mg/kg zearalenone) 0.2% esterified-glucomannan polymer derived from Saccharomyces cerevisiae1026 (E-GM). Body weight gain and feed consumption responded in a

significant quadratic fashion to the inclusion of contaminated grains during the finisher period. Efficiency of feed utilization, however, was not affected by diets. The feeding of contaminated grains in the finisher period also caused significant linear increases in blood erythrocyte count and serum uric acid concentration and a significant linear decline in the serum lipase activity. Dietary inclusion of contaminated grains resulted in a significant quadratic effect on serum albumin and y-glutamyltransferase activity. Blood hemoglobin and biliary IgA concentrations, however, responded in significant linear and quadratic fashions. Supplementation of E-GM counteracted most of the blood parameter alterations caused by the Fusarium mycotoxin-contaminated grains and reduced breast muscle redness. It was concluded that broiler chickens may be susceptible to Fusarium mycotoxicoses when naturally contaminated grains are fed containing a combination of mycotoxins.

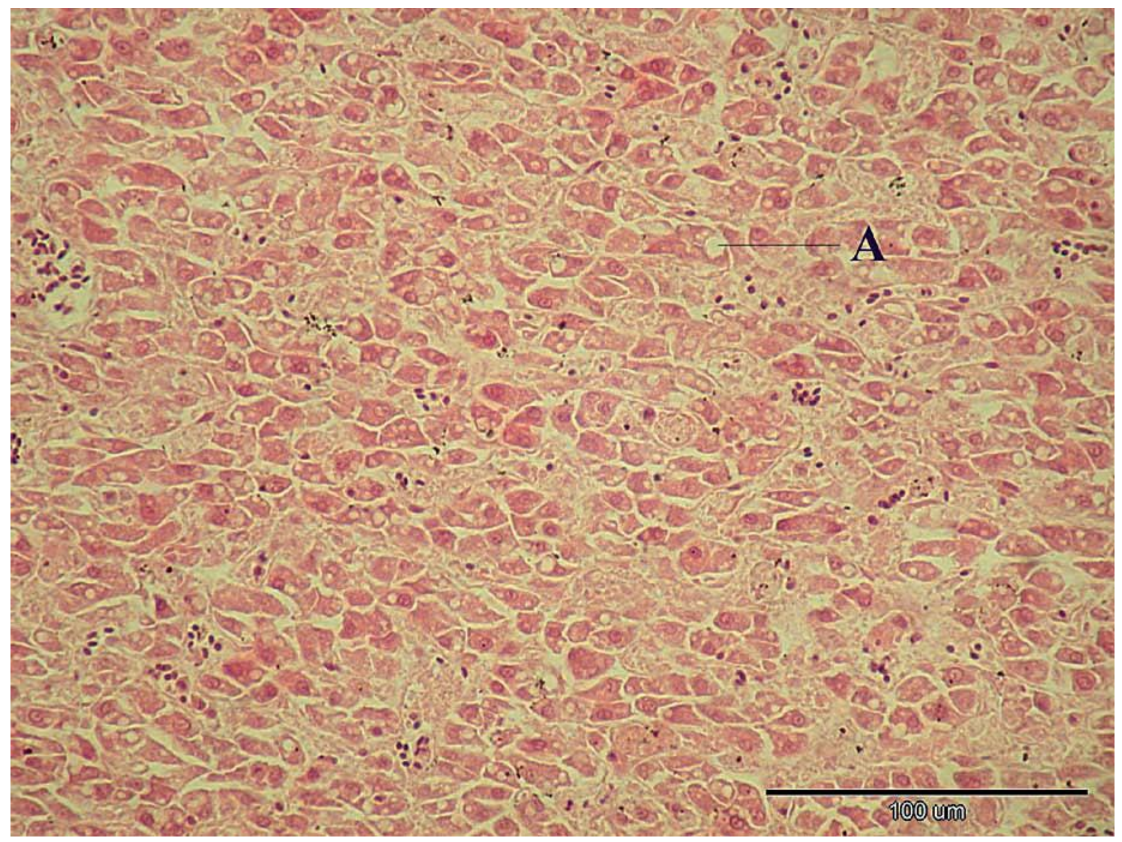
Dänicke *et al.* (2003) carried out a growth experiment with male broilers from d 1 to d 35 of age in order to evaluate the effects of the addition of a detoxifying agent (Mycofix Plus, Biomin GmbH, Herzogenburg, Austria) at different dietary proportions of wheat (0, 16.5, 33, 49.5 and 66%) contaminated with Fusarium mycotoxins (21.2 mg of deoxynivalenol and 406 microg of zearalenone, ZON, per kg of wheat) on growth performance, nutrient and zearalenone balance and clinical-chemical parameters. 2. An increase in dietary mycotoxin concentration resulted in a linearly related decrease in feed intake, a slight decrease in weight gain and an improvement in feed to gain ratio. 3. Apparent protein digestibility and net protein utilisation were higher in diets containing exclusively Fusarium toxin-contaminated wheat than control diets. 4. The proportions of beta-zearalenol, alpha-zearalenol and ZON of total ZON metabolites in excreta of broilers fed on the diets containing the Fusarium toxin-contaminated wheat were approximately 3, 21 and 76%. 5. Serum antibody titres to Newcastle disease virus decreased in a linear fashion with increasing mycotoxin concentration in the diets, whereas other clinical-chemical serum parameters (liver cell and muscle cell necrosis indicating enzymes, haemoglobin, haematocrit, magnesium, inorganic phosphate) were not influenced by increasing Fusarium toxin concentrations. 6. Supplementation of the diets with Mycofix Plus decreased performance in a manner independent of mycotoxin concentration. Moreover, some clinical-chemical serum parameters were significantly altered due to Mycofix Plus but also independently of the dietary mycotoxin concentration.

Awad *et al.* (2004) conducted a feeding trial to evaluate the effects of diets contaminated with deoxynivalenol (DON on the performance of broilers and on the electro-physiological parameters of the gut. The control group was fed the starter and finisher diets without addition of DON. Another group of broilers was fed the starter and finisher diets with 10 mg/kg DON, whereas another group was fed the DON-contaminated diets supplemented with a microbial feed additive (Eubacterium sp.). The diets were provided ad libitum for 6 wk. DON had no effect ($P > 0.05$) on feed consumption, feed conversion, or body weight. The effect of DON on the electrophysiological parameters of the jejunum was studied in vitro using isolated gut mucosa in Ussing chambers. At the end of the feeding period, 7 birds from each group were killed, and the basal and glucose stimulated transmural potential difference (PD), short-circuit current (Isc), and electrical resistance (R) were measured in the isolated gut mucosa to characterize the electrical properties of the gut. The transmural PD did not differ ($P > 0.05$) among groups. The tissue resistance was greater (P <

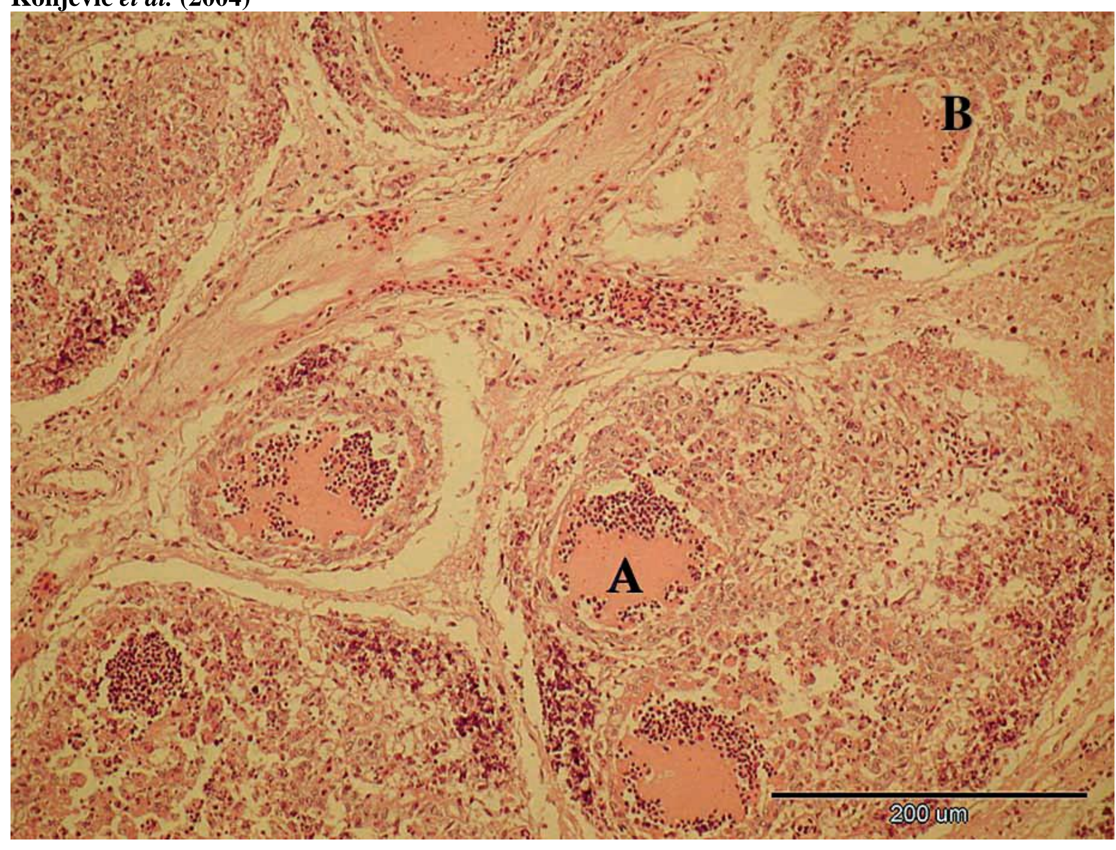
0.05) in birds receiving DON and the microbial feed additive than in the controls and DON group. Addition of D-glucose on the luminal side of the isolated mucosa increased ($P < 0.05$) Isc in the control and DON-probiotic (Eubacterium sp.; PB) groups, whereas it decreased ($P < 0.05$) in the DON group indicating that the glucose-induced Isc was altered by DON. Addition of the eubacteria to the DON contaminated feed of the broilers led to electrophysiological properties in the gut that were comparable with those of the control group. It could be concluded that 10 mg/kg DON in the diet impaired the Na(+)-D-glucose cotransport in the jejunum of broilers. In the absence of clinical signs, and without impaired performance, DON appeared to alter the gut function of broilers. The addition of Eubacterium sp. may be useful in counteracting the toxic effects of DON on intestinal glucose transport.

Dänicke ***et al.*** **(2004)** fed diets with increasing proportions of Fusarium-toxin-contaminated wheat to **Pekin ducks** for 49 d in order to titrate the lowest effect level. Dietary **deoxynivalenol** (DON) and **zearalenone** (ZON) concentrations were successively increased up to 6 to 7 mg/kg and 0.05 to 0.06 mg/kg, respectively. Feed intake, live weight gain and feed to gain ratio were not influenced by dietary treatment. Gross macroscopic inspection of the upper digestive tract did not reveal any signs of irritation, inflammation or other pathological changes. The weight of the bursa of Fabricius, relative to live weight, decreased in a dose-related fashion. Activities of glutamate dehydrogenase and gamma-glutamyl-transferase in serum were either unaffected or inconsistently affected by dietary treatments. Concentrations of DON and of its de-epoxydised metabolite in plasma and bile were lower than the detection limits of 6 and 16 ng/ml, respectively, of the applied high performance liquid chromatography (HPLC) method. ZON or its metabolites were not detectable in plasma and livers (detection limits of the HPLC method were 1, 0.5 and 5 ng/g for ZON, alpha-zearalenol (alpha-ZOL) and beta-zearalenol (beta-ZOL), respectively). Concentrations of ZON, alpha-ZOL and beta-ZOL in bile increased linearly with dietary ZON concentration. The mean proportions of ZON, alpha-ZOL and beta-ZOL of the sum of all three metabolites were 80, 16 and 4%, respectively. Taken together, it can be concluded that dietary DON and ZON concentrations up to 6 and 0.06 mg/kg, respectively, did not adversely affect performance and health of growing Pekin ducks.

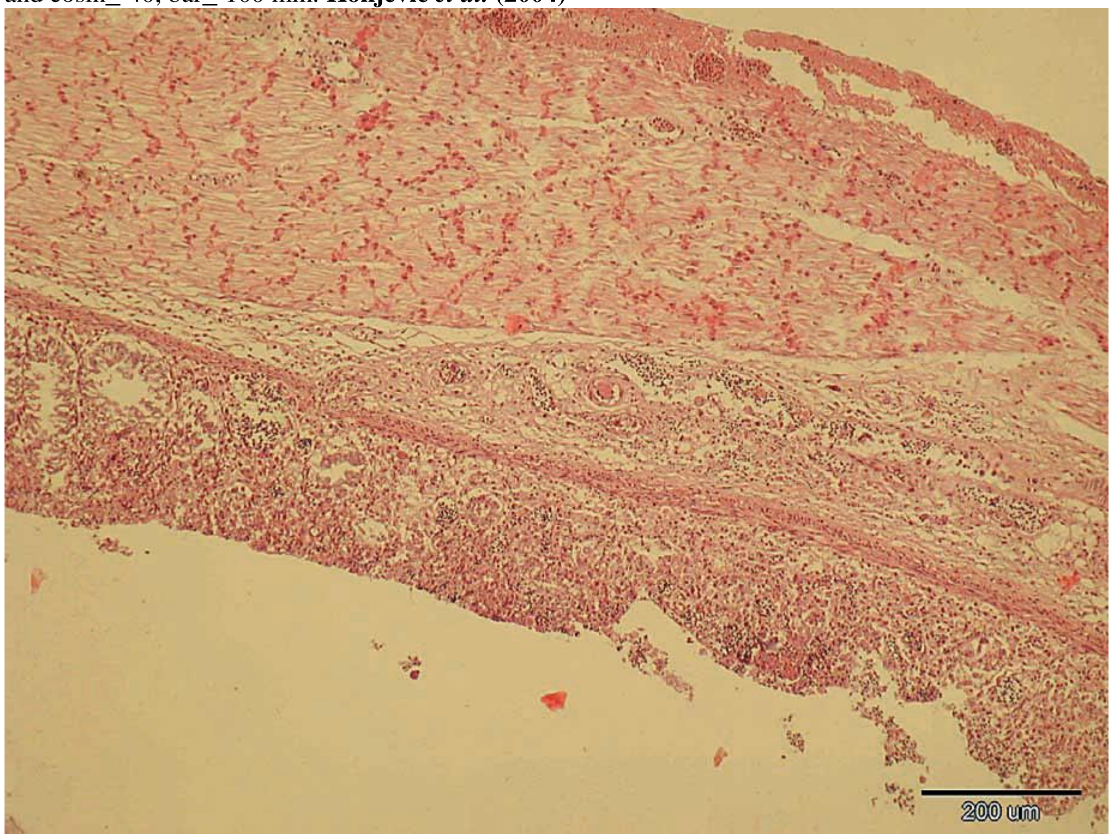
Konjevic *et al.* (2004) described the spontaneous poisoning of two Brahma chickens with T-2 toxin, diacetoxyscirpenol and deoxynivalenol. Two out of 10 chickens died under signs of depression and loss of appetite. Histopathological analysis revealed vacuolar dystrophy of the liver, necrosis and depletion of lymphocyte in the bursa of Fabricius as well as multiple necroses in the glandular stomach and gut. Even though quantities of 0.70 mg/kg T-2 in the food together with 0.50 mg/kg diacetoxyscirpenol significantly differ from the median lethal dose for chickens reported in literature (4.97 mg/kg), parasitological, virological and histopathological results indicate trichotecenes as the causative agents of this pathological condition.



The liver of a 2-month-old Brahma chicken affected by trichotecens. Advanced vacuolar dystrophy (A) of hepatocytes associated with passive hyperaemia. Haemotoxylin and eosin _ 20, bar_ 200 mm.
Konjevic *et al.* (2004)



The Bursa Fabricii of a 2-month-old Brahma chicken affected by trichotecens. Note the necrotic areas in follicles of Bursa Fabricii (A) and the markedly decreased number of lymphocyte (B). Haemotoxylin and eosin_ 40, bar_ 100 mm. **Konjevic *et al.* (2004)**



Severe necrotic typhlitis. Haemotoxylin and eosin _ 20, bar_ 200 mm. **Konjevic *et al.* (2004)**

Pestka *et al.* (2004) identified two critical upstream transducers of DON-induced mitogen-activated protein kinases (MAPKs) activation. One transducer is double-stranded RNA-(dsRNA)-activated protein kinase (PKR), a widely-expressed serine/theonine protein kinase that can be activated by dsRNA, interferon, and other agents. The second transducer is hematopoetic cell kinase (Hck), a non-receptor associated Src family kinase. Inhibitors and gene silencing studies have revealed that Hck and PKR play roles in DON induced gene expression and apoptosis. Future studies should focus on the molecular linkages between these kinases and trichothecene toxicity.

Swamy *et al.* (2004) conducted an experiment to investigate the effects of feeding grains naturally contaminated with Fusarium mycotoxins on growth and immunological parameters of broiler chickens. Three hundred sixty, 1- d-old male broiler chicks were fed 1 of 4 diets containing grains naturally contaminated with Fusarium mycotoxins for 56 d. The diets included (1) control; (2) low level of contaminated grains (5.9 mg/kg d**eoxynivalenol** (DON), 19.1 mg/kg **fusaric acid** (FA), 0.4 mg/kg **zearalenone**, and 0.3 mg/kg 15-acetyldeoxynivalenol; (3) high level of contaminated grains (9.5 mg/kg DON, 21.4 mg/kg FA, 0.7 mg/kg zearalenone, and 0.5 mg/kg 15-acetyldeoxynivalenol); and (4) high level of contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GM polymer). Body weight gains and feed consumption of chickens fed contaminated grains decreased linearly with the inclusion of contaminated grains during the grower phase (d 21 to 42). Efficiency of feed utilization, however, was not affected by diet. Production

parameters were not significantly affected by the supplementation of GM polymer to the contaminated grains. Peripheral blood monocytes decreased linearly in birds fed contaminated grains. The feeding of contaminated diets linearly reduced the B-cell count at the end of the experiment, whereas the T-cell count on d 28 responded quadratically to the contaminated diets. The feeding of contaminated diets did not significantly alter serum or bile immunoglobulin concentrations, contact hypersensitivity to dinitrochlorobenzene, or antibody response to SRBC. Supplementation with GM polymer in the contaminated diet nonspecifically increased white blood cell count and lymphocyte count, while preventing mycotoxin-induced decreases in B-cell counts. It was concluded that broiler chickens are susceptible during extended feeding of grains naturally contaminated with Fusarium mycotoxins.

Sypecka *et al.* (2004) assessed the potential for the *Fusarium* mycotoxins 4-deoxynivalenol (DON) and zearalenone (ZON) to enter the human food chain through contaminated eggs using a controlled feed study. Four groups of laying hens (eight in each group) were fed a diet that included differing amounts of naturally contaminated wheat containing DON (≈20 mg kg^{-1}) and ZON (0.5 mg kg^{-1}). Eggs were collected and pooled from each group on a daily basis. Pooled samples were analyzed by liquid chromatography with mass spectrometry detection (LC-MS/MS). The method allowed DON, other type B trichothecenes, ZON, and its metabolites to be determined in a single multi-residue analysis. The selectivity of the MS/MS procedure allowed cleanup to be minimized (for DON, cleanup by immunoaffinity column was used) or eliminated (for ZON). The limits of detection of 0.01 μg kg^{-1} for DON and 0.1 μg kg^{-1} for ZON in eggs were lower than previously published methods. None of the samples analyzed had detectable levels of ZON or its metabolites. Although maximum levels of DON contamination (10 mg kg^{-1} feed) were relatively high, no adverse effects were observed on egg production. On the basis of the determined DON levels in the hen's diet and the determined levels of DON in the corresponding eggs, transmission rates of 15 000:1, 18 000:1, and 29 000:1 for treatment levels 5, 7.5, and 10 mg DON kg^{-1} feed, respectively, were found. These results show that, although eggs could be a human exposure route for DON, the levels are insignificant compared to the other sources, although the presence of metabolites of DON was not studied.

Awad *et al.* (2005) conducted a study to characterize the in vitro effects of DON in the presence of mucosal amino acids, using L-proline as a model, on the electrophysiological parameters in the jejunums of laying hens. L-Proline (mucosal concentration of 1 mmol/L) was added to a stripped proximal part of jejunum sheets mounted in Ussing chambers in Ringer buffer, and the electrical properties were measured. The transmural potential difference (PD) was nearly constant between the treatments. The tissue resistance (Rt) was higher ($P < 0.05$) in the tissues exposed to DON compared with basal values and the values after addition of L-proline. Addition of L-proline on the luminal side of the isolated mucosa increased ($P < 0.05$) the short circuit-current (Isc), and it decreased ($P < 0.05$) after addition of DON, indicating that the proline-induced Isc was altered by DON. The addition of proline after incubation of the tissues with DON had no effect ($P > 0.05$) on PD or Rt. Proline did not increase the Isc under these conditions. DON decreased ($P < 0.1$) the Isc after addition of proline, indicating that DON inhibited the Na+-amino acid co-transport. We concluded from the present study that the amino acid cotransporter activity appears to be highly sensitive to DON suppression.

Chowdhury ***et al.*** **(2005)** examined the effects of feeding grains naturally contaminated with Fusarium mycotoxins on hematology and immunological indices and functions of laying hens and the possible protective effect of feeding a polymeric glucomannan mycotoxin adsorbent (GMA). One hundred forty-four laying hens were fed for 12 wk with diets formulated with (1) uncontaminated grains, (2) contaminated grains, or (3) contaminated grains + 0.2% GMA. Fusarium mycotoxins such as **deoxynivalenol** (DON, 12 mg/kg), 15-acetyl-DON (0.5 mg/kg), and **zearalenone** (0.6 mg/kg) were identified in the contaminated diets arising from contaminated grains grown in Ontario, Canada. The concentrations of DON arising from naturally contaminated grains in this study were similar to purified mycotoxin fed to experimental mice. The chronic feeding of Fusarium mycotoxins induced small decreases in hematocrit values, total numbers of white blood cells, lymphocytes including both CD4+ and CD8+ T lymphocytes and B lymphocytes, and biliary IgA concentration. Supplementation of diets containing feedborne mycotoxins with GMA prevented the reduction in total number of B lymphocytes in the peripheral blood and the reduction in biliary IgA concentration. In addition, the delayed-type hypersensitivity response to dinitrochlorobenzene was increased by feed-borne mycotoxins, whereas IgG and IgM antibody titers to sheep red blood cells were not affected by diet. We concluded that chronic consumption of grains naturally contaminated with Fusarium mycotoxins at levels likely to be encountered in practice were not systemically immunosuppressive or hematotoxic; however, mucosal immunocompetence needs to be explored further.

Labuda ***et al. (*****2005)** analyzed a total of 50 samples of poultry feed mixtures of Slovakian origin for eight toxicologically significant Fusarium mycotoxins, namely **zearalenone** (ZON), A-trichothecenes: **diacetoxyscirpeno**l (DAS), T-2 toxin (T-2) and HT-2 toxin (HT-2) and B-trichothecenes: deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON) and nivalenol (NIV). The A-trichothecenes and the B-trichothecenes were detected by means of high pressure liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS) and gas chromatography electron capture detection (GC-ECD), respectively. Reversed phase-high performance liquid chromatography with a fluorescence detector (RP-HPLC-FLD) was used for ZON detection. The most frequent mycotoxin detected was T-2, which was found in 45 samples (90%) in relatively low concentrations ranging from 1 to 130 microg kg(-1) (average 13 microg kg(-1)), followed by ZON that was found in 44 samples (88%) in concentrations ranging from 3 to 86 microg kg(-1) (average 21 microg kg(-1)). HT-2 and DON were detected in 38 (76%) and 28 (56%) samples, respectively, in concentrations of 2 to 173 (average 18 microg kg(-1)) for HT-2 and 64 to 1230 microg kg(-1) sample (average 303 microg kg(-1)) for DON. The acetyl-derivatives of DON were in just four samples, while NIV was not detected in any of the samples investigated. In as many as 22 samples (44%), a combination of four simultaneously co-occurring mycotoxins, i.e. T-2, HT-2, ZON and DON, was revealed. Despite the limited number of samples investigated during this study poultry feed mixtures may represent a risk from a toxicological point of view and should be regarded as a potential source of the Fusarium mycotoxins in Central Europe.

Valenta ***et al.*** **(2005)** performed an experiment to study the transmission of deoxynivalenol (DON) and of its metabolite de-epoxy-DON into eggs. This question

was addressed within the scope of a 16-week experiment with laying hens which were fed a maize-based diet with a DON concentration of 11.9 mg x kg(-1)dry matter. Eggs were collected during weeks 2, 4, 8, and 16 of the experiment, and DON and its metabolite de-epoxy-DON were analyzed in freeze-dried yolk and albumen. In order to cover possible conjugates, all samples were incubated with beta-glucuronidase prior to extraction. Yolk and albumen were extracted with acetonitrile-water, and the extracts were purified with immunoaffinity columns (IACs) after a precleaning step. The toxins were determined by high-performance liquid chromatography (HPLC) with UV detection. The detection limits of both toxins were 5 and 8 microg x kg(-1) in freeze-dried yolk and albumen, respectively, corresponding to approximately 2.5 and 1 microg x kg(-1) in fresh samples. The recovery of DON and de-epoxy-DON in yolk was 80% and 78%, respectively, and in albumen 77 and 72%. Neither DON nor de-epoxy-DON or glucuronide conjugates of both substances could be detected in any of the samples. These results indicate that eggs do not contribute significantly to the dietary DON intake of humans.

Awad *et al.* (2006a) conducted an experiment to study the effects of deoxynivalenol (DON) on the performance of broilers, organ weights, and intestinal histology and to evaluate the efficacy of a probiotic feed additive (PB, Eubacterium sp.) with the ability to deepoxidize DON. Two hundred seventy-seven 1-d-old broiler chicks were randomly assigned to 1 of the 3 dietary treatments for 6 wk. The dietary treatments were 1) control; 2) artificially contaminated diets with 10 mg of DON/kg of diet; 3) DON-contaminated diets plus probiotic feed additive (DON-PB). The BW and the efficiency of feed utilization were not adversely affected ($P > 0.05$) by the inclusion of DON in the diets. A slight improvement in feed intake and BW gain over the course of the experiment was observed in broilers fed DON-PB with no change in feed efficiency. The absolute or relative organ weights were not altered ($P > 0.05$) in broilers fed the diet containing DON compared with controls and the DON-PB group. The absolute liver weights were numerically increased ($P < 0.1$) for broilers receiving the diet containing DON-PB. There were no significant differences in the absolute and relative weights of the gizzard, duodenum, pancreas, heart, and spleen. However, the absolute and relative weights of the jejunum and cecum were increased for DON-PB-fed broilers compared with the controls and DON group. No pathological lesions were found in the gut of birds fed DON-contaminated diets during the feeding trial, but mild intestinal changes were observed. The DON altered small intestinal morphology, especially in the duodenum and jejunum, where villi were shorter and thinner ($P < 0.05$). The addition of the eubacteria to the DON-contaminated feed of the broilers effectively alleviated the histological alterations caused by DON and led to comparable villus length as in the control group. In conclusion, diets with DON contamination below levels that induce a negative impact on health and performance could affect small intestinal morphology in broilers. The histological alterations caused by DON were reduced by supplementing the DON-containing diets with PB. This indicates that in case of DON contamination of feedstuffs, the addition of PB would be a proper way to counteract the possible effects caused by this mycotoxin.

Awad *et al.* (2006b) conducted a feeding trial to evaluate the effects of moderate dietary concentrations of deoxynivalenol (DON) during a 21-day feeding experiment on the performance of broilers. Fifteen 1-day-old broiler chicks were randomly divided into two groups. The control group was fed non-contaminated diet. Another group of broilers was fed a diet naturally contaminated with 5 mg DON/kg diet. Deoxynivalenol had no effect ($p > 0.05$) on feed consumption, feed conversion, body-

weight gain, live body weight or mortality. The absolute and relative weight of the organs (gizzard, pancreas, heart, spleen, colon and caecum) were not altered by the dietary inclusion of DON contaminated grain. However, both the absolute and relative weight of small intestine was decreased ($p < 0.01$) in DON fed broilers compared to the controls. No gross lesions were detected in any of the organs of birds fed contaminated wheat during the feeding trial. The microscopic examination revealed that, the height and the width of villi in duodenum decreased ($p < 0.05$) in birds fed DON contaminated wheat compared to controls. On the other hand the height and the width of jejunum villi were not affected ($p > 0.05$). This study indicates that feeding DON for 21 days to broiler chickens at a concentration of up to 5 mg/kg of diet influenced the weight of the small intestine as well as intestinal histology, especially the duodenum, as evidenced by shorter and thinner villi. In conclusion, diets with DON contamination below levels that induce negative impact on health and performance cauld affect small intestinal morphology in broilers.

FAIXOVÁ *et al.* (2006) evaluated the effects of modified glucomannan (Mycosorb®) on plasma chemistry of broiler chicks after deoxynivalenol (DON) inclusion in the diet from hatching to 6 weeks of age. Three groups of broiler chicks were formed with 14 birds in each group. The three diets included control (0.2 ppm deoxynivalenol), deoxynivalenol-contaminated (3 ppm deoxynivalenol) and deoxynivalenol-contaminated (3 ppm deoxynivalenol) plus Mycosorb®(2 g/kg diet). After 6 weeks of feeding all birds were sacrificed and blood samples for chemical analyses were collected. Serum calcium and alanine aminotransferase activity were significantly elevated and magnesium, total protein, triglycerides and free glycerol were decreased in chicks fed deoxynivalenol-contaminated diet compared with those fed the control diet. Inclusion of Mycosorb®in the diet decreased plasma alkaline phosphatase and alanine aminotransferase activities and reversed plasma levels of magnesium, triglycerides, free glycerol and total protein in chicks induced by dietary deoxynivalenol. Chloride level was not affected by diets. The inclusion of Mycosorb® to DONcontaminated diet, however, did not prevent or alleviate toxic effect on calcium metabolism. Supplementation of modified glucomannan Mycosorb® counteracted most of the plasma parameter alterations caused by deoxynivalenol-contaminated diet in chicks.

Frankic *et al.* (2006) determined the effect of T-2 toxin and deoxynivalenol (DON) on DNA fragmentation in spleen leukocytes and oxidative stress in chickens, and furthermore, evaluated the potential of dietary nucleotides in reduction of toxin-induced DNA damage. Male broiler chickens were exposed to 10mg/kg feed of either T-2 toxin or DON with or without addition of dietary nucleotides. After 17 days of treatment DNA damage of spleen leukocytes was measured by Comet assay, lipid peroxidation was studied by malondialdehyde (MDA), total antioxidant status (TAS) of plasma and glutathione peroxidase (GPx) assays, and the hepatotoxicity was studied by measuring plasma liver enzyme levels (ALT, AST and GGT) levels. T-2 toxin and DON induced DNA fragmentation in chicken spleen leukocytes and supplementation with nucleotides reduced the amount of damage only when added to T-2 toxin. In comparison to control group, values of TAS and AST decreased significantly in the groups fed T-2 toxin with or without nucleotide supplementation. Plasma and liver MDA content in groups fed T-2 toxin and DON did not differ significantly from the control. Dietary nucleotides did not affect MDA formation when added to the diets with mycotoxins. The results obtained suggest that dietary

nucleotides have the potency to reduce the extent of DNA damage induced by the action of T-2 toxin in immune cells. This underlines their possible beneficial effect on the immune system in mycotoxin intoxication.

Martins et al. (2006) carried out a study to investigate the co-occurrence of zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FB1 and FB2) in 52 samples of mixed-feed for poultry contaminated with Fusarium verticillioides. The zearalenone and deoxynivalenol were checked using immunoaffinity column and the extraction of fumonisin was performed by strong anion exchange (SAX) solid phase column. Detection and quantification were determined by high performance liquid chromatography (HPLC). The limit of detection was 5 µg/kg for ZEN, 100 µg/kg for DON and 50 and 100 µg/kg for FB1 and FB2 respectively.Fusarium toxins were detected in 20 samples. Sixteen samples were positive for ZEN (30.7%) presenting levels that ranged from 7.4 µg/kg to 61.4 µg/kg (mean=27.0 µg/kg). 13.5% of the samples presented contaminations of DON, with levels ranging from 100.0 µg/kg to 253 µg/kg (mean=l18.07 µg/kg). FB1 was detected in 19.2% of samples, with levels ranging from 50.0 µg/kg to 110.0 µg/kg (mean=73.6 µg/kg). FB2 was not detected in any sample. In positive samples simultaneously contamination with two or three mycotoxins were detected in 9 of them (17.3%).

Yegani *et al.* (2006b) conducted a study to investigate the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of broiler breeders. Forty-two 26-wk-old broiler breeder hens and nine 26-wk-old roosters were fed the following diets: (1) control, (2) contaminated grains, and (3) contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GMA) for 12 wk. The major contaminant was **deoxynivalenol** (12.6 mg/kg of feed), with lesser amounts of **zearalenone** and 15-acetyl-deoxynivalenol. Feed consumption and BW were not affected by diet. The feeding of contaminated grains did not significantly affect egg production. Decreased eggshell thickness was seen, however, at the end of wk 4, and dietary supplementation with GMA prevented this effect. There was no effect of diet on other egg parameters measured. There was a significant increase in early (1 to 7 d) embryonic mortality in eggs from birds fed contaminated grains at wk 4, but mid- (8 to 14 d) and late- (15 to 21 d) embryonic mortalities were not affected by diet. There were no differences in newly hatched chick weights or viability. The ratio of chick weight to egg weight was not affected by the feeding of contaminated grains. Weight gains of chicks fed a standard broiler starter diet at 7, 14, and 21 d of age were not significantly affected by previous dietary treatments for the dam. It was found that rooster semen volume and sperm concentration, viability, and motility were not affected by the feeding of contaminated diets. There was no effect of diet on the relative weights of liver, spleen, kidney, and testes. The feeding of contaminated grains decreased antibody titers against infectious bronchitis virus at the end of wk 12, and this was prevented by dietary supplementation with GMA. There was no effect of the diet on serum antibody titers against Newcastle disease virus. It was concluded that the feeding of blends of grains contaminated with *Fusarium* mycotoxins could affect performance and immunity in broiler breeder hens.

Awad *et al.* (2007) investigated the effects of DON on the glucose transport capacity in chickens' jejunum and the permeation of DON itself by the Ussing chamber technique. Glucose uptake into chicken jejunal epithelia was measured after the addition of 200 mumol/L of (14)C-labeled glucose to the mucosal solution. Glucose uptake under control condition was 3.28 +/- 0.53 nmol/cm(2) x min. The contribution

of sodium glucose-linked transporter 1 (SGLT-1) to total glucose uptake was estimated by inhibiting SGLT-1 with phlorizin (100 micromol/L). In the presence of phlorizin, glucose uptake was reduced ($P < 0.05$) to 1.21 +/- 0.19 nmol/cm(2) x min. Deoxynivalenol decreased ($P < 0.05$) the glucose uptake in the absence of phlorizin to 1.81 +/- 0.24 nmol/cm(2) x min but had no additional effect on the glucose uptake in the presence of phlorizin (0.97 +/- 0.17 nmol/cm(2) x min). Mucosal-to-serosal permeation of DON was proportional to the initial DON concentration over a concentration range from 1 to 10 mug/mL on the mucosal side. Apparent permeability at 10 microg/mL of DON measured 60 to 90 min after DON application was 1.7 x 10(-05) cm/s. It can be concluded that DON (10 mg/L) decreases glucose uptake almost as efficiently as phlorizin. The similarity between the effects of phlorizin and DON on glucose uptake evidences their common ability to inhibit Na(+)-D-glucose cotransport. In addition to local effects, DON can be absorbed from the jejunum. A predominant part of DON passes across the chicken intestinal epithelium by passive diffusion, which is likely on the paracellular pathway. The results imply that the exposure to DON-contaminated feeds may negatively affect animal health and performance by local (i.e., inhibition of intestinal SGLT-1) and systemic effects.

Dänicke *et al.* (2007) inoculated wheat with Fusarium culmorum. Broiler diets were formulated to contain this Fusarium-infected wheat (FIW) or control wheat (CW) at a proportion of 60% and were prepared without and with an exogenous nonstarch polysaccharide (NSP) hydrolyzing enzyme preparation [endo-1,4-beta-xylanase (EC 3.2.1.8) 1,000 FXU/g; ZY68, Lohmann Animal Health GmbH & Co. KG, Cuxhaven, Germany] to test the hypothesis that Fusarium infection-related increases in NSP hydrolyzing enzyme activities could compensate for the deleterious effects of the fungal-origin mycotoxins such as deoxynivalenol (DON). Deoxynivalenol concentration of CW and FIW amounted to 0.045 and 2.5 mg/kg of DM, respectively. After 35 d, the level of feed intake was generally lower in broilers fed the diets containing the FIW. Feed intake was stimulated by the addition of the NSP enzyme to both diet types. Similar relationships were observed for live weight gain, although the enzyme effect was much more pronounced for the CW-fed broilers, who performed even worse than the broilers fed the unsupplemented FIW. Viscosity was significantly reduced in the jejunum and the ileum by supplemental exogenous NSP hydrolyzing enzyme. However, this effect was more pronounced when the enzyme was added to the control diet, as indicated by the significant interactions between wheat and NSP enzyme. Concentrations of DON and its metabolite deepoxy-DON in plasma, bile, liver, and breast meat were lower than the detection limits of the applied HPLC-method. Overall, it can be concluded that feeding FIW might positively influence broiler performance and nutritional physiology, as indicated by the reduced intestinal viscosity and the less pronounced effects of addition of an exogenous NSP hydrolyzing enzyme preparation.

FAIXOVÁ *et al.* (2007) conducted an experiment to investigate the effect of different doses of deoxynivalenol on plasma indices of broiler chickens. Forty-two one-day-old male broiler chicks were fed 1 of 3 diets containing deoxynivalenol (DON) for 42 d. The diets included: (1) control (0.2 ppm of deoxynivalenol), (2) low level of deoxynivalenol (1 ppm of DON), and (3) high level of deoxynivalenol (3 ppm of DON). Then, all the birds were sacrificed and blood samples for biochemical analyses were collected. The mycotoxin doses in diets were verified using gas chromatography-mass spectrometry. The administration of 1 ppm of DON altered

total protein, triglycerides, free glycerol, and potassium levels. Dietary addition of 3 ppm of DON resulted in altered calcium, potassium, total protein, triglycerides, along with free glycerol levels, and aspartate aminotransferase activity. No biochemical parameter, however, responded to increased DON concentration in the diet. The feeding of DON-containing diets did not significantly alter plasma chloride, cholesterol, and albumin levels or aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase activities. It was concluded that both levels of deoxynivalenol in the diets tested significantly affected protein and lipid metabolism in broiler chicks.

Young ***et al.*** **(2007)** monitored the degradation of 12 trichothecene mycotoxins by chicken intestinal microbes by liquid chromatography-ultraviolet-mass spectrometry under positive ion atmospheric pressure chemical ionization. Two pathways were observed: deacylation and deepoxidation. Essentially complete conversions to the deepoxy metabolites were observed for the non-acylated trichothecenes 4-deoxynivalenol, nivalenol, and verrucarol. However, deacetylation was the predominant pathway for the monoacetyl trichothecenes 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol (15ADON), and fusarenon X. Small amounts of the deepoxy metabolites were observed from 15ADON and large amounts from 15-monoacetoxyscirpenol where steric hindrance protected the C-15 acetyl groups from enzymatic attack. Diacetylated trichothecenes diacetoxyscirpenol and neosolaniol exhibited only deacetylation. The larger isovaleryl functionality was resistant to removal and deepoxidation was the prevalent reaction in HT-2 toxin and T-2 triol, whereas T2 toxin showed only deacetylation.

Awad *et al.* (2008) mentioned that Deoxynivalenol (DON), a trichothecene, is prevalent worldwide in crops used for food and feed production. The presence of mycotoxins in poultry feeds is a significant factor for financial losses to animal industries. Although DON is one of the least acutely toxic trichothecenes, it should be treated as an important food safety issue because it is a common contaminant of grains. Special care must be taken in so-called “Fusarium years”. As poultry is regarded to be less sensitive to DON compared to other species it is suspected to divert the infected cereal batches to poultry feeding. This review focused on the ability of DON to induce toxicologic and immunotoxic effects in chickens. Chickens and laying hens respond to increasing dietary DON concentrations with a reduction in productivity only at high levels above 5mg/kg but there is no clear evidence of a dose-response relationship. The main effect at low dietary concentrations appears to be a reduction in food consumption (anorexia), while higher doses induce severe reduction in weight and impaired resistance to infection, particularly bacterial infection. One important aspect of DON toxicity is injury to the gastrointestinal tract. DON has an influence intestinal morphology of chickens, especially in the duodenum and jejunum, as evidenced by shorter and thinner villi. Additionally, DON decreased the intestinal nutrients absorption (glucose and amino acid) in the chicken small intestine in vivo and In vitro. The capacity of DON to alter normal immune function has been of particular interest. There is extensive evidence that DON impairs the immune function in broiler and Leghorn chicks. DON induced changes in the haematopoietic system of chicks and altered the mitogen-induced proliferation of lymphocytes. The feeding of DON contaminated grains decreases serum antibody titers against Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) in laying hens and broilers. Other effects include superinduction of cytokine production by T helper cells (In vitro) and activation of T cells to produce a proinflammatory cytokine. To what extent the

elevation of cytokines contributes to metabolic effects such as decreased feed intake remains to be established. Further toxicological studies on the impact of DON in the immune system and gastrointestinal tract of poultry are warranted.

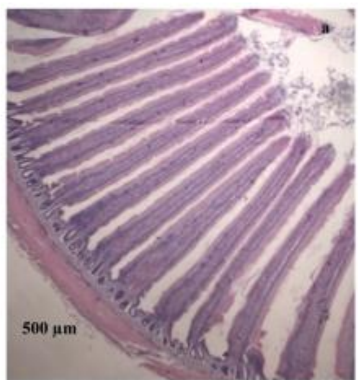
Borutova *et al.* (2008) investigated the effects of dietary contamination with various levels of deoxynivalenol (DON) and zearalenone (ZEA) on Ross 308 hybrid broilers of both sexes. After hatching, all chickens were fed an identical control diet for two weeks. Then chickens of Group 1 received a diet contaminated with DON and ZEA, both being 3.4 mg kg(-1), while Group 2 received DON and ZEA at 8.2 and 8.3 mg kg(-1), respectively. The diet of the control group contained background levels of mycotoxins. Samples of blood and tissues were collected after two weeks. Intake of both contaminated diets resulted in a significantly decreased activity of glutathione peroxidase (GPx) and increased level of malondialdehyde (MDA) in liver tissue, while in kidneys the concentration of MDA was significantly increased only in Group 1. On the other hand, activities of blood GPx and plasma gamma-glutamyltransferase (GGT) were elevated in Group 2 only. Activities of thioredoxin reductase in liver and GPx in duodenal mucosa tissues, superoxide dismutase (SOD) in erythrocytes as well as levels of MDA in duodenal mucosa and alpha-tocopherol in plasma were not affected by dietary mycotoxins. Blood phagocytic activity was significantly depressed in Group 1 and 2. These results demonstrate that diets contaminated with DON and ZEA at medium levels are already able to induce oxidative stress and compromise the blood phagocytic activity in fattening chickens.

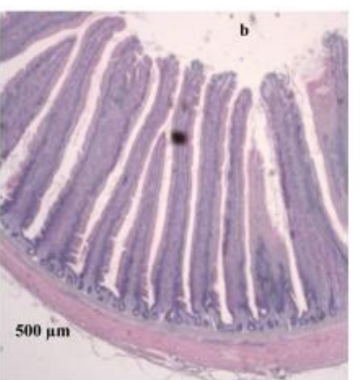
Girish *et al.* (2008) conducted an experiment to investigate the effects of feeding grains naturally contaminated with Fusarium mycotoxins on brain regional neurochemistry of turkeys. The possible preventative effect of a poly-meric glucomannan mycotoxin adsorbent (GMA) was also determined. Forty-five 1-d-old male turkey poults were fed wheat-, corn-, and soybean meal-based diets up to wk 6, formulated with control grains, contaminated grains, or contaminated grains + 0.2% GMA. Deoxynivalenol was the major contaminant, and the concentrations were 2.2 and 3.3 mg/kg of feed during starter and grower phases, respectively. Concentrations of brain monoamine neurotransmitters and metabolites were measured in discrete regions of the brain including the pons, hypothalamus, and cortex by HPLC with electrochemical detection. Neurotransmitters and metabolites analyzed included norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid, serotonin (5-hydroxytryptamine, 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA). The concentration of 5-HIAA and the 5-HIAA:5-HT-ratio were significantly decreased in pons after feeding contaminated grains. Dietary supplementation with GMA prevented these effects. In the pons, a significant positive correlation ($r = 0.52$, $P < 0.05$) was observed between the concentration of 5-HT and BW gain after feeding contaminated diets. The feeding of contaminated diet had no significant effects on the concentrations of neurotransmitters and metabolites in hypothalamus and cortex. It was concluded that consumption of grains naturally contaminated with Fusarium mycotoxins adversely altered the pons serotonergic system of turkeys. Supplementation with GMA partially inhibited these effects.

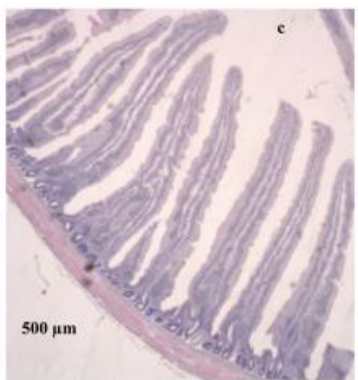
Yunus *et al.* (2010) carried out an experiment to evaluate the transfer of deoxynivalenol (DON) and its de-epoxy metabolite (de-epoxy-DON) in the plasma of chicken. Mashed oats naturally contaminated with 9.5 mg DON/kg were fed to four broilers (35 days age) at a dose of 20 g/bird. Blood samples were then collected from two birds at 1 h, 3 h, and 5 h post-feeding, while from the other two birds at 2 h, 4 h, and 6 h post-feeding. Analysis of DON and de-epoxy-DON was carried out by using

liquid chromatography-tandem mass spectrometry after clean-up with immunoaffinity columns. At 1 h, 3 h, and 5 h post-feeding, the average values of plasma DON were 0.35 ng/ml, 0.20 ng/ml, and 0.15 ng/ml, respectively. The corresponding average values of de-epoxy-DON at these time points were 0.70 ng/ml, 0.80 ng/ml, and 0.25 ng/ml, respectively. The sum of DON and de-epoxy-DON appearing in the plasma at 1 h post-feeding in these birds was estimated to be 0.044% of the total DON fed. At 2 h, 4 h, and 6 h post-feeding, the average values of plasma DON were 0.85 ng/ml, 0.45 ng/ml, and 0.30 ng/ml. De-epoxy-DON could not be detected in the birds sampled at 2 h, 4 h, and 6 h post-feeding. The total amount of DON appearing in the plasma at 2 h post-feeding in these birds was estimated to be 0.036% of the DON fed. These data show that the absorption rate of DON is very low in broilers and that there is also a rapid transformation, and clearance from plasma. Furthermore, there appeared to be individual variability in the capacity of birds to de-epoxidise DON.

Awad *et al.* (2011) performed an experiment to investigate the effects of feeding grains naturally contaminated with Fusarium mycotoxins on morphometric indices of jejunum and to follow the passage of deoxynivalenol (DON) through subsequent segments of the digestive tract of broilers. A total of 45 1-d-old broiler chickens (Ross 308 males) were randomly allotted to three dietary treatments (15 birds/treatment): (1) control diet; (2) diet contaminated with 1 mg DON/kg feed; (3) diet contaminated with 5 mg DON/kg feed for five weeks. None of the zootechnical traits (body weight, body weight gain, feed intake, and feed conversion) responded to increased DON levels in the diet. However, DON at both dietary levels (1 mg and 5 mg DON/kg feed) significantly altered the small intestinal morphology. In the jejunum, the villi were significantly ($P < 0.01$) shorter in both DON treated groups compared with the controls. Furthermore, the dietary inclusion of DON decreased ($P < 0.05$) the villus surface area in both DON treated groups. The absolute or relative organ weights (liver, heart, proventriculus, gizzard, small intestine, spleen, pancreas, colon, cecum, bursa of Fabricius and thymus) were not altered ($P > 0.05$) in broilers fed the diet containing DON compared with controls. DON and de-epoxy-DON (DOM-1) were analyzed in serum, bile, liver, feces and digesta from consecutive segments of the digestive tract (gizzard, cecum, and rectum). Concentrations of DON and its metabolite DOM-1 in serum, bile, and liver were lower than the detection limits of the applied liquid chromatography coupled with mass spectrometry (LC-MS/MS) method. Only about 10 to 12% and 6% of the ingested DON was recovered in gizzard and feces, irrespective of the dietary DON-concentration. However, the DON recovery in the cecum as percentage of DON-intake varied between 18 to 22% and was not influenced by dietary DON-concentration. Interestingly, in the present trial, DOM-1 did not appear in the large intestine and in feces. The results indicate that deepoxydation in the present study hardly occurred in the distal segments of the digestive tract, assuming that the complete de-epoxydation occurs in the proximal small intestine where the majority of the parent toxin is absorbed. In conclusion, diets with DON contamination below levels that induce a negative impact on performance could alter small intestinal morphology in broilers. Additionally, the results confirm that the majority of the ingested DON quickly disappears through the gastrointestinal tract







Histomorphometric analysis of the jejunum of a 5-wk-old broiler chickens fed diets with or without DON with magnification 200 (*n* = 6): (**a**) the Villus height of the jejunum of the control birds; (**b**) birds fed with 1 mg DON/kg diet; (**c**) birds fed with 5 mg DON/kg diet. **Awad *et al.* (2011)**

Xu *et al.* (2011) conducted 2 experiments to determine the effects of increasing dietary concentrations of deoxynivalenol (DON) on performance, intestinal morphology, and measures of innate immunity in broilers and turkeys. For experiment 1, the 3-wk study used 5 concentrations of DON (up to 18 or 10 mg of DON/kg of feed in broilers or turkeys, respectively) from naturally contaminated corn. The BW gains were cubically or quadratically affected by the increasing dietary concentrations of DON for broilers and turkeys, respectively; however, feed consumption was not affected. For experiment 2, the birds were subsequently injected or not injected with lipopolysaccharide (LPS) 24 h before tissue and blood sample collection. Dietary DON had no effect on intestinal crypt depth, but linearly increased the mid-ileal villus height in broilers ($P = 0.04$). An interaction was observed between the LPS challenge and the dietary DON with regards to heterophil to lymphocyte ratio ($P < 0.05$) in broilers, but not in turkeys. The cecal tonsil cell phagocytosis of microbeads was not affected by the dietary concentration of DON either with or without the subsequent LPS challenge for both broilers and turkeys. Conversely, the phagocytic capacity of cecal tonsil cells to engulf killed Staphylococcus aureus was significantly reduced (over 2.5-fold) when broilers were fed the highest concentration of dietary DON (non-LPS-challenged; $P < 0.05$). However, diets containing DON showed no effects on broilers when they were challenged with LPS. Antibody-dependent phagocytosis (S. aureus) was not affected in turkeys fed DON. Overall, corn naturally contaminated with up to 18 or 10 mg/kg of DON (broiler or turkey, respectively) reduced bird BW gain at 21 d of age, reduced antibody-dependent phagocytosis of previously killed S. aureus by cecal tonsil cells in non-LPS-challenged broilers, and greatly decreased heterophil to lymphocyte ratios in LPS-challenged broilers.

Awad *et al.* (2012a) mentioned in their review, that Deoxynivalenol (DON) is prevalent worldwide in crops used for food and feed production. The presence of mycotoxins in poultry feeds is a significant factor contributing to financial losses in animal industries. DON causes losses in livestock production and poses a health problem to livestock and humans consuming contaminated cereal products. Although DON is one of the least acutely toxic trichothecenes, it should be treated as an important food safety issue because it is a very common contaminant of grains. Poultry respond to increasing dietary DON concentrations with a reduction in productivity only at high levels (above 5 mg/kg) but there is no evidence of a clear dose-response relationship. Poultry fed low to moderate doses are able to recover from initial weight losses, while higher doses induce more long-term changes in feeding behaviour. At low dosages of DON, haematological, clinical and

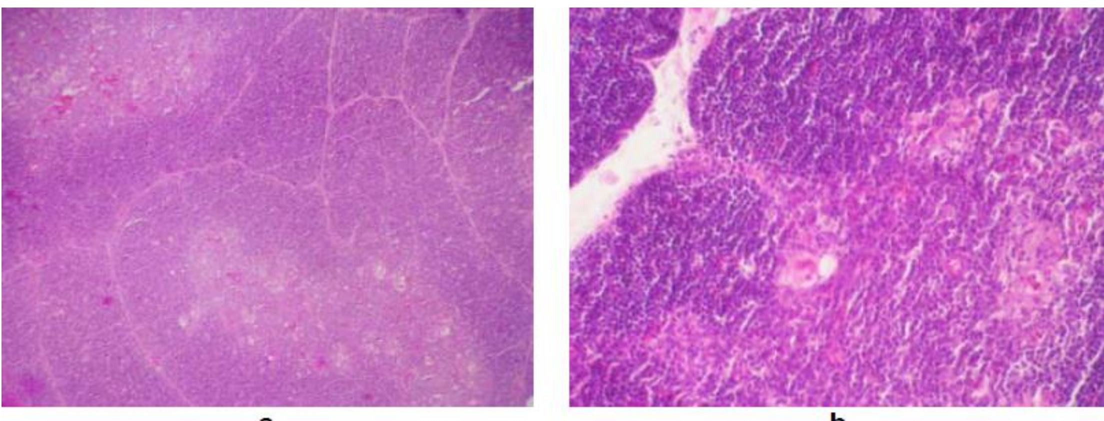
immunological changes are transitory and decrease as compensatory/adaptation mechanisms are established. The exposure to higher dose levels of DON are mainly expressed as severe reductions in body weight and impaired resistance to infection, particularly bacterial infection. Common symptoms of acute toxicity of DON are nausea, vomiting, dermal irritation and lesions, haemorrhagic lesions and pathological changes in the haemopoietic organs. The capacity of DON to alter normal gut and immune function has been of particular interest. One important aspect of DON toxicity is an injury of the gastrointestinal tract. It was found that DON had an influence on intestinal nutrient absorption and the intestinal morphology of chickens, especially in the duodenum and jejunum, as evidenced by shorter and thinner villi. DON decreases glucose and amino acid absorption in the chicken's small intestine *in vivo* and *in vitro* and this effect is apparently mediated by the inhibition of the sodium D-glucose co-transporter. It was found that immune function decreased in broiler Leghorn chicks that were fed DON-contaminated diets. It induces changes in the hematopoietic system of chicks and alters immune response, whereas, DON impairs mitogen-induced proliferation of lymphocytes. The feeding of DON-contaminated grains decreased serum antibody titers against Newcastle disease virus (NDV) and infectious bronchitis (IB) in laying hens and broilers. The impact of DON on the immune system and gastrointestinal tract are important to define the maximum tolerable levels of DON in animal feedstuffs. The purpose of this review is to summarise the information to date regarding the toxicological and immunological effects of DON on poultry.

Awad *et al.* (2012b) performed a study to establish the effect of DON on lipid peroxidation and lymphocyte DNA fragmentation in broilers and to evaluate the potential of Mycofix select in the prevention of toxin-mediated changes. Thirty-two 1-d-old (Ross 308 male) broiler chicks were randomly divided into 4 groups. The control group was fed a noncontaminated diet, and a second group was fed the same diet but supplemented with Mycofix select (0.25%). A third group of broilers was fed a diet artificially contaminated with 10 mg of feed-grade DON/kg of diet, and a fourth group was fed a DON-contaminated diet supplemented with Mycofix select. At the end of the feeding trial, blood was collected and the degree of lymphocyte DNA damage was measured in the plasma by comet assay. Deoxynivalenol increased (P = 0.016) the amount of DNA damage in chicken lymphocytes by 46.8%. Mycofix select protected lymphocyte DNA from the DON effects. To our knowledge, these are the first data on genotoxic effects of a moderate dose of DON on chicken lymphocytes. However, the thiobarbituric acid reactive substances level in liver and liver enzyme activity did not differ among the groups. In conclusion, the present study demonstrated that the diets contaminated with the mycotoxin DON at moderate levels in combination with low-protein feed are able to induce lymphocyte DNA damage in chickens. Supplementation with Mycofix select protected lymphocyte DNA and it was beneficial for maintaining the lymphocyte DNA integrity.

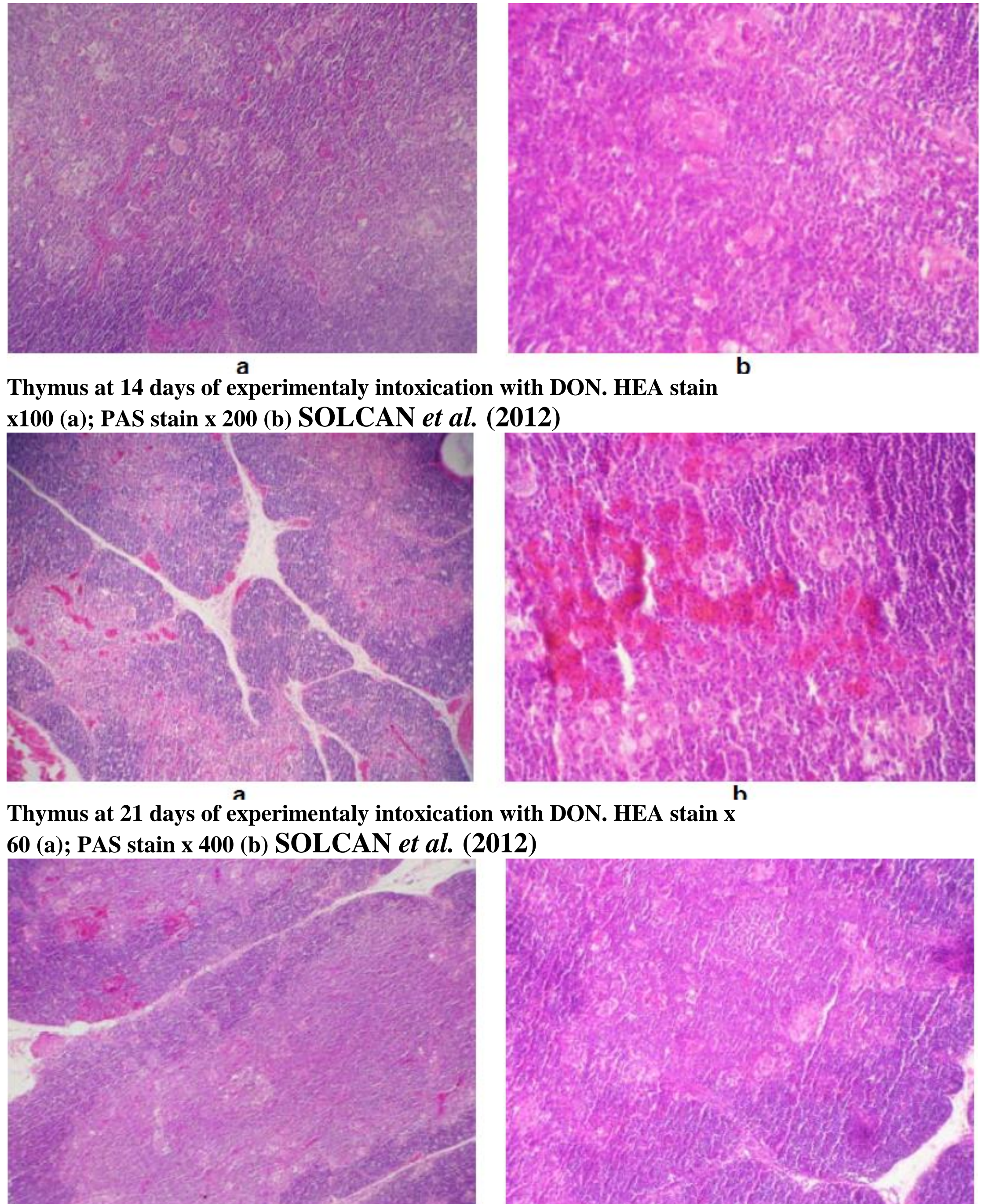
Ghareeb *et al.* (2012) performed an experiment with 1-d-old male broilers (Ross 308) to examine the effects of feeding DON-contaminated low-protein grower diets on performance, serum biochemical parameters, lymphoid organ weight, and antibody titers to infectious bronchitis vaccination in serum and to evaluate the effects of Mycofix select dietary supplementation in either the presence or absence of DON in broilers. In total, thirty-two 1-d-old broiler chicks were randomly assigned to 1 of the 4 dietary treatments for 5 wk. The dietary treatments were 1) control; 2) artificially contaminated diets with 10 mg of DON/kg of diet; 3) DON-contaminated diets

supplemented with Mycofix select; and 4) control diet supplemented with Mycofix select. Feeding of contaminated diets decreased (P = 0.000) the feed intake, BW (P = 0.001), BW gain (P = 0.044), and feed efficiency during the grower phase. Deoxynivalenol affected the blood biochemistry, whereas plasma total protein and uric acid concentrations in birds fed contaminated grains were decreased compared with those of the controls. Moreover, in birds fed contaminated feeds, there was a tendency to reduce triglycerides in the plasma (P = 0.090), suggesting that DON in the diets affected protein and lipid metabolism in broiler chickens. The feeding of contaminated diets altered the immune response in broilers by reducing the total lymphocyte count. Similarly, the antibody response against infectious bronchitis vaccination antigens was decreased (P = 0.003) after feeding contaminated diets, compared with the controls. Moreover, contamination of the broiler diet with DON increased the heteropil:lymphocyte ratio (stress index), suggesting that DON elevated the physiological stress responses of broilers. However, feeding of DON-containing diets did not alter the other plasma constituents, including activities of enzymes. Mycofix select addition to the DON-contaminated feed led to normal immunological and physiological functions in broilers

SOLCAN *et al.* (2012) conducted a study to prove the immunosuppressant action of deoxynivalenol in chickens experimentally treated each day, from the 7th day of life, using 5,4 mg/kg b.w in E group for 28 days (since 35 days of life). Histopathology studies of thymus were made on 7th, 14th, 21st and 28th days of experiment. In E group small lesions of thymus were observed even after 7th day of poisoning but intense lesions, hydropic degeneration, necrotic foci and moderate lymphoid depletion was observed after the 14th and 21st day of poisoning. After 28th day a marked proliferation of stromal cells in the reticulum network, in medulla zone, presence of mucous cells, small mucous cysts and haemorages were observed.



Thymus at 7th day of experimentaly intoxication with DON. HEA stain x60 (a); PAS stain x 400 (b) **SOLCAN *et al.* (2012)**



Thymus at 14 days of experimentaly intoxication with DON. HEA stain x100 (a); PAS stain x 200 (b) SOLCAN *et al.* (2012)

Thymus at 21 days of experimentaly intoxication with DON. HEA stain x 60 (a); PAS stain x 400 (b) SOLCAN *et al.* (2012)

Thymus at 28 days of experimentaly intoxication with DON. HEA stain x100 (a); x 200 (b) SOLCAN *et al.* (2012)

Yunus *et al.* (2012) investigated the effects of deoxynivalenol (DON), a type-B trichothecene, on broilers. Male broilers at 7 d of age were fed either a basal diet (0.265 ± 0.048 mg of DON; 0.013 ± 0.001 mg of zearalenone/kg), a low DON diet (1.68 mg of DON/kg; 0.145 ± 0.007 mg of zearalenone/kg), or a high DON diet (12.209 ± 1.149 mg of DON/kg; 1.094 ± 0.244 mg of zearalenone/kg). Increasing levels of DON decreased the weekly weight gain linearly ($P \leq 0.041$) during the first 3 wk of exposure; there were no significant differences in the weight gain of the birds after wk 3. With increasing levels of DON, the titers against Newcastle disease virus increased linearly during wk 2 ($P = 0.022$) and wk 4 ($P = 0.033$) of exposure, whereas

the titers against infectious bronchitis virus decreased linearly (P = 0.006) during wk 5 of exposure. The serum protein concentration increased linearly (P = 0.017) during wk 2 and quadratically (P = 0.002) during wk 4 of exposure. Under these experimental conditions, the performance and vaccine response of the broilers were modulated to varying degrees at concentrations of DON that are currently permitted (up to 5 mg/kg of diet) in many countries. Further studies are therefore required to clarify the implications of these results on the welfare of chickens.

Awad *et al.* (2013), in their review, highlighted the impacts of DON intoxication on cell mediated immunity, humoral immunity, gut immunity, immune organs and pro-inflammatory cytokines in chickens. Deoxynivalenol (DON) is a common *Fusarium* toxin in poultry feed. Chickens are more resistant to the adverse impacts of deoxynivalenol (DON) compared to other species. In general, the acute form of DON mycotoxicosis rarely occurs in poultry flocks under normal conditions. However, if diets contain low levels of DON (less than 5 mg DON/kg diet), lower productivity, impaired immunity and higher susceptibility to infectious diseases can occur. The molecular mechanism of action of DON has not been completely understood. A significant influence of DON in chickens is the impairment of immunological functions. It was known that low doses of DON elevated the serum IgA levels and affected both cell-mediated and humoral immunity in animals. DON is shown to suppress the antibody response to infectious bronchitis vaccine (IBV) and to Newcastle disease virus (NDV) in broilers (10 mg DON/kg feed) and laying hens (3.5 to 14 mg of DON/kg feed), respectively. Moreover, DON (10 mg DON/kg feed) decreased tumor necrosis factor alpha (TNF-α) in the plasma of broilers. DON can severely affect the immune system and, due to its negative impact on performance and productivity, can eventually result in high economic losses to poultry producers.

Dänicke *et al.* (2013) performed a study to examine thes interactions between A. galli infection and DON contamination of feed on 4 groups of 9 pullets. Nnon-infected groups were fed either an uncontaminated control (CON-) or a Fusarium toxin contaminated and mainly DON-containing diet (FUS-), and the corresponding A. galli inoculated groups were fed accordingly (CON+, FUS+). A. galli infection significantly reduced the jejunal villi height and increased the thickness of the tunica muscularis with the effect being more pronounced when the DON-containing diet was fed (Group FUS+). Only in this group significantly increased weights of jejunal and ileal tissues and of livers were noticed. Moreover, DON was detected in plasma of the pullets at higher frequencies when they were infected suggesting a facilitated absorption of DON. Group FUS+ was characterized by a significantly higher excretion of A. galli eggs and a concomitant lower proportion of pullets with detectable antibodies against a somatic antigen of A. galli while worm burden and worm characteristics were not affected by diet. Other effects of feeding the FUS diet to the infected pullets included an increased mass per length of male worms. In conclusion, infection of pullets with A. galli might increase the susceptibility towards DON as indicated by an increased DON absorption rate and a compromised antibody formation. The effects of DON on fecundity and worm morphology require further examination.

Ghareeb *et al.* (2013) conducted an experiment to investigate the individual and combined effects of dietary deoxynivalenol (DON) and a microbial feed additive on plasma cytokine level and on the expression of immune relevant genes in jejunal tissues of broilers. A total of 40 broiler chicks were obtained from a commercial hatchery and divided randomly into four groups (10 birds per group). Birds were

reared in battery cages from one day old for 5 weeks. The dietary groups were 1) control birds fed basal diet; 2) DON group fed basal diet contaminated with 10 mg DON/ kg feed; 3) DON + Mycofix group fed basal diet contaminated with 10 mg DON/ kg feed and supplemented with a commercial feed additive, Mycofix® Select (MS) (2.5 kg/ton of feed); 4) Mycofix group fed basal diet supplemented with MS (2.5 kg/ton of feed). At 35 days, the plasma levels of tumor necrosis factor alpha (TNF-α) and interleukin 8 (IL-8) were quantified by ELISA test kits. Furthermore, the mRNA expression of TNF-α, IL-8, IL-1β, interferon gamma (IFNγ), transforming growth factor beta receptor I (TGFBR1) and nuclear factor kappa-light-chain-enhancer of activated B cells 1 (NF-κβ1) in jejunum were quantified by qRT-PCR. The results showed that the plasma TNF-α decreased in response to DON, while in combination with MS, the effect of DON was reduced. DON down-regulated the relative gene expression of IL-1β, TGFBR1 and IFN-γ, and addition of MS to the DON contaminated diet compensates these effects on IL-1β, TGFBR1 but not for IFN-γ. Furthermore, supplementation of MS to either DON contaminated or control diet up-regulated the mRNA expression of NF-κβ1. In conclusion, DON has the potential to provoke and modulate immunological reactions of broilers and subsequently could increase their susceptibility to disease. The additive seemed to have almost as much of an effect as DON, albeit on different genes.

Khmelnitskiy and Korzunenko (2013) carried out a study to determine the detoxification activity of combined sorbent preparation consisted of anthracite, saponite and inactivated yeasts on the mixed chickens' mycotoxicosis, thirty, two-weeks-old chickens cross "Ross 308" were divided into three groups: A (control); B (T-2 toxin and deoxynivalenol); C (T-2 toxin, deoxynivalenol and the combined sorbent preparation). Chickens were weighed every week, hematological and serum biochemical investigations were provided at 28-th and 42-nd day of chicken`s age. Applying of the combined sorbent preparation in T-2 toxin and deoxynivalenol mixed chickens toxicosis at 3 % by weight of the feed, neutralizes the negative effects of mycotoxins on the bird. It manifests high yield carcass weight and lowers the feed conversion, with almost no variations in hematological and serum biochemical parameters of blood

Osselaere (2013) studied the absolute oral bioavailability and the toxicokinetic parameters of deoxynivalenol, T-2 and zearalenone in broilers. Toxins were administered intravenously and orally in a two-way cross-over design. For deoxynivalenol a bolus of 0.75 mg/kg BW was administered, for T-2 toxin 0.02 mg/kg BW and for zearalenone 0.3 mg/kg BW. Blood was collected at several time points. Plasma levels of the mycotoxins and their metabolite(s) were quantified using LC-MS/MS methods and toxicokinetic parameters were analyzed. Deoxynivalenol has a low absolute oral bioavailability (19.3%). For zearalenone and T-2 no plasma levels above the limit of quantification were observed after an oral bolus. Volumes of distribution were recorded, i.e. 4.99 L/kg, 0.14 L/kg and 22.26 L/kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. Total body clearance was 0.12 L/min.kg, 0.03 L/min.kg and 0.48 L/min.kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. After IV administration, T-2 toxin had the shortest elimination half-life (3.9 min), followed by deoxynivalenol (27.9 min) and zearalenone (31.8 min)

Osselaere **et al. (2013b)** investigated the effects of three weeks of feeding deoxynivalenol on the gut wall morphology, intestinal barrier function and

inflammation in broiler chickens. In addition, oxidative stress was evaluated in both the liver and intestine. Besides, the effect of a clay-based mycotoxin adsorbing agent on these different aspects was also studied. Our results show that feeding deoxynivalenol affects the gut wall morphology both in duodenum and jejenum of broiler chickens. A qRT-PCR analysis revealed that deoxynivalenol acts in a very specific way on the intestinal barrier, since only an up-regulation in mRNA expression of claudin 5 in jejunum was observed, while no effects were seen on claudin 1, zona occludens 1 and 2. Addition of an adsorbing agent resulted in an up-regulation of all the investigated genes coding for the intestinal barrier in the ileum. Up-regulation of Toll-like receptor 4 and two markers of oxidative stress (heme-oxigenase or HMOX and xanthine oxidoreductase or XOR) were mainly seen in the jejunum and to a lesser extent in the ileum in response to deoxynivalenol, while in combination with an adsorbing agent main effect was seen in the ileum. These results suggest that an adsorbing agent may lead to higher concentrations of deoxynivalenol in the more distal parts of the small intestine. In the liver, XOR was up-regulated due to DON exposure. HMOX and HIF-1α (hypoxia-inducible factor 1α) were down-regulated due to feeding DON but also due to feeding the adsorbing agent alone or in combination with DON.

Antonissen *et al.* (2014) carried out a study that aimed at examining the predisposing effect of DON on the development of necrotic enteritis in broiler chickens. An experimental *Clostridium perfringens* infection study revealed that DON, at a contamination level of 3,000 to 4,000 µg/kg feed, increased the percentage of birds with subclinical necrotic enteritis from 20±2.6% to 47±3.0% ($P<0.001$). DON significantly reduced the transepithelial electrical resistance in duodenal segments ($P<0.001$) and decreased duodenal villus height ($P=0.014$) indicating intestinal barrier disruption and intestinal epithelial damage, respectively. This may lead to an increased permeability of the intestinal epithelium and decreased absorption of dietary proteins. Protein analysis of duodenal content indeed showed that DON contamination resulted in a significant increase in total protein concentration ($P=0.023$). Furthermore, DON had no effect on *in vitro* growth, alpha toxin production and *netB* toxin transcription of *Clostridium perfringens*. In conclusion, feed contamination with DON at concentrations below the European maximum guidance level of 5,000 µg/kg feed, is a predisposing factor for the development of necrotic enteritis in broilers. These results are associated with a negative effect of DON on the intestinal barrier function and increased intestinal protein availability, which may stimulate growth and toxin production of *Clostridium perfringens*.

Awad *et al.* (2014) performed an experiment to study the effects of DON and/or a microbial feed additive on the DNA damage of blood lymphocytes and on the level of thiobarbituric acid reactive substance (TBARS) as an indicator of lipid peroxidation and oxidative stress in broilers. A total of forty 1-d-old broiler chicks were randomly assigned to 1 of 4 dietary treatments (10 birds per group) for 5 wk. The dietary treatments were 1) basal diet; 2) basal diet contaminated with 10 mg DON/kg feed; 3) basal diet contaminated with 10 mg DON/kg feed and supplemented with 2.5 kg/ton of feed of Mycofix Select; 4) basal diet supplemented with Mycofix Select (2.5 kg/ton of feed). At the end of the feeding trial, blood were collected for measuring the level of lymphocyte DNA damage of blood and the TBARS level was measured in plasma, heart, kidney, duodenum and jejunum. The dietary exposure of DON caused a significant increase (P=0.001) of DNA damage in blood lymphocytes (31.99±0.89%) as indicated in the tail of comet assay. Interestingly addition of Mycofix Select to

DON contaminated diet decreased (P=0.001) the DNA damage (19.82±1.75%) induced by DON. In order to clarify the involvement of lipid peroxidation in the DNA damage of DON, TBARS levels was measured. A significant increase (P=0.001) in the level of TBARS (23±2 nmol/mg) was observed in the jejunal tissue suggesting that the lipid peroxidation might be involved in the DNA damage. The results indicate that DON is cytotoxic and genotoxic to the chicken intestinal and immune cells and the feed additive have potential ability to prevent DNA damage induced by DON.

Ebrahem ***et al.*** **(2014a)** investigated the potential for carry-over of deoxynivalenol (DON) into eggs and DON residues in plasma and bile of laying hens of different genetic backgrounds after long-term feeding trial. A total of 80, 23-week-old laying hens were assigned to a feeding trial with two diets, a control diet and a Fusarium toxin-contaminated diet (FUS) (0.4 and 9.9 mg DON kg(-1), respectively). In the 60th week of hen's life, 10 eggs from each group were collected. In the 70th week of hen's life, all hens were slaughtered and samples of blood and bile were collected. The samples were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) for DON and de-epoxy-DON. DON was only detected in samples of hens which fed the FUS diet while none of the samples analysed had detectable levels of de-epoxy-DON. In plasma and bile samples, DON levels ranged from 0.2 to 0.6 ng ml(-1) and from 1.8 to 4.1 ng ml(-1), respectively. DON levels in egg yolk and albumen ranged between 0.0-0.46 ng g(-1) and 0.0-0.35 ng g(-1), respectively, corresponding to carry-over rates of DON into eggs from 0.0 to 0.000016. Moreover, no differences in DON levels or carry-over rates were noticed between the two tested breeds. These results show that very low levels of DON were transferred into eggs and indicate that although eggs could contribute to human exposure to DON, the levels are very low and insignificant.

Ebrahem *et al.* (2014b) assigned 216 23-week-old laying hens from two different genetic backgrounds (half of the birds were Lohmann brown [LB] and [LSL] hens, respectively) and 24 adult roosters to a feeding trial to study the effect of increasing concentrations of deoxynivalenol (DON) in the diet (0, 5, 10 mg/kg) on the reproductive performance of hens and roosters, and the health of the newly hatched chicks. Hatchability was adversely affected by the presence of DON in LB hens' diet, while the hatchability of the LSL chicks was significantly higher than LB chicks. An interaction effect between DON in the hens' diet and the breed was noticed on fertility, as the fertility was decreased in the eggs of LB hens receiving 10 mg/kg DON in their diet and increased in the eggs of LSL hens fed 10 mg/kg DON. Moreover, spleen relative weight was significantly decreased in the chicks hatched from eggs of hens fed contaminated diets, while gizzard relative weight was significantly decreased in LB chicks with 10 mg/kg DON in their diet compared with the control group. On the other hand, the chicks' haematology and organ histopathology were not affected by the dietary treatment. Additionally, the presence of DON in the roosters' diet had no effect on fertility (the percentage of fertile eggs of all laid eggs). Consequently, the current results indicate a negative impact of DON in LB hens' diet on fertility and hatchability, indicating that the breed of the hens seems to be an additional factor influencing the effect of DON on reproductive performance of the laying hens.

Ebrahem *et al.* (2014c) carried out a 12-laying months experiment with laying hens of two different genetic backgrounds to evaluate the effect of feeding of DON contaminated wheat on performance, egg components and health of the hens and the

effect of the breed of the laying hens on the sensitivity towards DON. A total of 216, 23 weeks old laying hens (108 Lohmann Brown, LB, and Lohmann Selected Leghorn, LSL, respectively) were assigned to the feeding trial with increasing concentrations of DON (0, 3.4, 9.9 mg/kg) resulting in 6 experimental groups of 36 hens each. All birds were caged individually and had free access to feed and water. Eggs were collected three times during the experiment for the evaluation of egg quality. At the end of the experiment 20 laying hens per group were slaughtered. Blood was collected for haematology. Liver, spleen, heart, breast muscle, glandular stomach and gizzard were dissected, emptied (glandular stomach and gizzard), and weighed. Tissues for histological examination were collected directly after slaughtering. Significant adverse effect of DON was noticed on the laying intensity, body weight and weight gain of the laying hens; laying intensity was significantly decreased due to the presence of DON in the diet in the second laying period while laying intensity of the LSL hens was significantly higher than the LB hens. Moreover, a decrease in life body weight and lower weight gain ratio was detected in the LB hens fed 9.9 mg/kg DON, while the LSL hens were not significantly affected by the dietary treatment. The relative weight of breast muscle of the LB hens fed 9.9 mg/kg DON was significantly lower than that of other LB groups, while the relative weight of liver was significantly higher. On the other hand, breast muscle and liver relative weights of the LSL hens were not significantly affected by the dietary treatment. Haematocrit and concentrations of white blood cells were not significantly affected by the dietary treatment while significant breed differences were observed. Moreover, DON contaminated wheat resulted in reduction in the eggshell proportion of the eggs of the LB hens fed 9.9 mg DON/kg diet at the 40th and 60th week of life while LSL eggs were not significantly affected. Overall, it can be concluded that the performance and health of the laying hens was adversely affected by the presence of DON in hen's diet at the highest level (9.9 mg/kg) but to a different extent and depending on the breed of the laying hens. Keywords: (Deoxynivalenol, laying hens, different genetic background, performance)

Antonissen ***et al.*** **(2015)** performed a toxicokinetic study with two groups of 6 broiler chickens to investigate whether chronic exposure to DON could influence the intestinal absorption of FBs leading to an altered exposure and increased toxic effects of this mycotoxin in broiler chickens. All broiler chickens were administered an oral bolus of 2.5 mg FBs/kg BW after three-week exposure to either uncontaminated feed (group 1) or feed contaminated with 3.12 mg DON/kg feed (group 2). No significant differences in toxicokinetic parameters of FB1 could be demonstrated between the groups. Also, no increased or decreased body exposure to FB1 was observed, since the relative oral bioavailability of FB1 after chronic DON exposure was 92.2%. The plasma concentration-time profile revealed that FB1 reached the maximum plasma concentration (Tmax) at 20 min after oral dosing in both control and DON contaminated group. This rapid appearance of FB1 in the systemic circulation indicated that the ingested toxin is absorbed mainly in the proximal part of the intestinal tract

Devreese ***et al.*** **(2015)** carried out a study to reveal the toxicokinetic properties and absolute oral bioavailability of deoxynivalenol (DON) in turkey poults. Six turkey poults were administered this *Fusarium* mycotoxin *per os*and intravenously in a two-way cross-over design. Based on non-compartmental analysis, DON was absorbed

rapidly (T_{max}= 0.57 h) but incomplete, as the oral bioavailability was only 20.9%. DON was rapidly eliminated as well, both after oral ($T_{1/2elimination\ PO}$=0.86 h) as well as intravenous (IV) ($T_{1/2elimination\ IV}$ = 0.62 h) administration. Furthermore, semi-quantitative analysis using high-resolution mass spectrometry revealed that DON-3α-sulphate is the major metabolite of DON in turkeys after IV as well as oral administration, with DON-3α-sulphate/DON ratios between 1.3-12.6 and 32.4-140.8 after IV and oral administration, respectively. Glucuronidation of DON to DON-3α-glucuronide is a minor pathway in turkey poults, with DON-3α-glucuronide/DON ratios between 0.009-0.065 and 0.020-0.481 after IV and oral administration, respectively. Only trace amounts of other metabolites were found including 10-DON-sulphonate, de-epoxydeoxynivalenol and 10-de-epoxydeoxynivalenol-sulphonate. In addition, a similar two-way cross-over study was performed in three broiler chickens, in order to compare the biotransformation of DON in both poultry species. High-resolution mass spectrometry revealed that DON-3α-sulphate was the major metabolite of DON in broiler chickens as well, with DON-3α-sulphate/DON ratios between 243-453 and 1,365-29,624 after IV and oral administration, respectively. These ratios indicate that broiler chickens metabolise DON even more extensively to the sulphate conjugate compared to turkey poults. Only trace amounts of other metabolites were detected in broiler chickens. In conclusion, it can be stated that the toxicokinetic behaviour of DON in broiler chickens and turkey poults is comparable (low absolute oral bioavailability, rapid absorption and elimination, extensive biotransformation to DON-3α-sulphate), however, relative differences in DON-3α-sulphate/DON ratios exist between both species which might explain the hypothesised difference in sensitivity of both poultry species to DON.

Ghareeba *et al.* (2015) suggested that DON produces its toxicity primarily via activation of the mitogen-activated protein kinases (MAPKs) signalling pathway and alteration in the expression of genes responsible for key physiological and immunological functions of the intestinal tissue of chickens and pigs. The activation of MAPKs signalling cascade results in disruption of the gut barrier function and an increase in the permeability by reducing expression of the tight junction proteins. Exposure to DON also down-regulates the expression of multiple transporter systems in the enterocytes with subsequent impairment of the absorption of key nutrients. Other major intestinal cytotoxic effects of DON described herein are modulation of mucosal immune responses, leading to immunosupression or stimulation of local immune cells and cytokine release, and also facilitation of the persistence of intestinal pathogens in the gut. Both of the last events potentiate enteric infections and local inflammation in pigs and poultry, rendering enterocytes and the host more vulnerable to luminal toxic compounds.

Guerre (2015) mentioned that, despite the fact avian species are highly exposed to fusariotoxins, the avian species are considered as resistant to their toxic effects, partly because of low absorption and rapid elimination, thereby reducing the risk of persistence of residues in tissues destined for human consumption. This review focuses on the main fusariotoxins deoxynivalenol, T-2 and HT-2 toxins, zearalenone and fumonisin B1 and B2. The key parameters used in the toxicokinetic studies are presented along with the factors responsible for their variations. Then, each toxin is analyzed separately. Results of studies conducted with radiolabelled toxins are compared with the more recent data obtained with HPLC/MS-MS detection. The metabolic pathways of deoxynivalenol, T-2 toxin, and zearalenone are described, with attention paid to the differences among the avian species. Although no metabolite of

fumonisins has been reported in avian species, some differences in toxicokinetics have been observed. All the data reviewed suggest that the toxicokinetics of fusariotoxins in avian species differs from those in mammals, and that variations among the avian species themselves should be assessed.

Schwartz-Zimmermann (2015) reported that, deoxynivalenol-3-sulfate (DON-3-sulfate) was proposed recently as a major DON metabolite in poultry. In the present work, the first LC-MS/MS based method for determination of DON-3-sulfate, deepoxy-DON-3-sulfate (DOM-3-sulfate), DON, DOM, DON sulfonates 1, 2, 3, and DOM sulfonate 2 in excreta samples of chickens and turkeys was developed and validated. To this end, DOM-3-sulfate was chemically synthesized and characterized by NMR and LC-HR-MS/MS measurements. Application of the method to excreta and chyme samples of four feeding trials with turkeys, chickens, pullets, and roosters confirmed DON-3-sulfate as the major DON metabolite in all poultry species studied. Analogously to DON-3-sulfate, DOM-3-sulfate was formed after oral administration of DOM both in turkeys and in chickens. In addition, pullets and roosters metabolized DON into DOM-3-sulfate. *In vitro* transcription/translation assays revealed DOM-3-sulfate to be 2000 times less toxic on the ribosome than DON. Biological recoveries of DON and DOM orally administered to broiler chickens, turkeys, and pullets were 74%–106% (chickens), 51%–72% (roosters), and 131%–151% (pullets). In pullets, DON-3-sulfate concentrations increased from jejunum chyme samples to excreta samples by a factor of 60. This result, put into context with earlier studies, indicates fast and efficient absorption of DON between crop and jejunum, conversion to DON-3-sulfate in intestinal mucosa, liver, and possibly kidney, and rapid elimination into excreta via bile and urine.

Liu *et al.* (2016) conducted a survey to determine whether mycotoxins present in the foods consumed by red-crowned cranes (*Grus japonensis*) in the Yancheng Biosphere Reserve, China. A total of 113 food samples were collected in the reserve's core, buffer, and experimental zones during overwintering periods of 2013 to 2015. Samples were analyzed for aflatoxin B_1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A using high performance liquid chromatography (HPLC). The contamination incidences vary among different zones and the mycotoxins levels of different food samples also presented disparity. Average mycotoxin concentration from rice grain was greater than that from other food types. Among mycotoxin-positive samples, 59.3% were simultaneously contaminated with more than one toxin. This study demonstrated for the first time that red-crowned cranes were exposed to mycotoxins in the Yancheng Biosphere Reserve and suggested that artificial wetlands could not be considered good habitats for the birds in this reserve, especially rice fields.

References:

1. Antonissen, G., Van Immerseel, F., Pasmans, F., Ducatelle, R., Haesebrouck, F., Timbermont, L., Croubels, S. (2014). The Mycotoxin Deoxynivalenol Predisposes for the Development of *Clostridium perfringens*-Induced Necrotic Enteritis in Broiler Chickens. *PLoS ONE*, *9*(9), e108775. http://doi.org/10.1371/journal.pone.0108775
2. Antonissen, G., Devreese, M., Van Immerseel, F., De Baere, S., Hessenberger, S., Martel, A., & Croubels, S. (2015). Chronic Exposure to Deoxynivalenol Has No

Influence on the Oral Bioavailability of Fumonisin B_1 in Broiler Chickens. *Toxins*, *7*(2), 560–571. http://doi.org/10.3390/toxins7020560

3. Awad, WA, J Böhm, E Razzazi-Fazeli, HW Hulan, J Zentek. Effects of deoxynivalenol on general performance and electrophysiological properties of intestinal mucosa of broiler chickens, Poultry Science (2004) 83 (12):1964-1972.
4. Awad, WA, H Rehman, J Böhm, E Razzazi-Fazeli, J Zentek, Effects of luminal deoxynivalenol and L-proline on electrophysiological parameters in the jejunums of laying hens. Poultry science 84 (6), 928-932, 2005
5. Awad WA, Bohm J, Razzazi-Fazeli E, Ghareeb K, Zentek J (2006a) Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult Sci 85: 974–979
6. Awad, WA, J Böhm, E Razzazi-Fazeli, J Zentek. Effects of feeding deoxynivalenol contaminated wheat on growth performance, organ weights and histological parameters of the intestine of broiler chickens. Journal of animal physiology and animal nutrition 90 (1-2), 32-37,2006b
7. Awad WA, Aschenbach JR, Setyabudi FMCS, Razzazi-Fazeli E, Böhm J, et al. (2007) In vitro effects of deoxynivalenol on small intestinal D-glucose uptake and absorption of deoxynivalenol across the isolated jejunal epithelium of laying hens. Poult. Sci 86: 15–20
8. Awad, W.A., K. Ghareeb , J. Böhm , E. Razzazi , P. Hellweg and J. Zentek. The Impact of the Fusarium Toxin Deoxynivalenol (DON) on Poultry. International Journal of Poultry Science 7 (9): 827-842, 2008
9. Awad W.A., Hess M., Twarużek M., Grajewski J., Kosicki R., Böhm J., Zentek J. The impact of the*Fusarium* mycotoxin deoxynivalenol on the health and performance of broiler chickens. Int. J. Mol. Sci.2011;12:7996–8012.
10. Awad WA, Ghareeb K, and Bohm J. The toxicity of Fusarium mycotoxin deoxynivalenol in poultry feeding. Review Article. World's Poultry Science Journal / Volume 68 / Issue 04 / December 2012a, pp 651-668
11. Awad WA, Ghareeb K, Dadak A, Gille L, Staniek K, Hess M, Bohm J (2012b) Genotoxic effects of deoxynivalenol in broiler chickens fed with low protein diets. Poult Sci 91: 550–555
12. Awad WA, Ghareeb K, Böhm J, Zentek J (2013) The toxicological impacts of the fusarium mycotoxin, deoxynivalenol, in poultry flocks with special reference to immunotoxicity. Toxins 5: 912–925
13. Awad, W. A., Ghareeb, K., Dadak, A., Hess, M., & Böhm, J. (2014). Single and Combined Effects of Deoxynivalenol Mycotoxin and a Microbial Feed Additive on Lymphocyte DNA Damage and Oxidative Stress in Broiler Chickens. *PLoS ONE*, *9*(1), e88028. http://doi.org/10.1371/journal.pone.0088028
14. Borutova R, Faix S, Plachaa I, Gresakovaa L, Cobanovaa K, et al. (2008) Effects of deoxynivalenol and zearalenone on oxidative stress and blood phagocytic activity in broilers. Arch Anim Nutr 62: 303–312
15. BOSTON, S., G. WOBESER, and M. GILLESPIE (1996): Consumption of deoxynivalenol-contaminated wheat by mallard ducks under experimental conditions. J Wildl Dis. 32, 17-22 Branton, S.L.; Deaton, J.W.; Hagler, W.M.; Maslin, W.R., Jr.; Hardin, J.M. Decreased egg production in commercial laying hens fed zearalenone- and deoxynivalenol-contaminated grain sorghum. *Avian Dis.* 1989, *33*, 804–808

16. CHOWDHURY, S.R. and T.K. SMITH (2005): Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on hepatic fractional protein synthesis rates of laying hens and the efficacy of a polymeric glucomannan mycotoxin adsorbent. Poultry Science. 84, 1671-1674

17. Dänicke S., Ueberschär K.H., Valenta H., Matthes S., Matthäus K., Halle I. Effects of graded levels of Fusarium-toxin-contaminated wheat in Pekin duck diets on performance, health and metabolism of deoxynivalenol and zearalenone. Br. Poult. Sci. 2004;

18. Dänicke S., Beinekeb A., Rautenschlein S., Valenta H., Kersten S., Gaulyd M. *Ascaridia galli* infection affects pullets differently when feed is contaminated with the *Fusarium* toxin deoxynivalenol (DON) Vet. Parasitol. 2013;198:351–363.

19. Davis, G.S.; Anderson, K.E.; Parkhurst, C.R.; Rives, D.V.; Hagler, W.M. Mycotoxins and feed refusal by pekin ducks. *J. Appl. Poult. Res.* 1994, *3*, 190–192

20. Devreese M., Antonissen G., Broekaert N., de Mil T., de Baere S., Vanhaecke L. Toxicokinetic study and oral bioavailability of DON in turkey poults, and comparative biotransformation between broilers and turkeys. World Mycotoxin J. 2015;1:1–7.

21. Ebrahem M., Kersten S., Valenta H., Breves G., Dänicke S. Residues of deoxynivalenol (DON) and its metabolite de-epoxy-DON in eggs, plasma and bile of laying hens of different genetic backgrounds. Arch. Anim. Nutr. 2014a;68:412–422.

22. Ebrahem M., Kersten S., Valenta H., Breves G., Beineke A., Hermeyer K., Dänicke S. Effects of feeding deoxynivalenol (DON)-contaminated wheat to laying hens and roosters of different genetic background on the reproductive performance and health of the newly hatched chicks. Mycotox Res.2014b;30:131–140

23. Ebrahem, M. , S. Kersten , G. Breves , A. Beineke , K. Hermeyer and S. Dänicke, Effect of increasing concentrations of deoxynivalenol (DON) in diet on health and performance of laying hens of different genetic background Europ. Poult.Sci., 78. 2014c, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2013.4

24. FAIXOVÁ, ZITA, , FAIX, S., LENG L, VÁCZI P, SZABÓOVÁ RENÁTA and MAKOVÁ ZUZANA.EFFECTS OF FEEDING DIET CONTAMINATED WITH DEOXYNIVALENOL ON PLASMA CHEMISTRY IN GROWING BROILER CHICKENS AND THE EFFICACY OF GLUCOMANNAN MYCOTOXIN ADSORBENT. Acta Veterinaria (Beograd), Vol. 56, No. 5-6, 479-487, 2006.

25. FAIXOVÁ. ZITA, ŠTEFAN FAIX1 , RADKA BOŘUTOVÁ1 , AND ĽUBOMÍR LENG. EFFECT OF DIFFERENT DOSES OF DEOXYNIVALENOL ON METABOLISM IN BROILER CHICKENS. Bull Vet Inst Pulawy 51, 421-424, 2007

26. Frankic T, Pajk T, Rezar V, Levart A, Salobir J (2006) The role of dietary nucleotides in reduction of DNA damage induced by T-2 toxin and deoxynivalenol in chicken leukocytes. Food Chem Toxicol 44: 1838–1844

27. Ghareeb K, Awad WA, Bohm J (2012) Ameliorative effect of a microbial feed additive on infectious bronchitis virus antibody titer and stress index in broiler chicks fed deoxynivalenol. Poult Sci 91: 800–807

28. Ghareeb, K., Awad, W. A., Soodoi, C., Sasgary, S., Strasser, A., & Böhm, J. (2013). Effects of Feed Contaminant Deoxynivalenol on Plasma Cytokines and mRNA Expression of Immune Genes in the Intestine of Broiler Chickens. *PLoS ONE*, *8*(8), e71492. http://doi.org/10.1371/journal. pone.0071492

29. Ghareeba, Kh, Wageha A. Awadb,c, Josef Böhma and Qendrim Zebeli. Impacts of the feed contaminant deoxynivalenol on the intestine of monogastric animals: poultry and swine. J. Appl. Toxicol. 2015; 35: 327–337

30. Girish, C.K. E. J. MacDonald,† M. Scheinin,‡ and T. K. Smith. Effects of Feedborne Fusarium Mycotoxins on Brain Regional Neurochemistry of Turkeys. Poultry Science 87:1295–1302

31. Harvey, R. B., L. F. Kubena, G. E. Rottinghaus, J. R. Turk, H. H. Casper, and S. A. Buckley. 1997. Moniliformin from Fusarium fujikuroi culture material and deoxynivalenol from naturally contaminated wheat incorporated into diets of broiler chicks. Avian Dis. 41:957–963

32. Labuda, Roman , Alexandra Parich , Elisavet Vekiru. INCIDENCE OF FUMONISINS, MONILIFORMIN AND FUSARIUM SPECIES IN POULTRY FEED MIXTURES FROM SLOVAKIA. Ann Agric Environ Med 2005, 12, 81–86

33. He P., Young L.G., Forsberg C. Microbial transformation of deoxynivalenol (vomitoxin) Appl. Environ. Microbiol. 1992;58:3857–3863

34. Keshavarz, K. (1993) Corn Contaminated with Deoxynivalenol: Effects on Performance of PoultryJ Appl Poult Res (1993) 2 (1): 43-50
35. Konjevic, D., E. Srebocan, A. Gudan, I. Lojkic, K. Severin, and M. Sokolovic. A pathological condition possibly caused by spontaneous trichotecene poisoning in Brahma poultry; first report. *Avian Pathol.*33(3):377–380. 2004.
36. KUBENA, L.F., R. B. HARVEY, D. E. CORRIER, W. E. HUFF, and T. D. PHILLIPS. Effects of Feeding Deoxynivalenol (DON, Vomitoxin)-Contaminated Wheat to Female White Leghorn Chickens from Day Old Through Egg ProductionPoultry Science (1987) 66 (10): 1612-1618
37. Kubena LF, Edrington TS, Harvey RB, Buckley SA, Phillips TD, Rottinghaus GE, Casper HH. Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and T-2 toxin or deoxynivalenol in broiler chicks. Poult Sci. 1997 Sep;76(9):1239-47.
38. Labuda, R.; Parich, A.; Berthiller, F.; Tančinová, D. Incidence of trichothecenes and zearalenonein poultry feed mixtures from Slovakia. *Int. J. Food Microbiol.* 2005, *105*, 19–25.
39. Lun A.K., Moran E.T., Jr., Young L.G., McMillan E.G. Disappearance of deoxynivalenol from digesta progressing along the chicken's gastrointestinol tract after intubation with feed containing contaminated corn.Bull. Environ. Contam. Toxicol. 1988;40:317–324
40. MORRIS, C.M., Y.C. LI, D.R. LEDOUX, A.J. BERMUDEZ, and G.E. ROTTINGHAUS (1999): The individual and combined effects of feeding moniliformin, supplied by *Fusarium* fujikuroi culture material, and deoxynivalenol in young turkey poults. Poultry Science. 78, 1110-1115)

41. Osselaere A., Devreese M., Goossens J., Vandenbroucke V., De Baere S., De Backer P., Croubels S. Toxicokinetic study and absolute oral bioavailability of deoxynivalenol, T-2 toxin and zearalenone in broiler chickens. Food Chem. Toxicol. 2013;51:350–355

42. Osselaere A., Santos R., Hautekiet V., de Backer P., Chiers K., Ducatelle R., Croubels S. Deoxynivalenol impairs hepatic and intestinal gene expression of selected oxidative stress, tight junction and inflammation proteins in broiler chickens, but addition of an adsorbing agent shifts the effects to the distal parts of the small intestine. PLoS One. 2013b;8:e69014. doi: 10.1371/journal.pone.0069014

43. Prelusky D.B., Hamilton R.M.G., Trenholm H.L., Miller J.D. Tissue distribution and excretion of radioactivity following administration of 14C-labeled deoxynivalenol to white leghorn hens. Fundam. Appl. Toxicol. 1986;7:635–645.

44. Prelusky D.B., Hamilton R.M., Trenholm H.L. Transmission of residues to eggs following long-term administration of 14C-labelled deoxynivalenol to laying hens. Poult. Sci. 1989;68:744–748.

45. Schwartz-Zimmermann, H. E., Fruhmann, P., Dänicke, S., Wiesenberger, G., Caha, S., Weber, J., & Berthiller, F. (2015). Metabolism of Deoxynivalenol and Deepoxy-Deoxynivalenol in Broiler Chickens, Pullets, Roosters and Turkeys.*Toxins*, *7*(11), 4706–4729. http://doi.org/10.3390/toxins7114706

46. SOLCAN, Carmen, C. COTEA , Gh. SOLCAN. IMMUNOSUPPRESSIVE ACTION OF DEOXYNIVALENOL OF THYMUS IN CHICKENS. Cercetări Agronomice în Moldova Vol. XLV , No. 4 (152) / 2012.

47. Swamy HV[1], Smith TK, MacDonald EJ, Boermans HJ, Squires EJ. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J Anim Sci. 2002 Dec;80(12):3257-67.

48. SWAMY, H.V.L.N., T.K. SMITH, N.A. KARROW, and H.J. BOERMANS (2004): Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on growth and immunological parameters of broiler chickens. Poultry Science. 83, 533-543

49. Sypecka, Zuzana , Mitchell Kelly , and Paul Brereton, Deoxynivalenol and Zearalenone Residues in Eggs of Laying Hens Fed with a Naturally Contaminated Diet: Effects on Egg Production and Estimation of Transmission Rates from Feed to Eggs *J. Agric. Food Chem.*, 2004, *52* (17), pp 5463–5471

50. Trenholm HL , Hamilton RM , Friend DW , Thompson BK , Hartin KE Feeding trials with vomitoxin (deoxynivalenol)-contaminated wheat: effects on swine, poultry, and dairy cattle.Journal of the American Veterinary Medical Association [1984, 185(5):527-531]

51. Valenta H., Dänicke S. Study on the transmission of deoxynivalenol and de-epoxy-deoxynivalenol into eggs of laying hens using a high-performance liquid chromatography-ultraviolet method with clean-up by immunoaffinity columns. Mol. Nutr. Food Res. 2005;49:779–785

52. YEGANI, M., T.K. SMITH, S. LEESON, and H.J. BOERMANS (2006): Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of broiler breeders and efficacy of a polymeric glucomannan mycotoxins adsorbent. Poultry Science. 85, 16-16

53. Young J.C., Zhou T., Yu H., Zhu H., Gong J. Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food Chem. Toxicol. 2007;45:136–143.

54. Yunus A.W., Valenta H., Abdel-Raheem S.M., Döll S., Dänicke S., Böhm J. Blood plasma levels of deoxynivalenol and its de-epoxy metabolite in broilers after a single oral dose of the toxin. Mycotox Res.2010;26:217–220.

55. Yunus AW, Ghareeb K, Twaruzek M, Grajewski J, Böhm J (2012) Deoxynivalenol as a contaminant of broiler feed: effects on bird performance and response to common vaccines. Poult Sci 91: 844–851

56. Xu L, Eicher SD, Applegate TJ (2011) Effects of increasing dietary concentrations of corn naturally contaminated with deoxynivalenol on broiler and turkey poult performance and response to lipopolysaccharide. Poult Sci 90: 2766–2774

4.7.2. Fumonisin toxicosis

Fumonisins are mycotoxins with important implications in animal health. The main target organs for the toxic actions of fumonisins are the brain in horses and the lungs in the case of swine. Experimentally induced fumonisin toxicosis has been studied in poultry and cattle using naturally contaminated corn or corn screenings as the mycotoxin source. Results have shown a much lower sensitivity of these species to the toxic action of fumonisins when compared to horses and pigs. However, adverse effects on performance parameters of broiler chickens and turkey poults and on selected immune parameters of chickens and cattle were reported. The toxicodynamics (mechanism of action) of fumonisins appears to be a blockage in the synthesis of sphingolipids and thus constitute a unique toxicological action among the known mycotoxins.**(Diaz *et al*., 1995)**

Historical background of fumonisins

- In 1900, fumonisin toxic effects were observed for the first time after sporadic fatal conditions in horses in countries such as the United States, China, Japan, Europe, South Africa and Egypt
- In 1902, Butler named the disease equine leukoencephalomalacia (ELEM) after inducing its symptoms in tested horses fed with moldy feed. Other names used to describe it were blind staggers, foraging disease, moldy corn poisoning, leucoencephalitis, and cerebritis **(BUTLER , 1902, BUCK,1979)**
- In 1970, an outbreak of ELEM in horses in South Africa was associated with the contamination of corn by the fungus Fusarium verticillioides in certain areas **(BUTLER, 1902)**
- In 1971, Wilson confirmed that causative agents of ELEM are maize and cereals infected with genus Fusarium mould. In particular, Fusarium moniliforme was implicated **(WILSON and MARONPOT , 1971)**
- In 1988, the real causative agent of ELEM in South Africa was discovered by Marasas's group, when the two toxic metabolites (FB1 and FB2) were isolated from contaminated maize with ***Fusarium verticillioides*** **(MARASAS et al.,1984, MAJA, 2001, WALTER et al., 2001,**
- In 1990, Kellerman observed typical symptoms of ELEM after horses were exposed to purified FB1 by oral route **(KELLERMAN et al., 1990)**

- Numerous studies have been performed to better understand the adverse effects of FB1 on different animal species. The results obtained from those studies confirmed that FB1 was implicated in hepatic and renal toxicities in equines, pigs, sheep, rodents and poultry **(BUCCI et al.,1998, MARIJANOVIC et al., 1991, LEDOUX et al., 1992)**
- Poultry are quite resistant to fumonisin toxicity. Nevertheless, they may be at risk as well. In large areas in the world, the major part of their diet consists of maize, which can be highly contaminated **(Diaz and Boermans, 1994**).
    - High doses (up to 300 mg/kg feed) are needed to induce clinical toxicity including decreased weight gain and liver failure in broiler chickens **(Ledoux et al., 1992).**
    - In general, high doses are needed to induce toxicity as fumonisins have a very low oral bioavailability (**Martinez-Larranaga *et al*., 1999**).
- Turkeys are more susceptible than chickens (**Weibking *et al*., 1994**).

Signs of acute fumonisin intoxication:

- Non-species specific symptoms, such as hepatotoxicity and renal failure, as well as species specific symptoms on target organs
- The most toxigenic is fumonisin B1 (FB1) and causes different pathologies, such as
    - liver cancer in rodents **(Gelderblom *et al.,* 1994),**
    - pulmonary edema in pigs (**Diaz & Boermans, 1994),**
    - leukoencephalomalacia in horses **(Norred & Voss, 1994).**
    - **Broilers** contaminated with increasing dietary FB1 levels (up to 400 mg fumonisin/kg of feed) showed
        - poor live performance,
        - diarrhea,
        - lack of appetite,
        - increased liver size,
        - high proventriculus, gizzard and kidney weight, and high mortality **(Ledoux *et al*., 1992).**
        - In poultry, these serious symptoms were observed with doses greater than 150 mg fumonisin/kg of feed **(Norred & Voss, 1994).**
            - **In broiler** at 190 to 280 ppm **(Weibking et al, 1993)**
            - **In** duckling study at 120 ppm **(Bermudez et al, 1995**)
            - **In turkey poults** at 99 ppm **(Ledoux et al, 1996)**
- Iimmunosuppression has been demonstrated after chronic fumonisin exposure. This is economically important as adverse effects on the immune system may lead to increased pathogen susceptibility and lowered vaccinal response (**Voss et al., 2007).**
- Although chickens may be slightly more resistant to fumonisins than turkeys and ducklings, it appears that these three species should be considered fairly resistant to the toxic effects of fumonisins and should be grouped into one category (poultry fed for slaughter). **(FDA, 2001)**

Adverse effect of FB1 on chickens, BENLASHEHR, 2013

Dose and duration	Descriptions	References
Laying hens 100, 200 mg FB1/kg feed/ 420 days	No mortality, no BW decrease Weak effect on biochemistry Decrease egg production.Weak egg weight increase	KUBENA et al.,1999
Broiler 20- 80 mg FB1/kg feed/ 21 days	No signs of toxicity No effect on biochemistry Alteration of sphingolipids	HENRY et al., 2000
Broiler 125, 274 mg FB1/kg feed/ 14 days	Increased mortality (20 - 50%) Young more sensitive	JAVED et al., 1993
Broiler 100-400 mg FB1/kg feed/ 21 days	400 mg/kg decrease BW and increased body organ weights Necrotic hepatic foci Altered biochemistry	LEDOUX et al., 1992
Broiler 33 - 627 mg FBs/kg feed/ 21 days	Reduction in weight gain and feed conversionHepatocellular hyperplasia	EFSA (2005)
Broiler 75- 525 mg FB1/kg feed/ 21 days	> 450 mg/kg : decrease feed intake, BW gains, increase liver and kidney weights. > 150 mg/kg Hepatocellular hyperplasia All doses: alteration of sphingolipids	WEIBKING et al., 1993
Broiler 50-200 mg FB1/kg feed/ 21 days with infectious challenge	200 mg /kg: decrease lymphocyte proliferation enhance bacterial colonies in blood, spleen, and liver. decrease secondary antibody response.	LI et al., 1999
Broiler 25, 50 mg FB1/kg feed/ 42 days	No mortality, no BW decrease Unexpected effects on biochemistry All doses: alteration of sphingolipids in liver	BROOMHEAD et al., 2002

Adverse effect of FB1 on turkeys BENLASHEHR, 2013

Dose and duration	Descriptions	References
75-300 mg FB1/kg feed/ 21 days	Decrease feed intake , BW gain Increase in liver weight, hepatocellular, biliary hyperplasia >75 mg/kg: alteration sphingolipids serum	WEIBKING et al., 1995
75 mg FB1/kg feed/126 days	Decrease BW gain Increase liver weight	BERMUDEZ **et al., 1996**
25-475 mg FB1/kg feed/ 21 days	Dose depending hepatocellular hyperplasia >250 mg /kg: decrease feed intakes and B.W. gains ≥ 175 mg /kg increase liver, pancreas weight >325 mg/kg ateration of biochemistry >25 mg/kg: alteration sphingolipids liver	LEDOUX **et al., 1996**
25, 50 mg FB1/kg feed/91 days	50 mg /kg: decrease feed intake	BROOMHEAD **et al., 2002**
5-20 mg FB1+FB2/kg feed/63 days	>5 mg/kg: alteration sphingolipids liver and kidney	TARDIEU **et al., 2007**

Adverse effect of FB1 on ducks, BENLASHEHR, 2013

Dose and duration	Descriptions	References
100- 400 mg FB1/kg feed/21 days	400 mg/kg: increase mortality >100 mg/kg: decrease feed intake, B.W.gain >100 mg/kg: increase organ weights, hepatocellular hyperplasia	BERMUDEZ **et al., 1995**

	>100 mg/kg: alteration sphingolipids	
5- 45 mg FB1/kg b.w. /12 days.	>5 mg/kg: Increase liver weight All doses: alteration of biochemistry and sphingolipids serum	TRANS et al., 2003 **TRAN et al., 2005**
2- 128 mg FB1/kg feed/ 77 days.	>8 mg/kg: decrease BW, increase organs weight. >8 mg/kg: alteration of biochemistry All doses: alteration of sphingolipids (liver, kidney, serum	TRAN **et al., 2005** TRAN **et al., 2006** TARDIEU **et al., 2006**
10, 20 mg FB1/kg feed/ 12 days.	20 mg/kg: increase mortality All doses: alteration of sphingolipids liver, serum	TARDIEU **et al., 2004**

Chemical structures of fumonisins

Fumonisins consist of a linear 19 or 20-carbon, polyketide-derived backbone with one nitrogen, 3–4 hydroxyl, two methyl, and two tricarballylic ester functions at positions along the backbone

	R_1	R_2	Formula	CAS Number	Molecular mass
Fumonisin B_1	OH	OH	$C_{34}H_{59}NO_{15}$	116355-83-0	721.838
Fumonisin B_2	OH	H	$C_{34}H_{59}NO_{14}$	116355-84-1	705.839
Fumonisin B_3	H	OH	$C_{34}H_{59}NO_{14}$	136379-59-4	705.839
Fumonisin B_4	H	H	$C_{34}H_{59}NO_{13}$	136379-60-7	689.840

Chemical structures of fumonisins www.inchem.org

- **Fumonisin B_1** is the diester of propane-1,2,3-tricarboxylic acid and 2*S*-amino-12*S*,16*R*-dimethyl-*3S*,5*R*,10*R*,14*S*,*15R*-pentahydroxyeicosane in which the C-14 and C-15 hydroxy groups are esterified with the terminal carboxy group of propane-1,2,3-tricarboxylic acid.
- **Fumonisin B_2** is the C-10 deoxy analogue of fumonisin B_1 in which the corresponding stereogenic units on the eicosane backbone have the same configuration.

- **Fumonisin B_3 and B_4** full structure is unknown, but the amino terminal of fumonisin B_3 has the same absolute configuration as that of fumonisin B_1.
- A, B, C and P fumonisins differ in structure by differences in the nitrogen function and by the length of the carbon backbone. For example,
    - in A fumonisins it is an acetylated amine, and
    - In B fumonisins (FBs) the back-bone is 20 carbon atoms long
    - in C fumonisins (FCs) it is 19 carbon atoms long
    - in P fumonisins it is a 3-hydroxypyridinium (Musser and Plattner,1997; Sewram et al., 2005).

Physical characteristics of fumonisins

- The pure substance of FB1 is a white hygroscopic powder which is soluble in water, acetonitrile-water or methanol-water.
- It is stable in acetonitrile-water (1:1), food-processing temperature and light.
- FB1 is unstable in methanol

Fumonisin analogs:

- There are 28 fumonisin analogs that have been characterized since 1988 can be separated into four main groups, identified as the fumonisin A, B, C, and P series.
- The fumonisin B (FB) analogs, comprising toxicologically important FB_1, FB_2,
- FB_1 typically accounts for 70 to 80% of the total fumonisins produced
- FB_2 usually makes up 15 to 25%
- FB_3 usually makes up from 3 to 8% when cultured on corn or rice or in liquid medium

Fumonisin mode of action

- Fumonisins competitively inhibit sphinganine N-acyl transferase (ceramide synthase) and consequently disrupt the ceramide and sphingolipid metabolism **(Merrill *et al.,* 2001; Riley *et al.,* 2001)**.
- The inhibition of ceramide synthase consequently leads to an accumulation of free sphinganine (Sa), and to a lesser extent of sphingosine (So), and to a decrease of complex sphingolipids formation.
- The increase of free Sa leads to an increased Sa:So ratio in tissues and body fl uids, which has been demonstrated to be a suitable biomarker for fumonisin exposure in mammals and avian species (**Haschek *et al.,* 2001**).
- This increase is dose- and time-dependent, and is opted to occur rapidly and even at low levels **(Voss *et al.,* 2007).**
- The increased concentrations of Sa and So, their phosphate adducts and a reduced ceramide concentration all contribute to the apoptotic, cytotoxic and growth inhibitory effects of fumonisins (**Merrill et al., 2001)**

- The decrease of complex sphingolipids itself appears to contribute to the cellular effects of FB1 as well **(Yoo et al., 1996)**

Fumonisin toxicokinetics

- Poultry are often considered to be quite resistant toward the deleterious effects of FBs, although important differences are observed depending on the age [19] and species [20–24]. **Ledoux et al., 1992Weibking et al., 1993, Kubena et al., 1999, Tardieu et al., 2004)**
- Increased mortality due to FB1 has only been demonstrated in broiler chicks during the first three days of life (≥125 mg/kg feed) and in growing ducks of 12–14 weeks old (20 mg/kg feed) (Javed et al., 1993,**Tardieu et al. 2004)**
- No mortality has been recorded in laying hens, turkeys or older broiler chickens fed high doses of FB1 (≥200 mg/kg feed) for several weeks **(Ledoux et al., 1992, Weibking et al., 1993, Ledoux et al., 1996, Kubena et al., 1999**)
- FBs can reduce growth performance, and induce alterations in serum constituents and enzyme activities demonstrating hepatic toxicity in broilers, turkeys and ducks **(Ledoux et al., 1992**, **Tardieu et al. 2004, Tran et al., 2005, Tardieu et al. 2008)**
- In different animal species it is shown that FBs are absorbed very poorly after oral administration.
- Oral bioavailability (F) of 0.71% was determined in laying hens administered 2 mg [14C]FB1/kg bodyweight **(Vudathula *et al.*,1994)**
- In turkeys and ducks, a similar F was demonstrated after administering 100 mg FB1/kg BW, namely 2.0%–2.3% and 3.2%, respectively **Tardieu et al. 2008, 2009)**
- The toxicokinetics parameters of FB2 are not strongly different from these of FB1 in ducks and turkeys**.(Benlashehr *et al.,* 2011)**
- The intestinal absorption of FBs in avian species is comparable with mammalian species **(Prelusky et al.,1994, Shephard et al., 1995 Martinez-Larranaga et al., 1999)**
- This poor intestinal absorption of FBs has been appointed as the "fumonisin paradox" by Shier [34], or how a toxin can induce liver failure in poultry although it is not effectively absorbed after oral intake **(Shier, 2000).**

Embryotoxicity and teratogenic effects

The chick embryo is regularly used in mycotoxicology as a rapid and cost-effective assay model and the prospects are that interest will continue as more emphasis is placed on the elucidation of interactions among co-occurring mycotoxins.

- **Javed *et al.* (1993a)** observed embryo mortality on inoculating fertile chicken eggs with FB1, effects which were dependent upon duration of exposure and dose level and which were replicated in trials with broiler chicks. In addition, evidence of embryonic deformities was presented.

- **Zacharias *et al*. (1996)** observed dysfunction of sphingoid metabolism in chick embryos exposed to FB1, as in pigs and horses, and furthermore correlated these changes with gross morphological aberrations.

Cytotoxicity

Assays based on isolated cells have emerged as useful adjuncts to whole-animal toxicology, yielding supplementary information on physiological and biochemical modes of action.

- FB1 causes morphological and functional abnormalities in chicken macrophages in vitro, indicative of an immunosuppressive effect . Chicken macrophage viability may be reduced by exposure to T-2 tetraol, a derivative of T-2 toxin **(Kidd *et al.,* 1997**).

Interactions

Under commercial conditions, livestock are exposed to a complex mixture of mycotoxins derived not only from fusaria but from the aspergilli as well. If the net effect is additive then it might be possible to predict the outcome in terms of productivity.

- Reports indicated that most interactions involving Fusarium mycotoxins are less than additive or additive for responses ranging from mortality **(Javed *et al.*, 1993b)** to feed intake and growth **(Harvey *et al.*, 1996; Kubena *et al.*, 1997).**
- Reports indicated synergistic effects of FB1 and DON and FB1 and fusaric acid; FB1 and aflatoxins.
- Thus, a toxic interaction between fusaric acid and FB1 has been demonstrated in the fertile chicken egg.
- In combination, high lethality was observed, whereas individually the mycotoxins had virtually no effect on mortality **(D'Mello *et al*., 1997)**.
- **Kubena *et al*. (1997)** observed that serum protein and urea nitrogen in broilers were increased only by the FB1 and DON combination, while serum Ca levels were increased only by the FB1 and T-2 toxin combination

Fumonisin-producing fungi

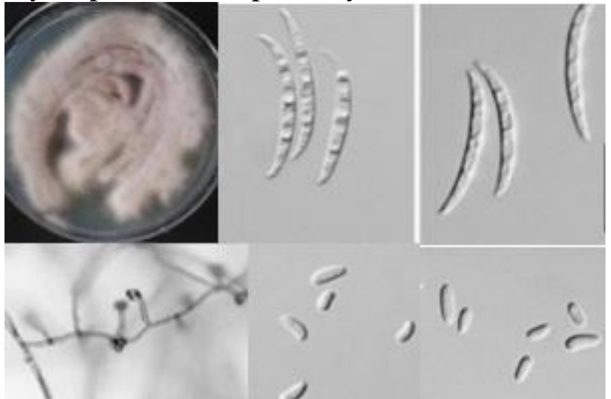
1. *Fusarium acutatum*
2. *Fusarium anantum*
3. *Fusarium andiyazi*
4. *Fusarium anthophilum*
5. *Fusarium begonia*
6. *Fusarium brevicatenulatum*
7. *Fusarium concentricum*
8. *Fusarium dlaminii*
9. *Fusarium konzum*

10. Fusarium napiforme
11. Fusarium nygamai
12. Fusarium phyllophilum
13. Fusarium polyphialidicum
14. Fusarium pseudocircinatum
15. Fusarium pseudonygamai
16. Fusarium ramigenum
17. Fusarium redolens
18. Fusarium sacchari
19. Fusarium subglutinans
20. Fusarium temperatum
21. Fusarium thapsinum
22. Fusarium verticillioides
23. Alternaria alternata f sp. Lycopersici (**Chen et al., 1992)**
***24. Aspergillus niger* (Frisvad *et al.,* 2007)**

Description of some fumonisin-producing Fusarium species

1. Fusarium acutatum Nirenberg & O'Donnell, Mycologia 90: 435 (1998)

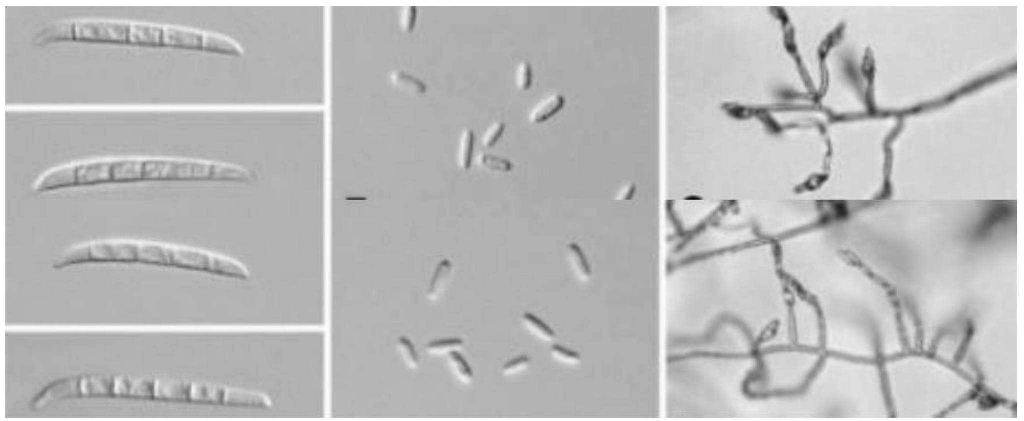
Colonies produce white to pinkish-white mycelium with light orange pigments in the agar. Macroconidia sparse, fulacate, thin-walled, 3-septate, apical cell bent, basal cell foot-shaped. Microconidia abundant, oval-fusoid, conidiogenous cell mono- or polyphialides. Chlamydospores develop slowly, in chains and clusters



Fusarium acutatum colony www.boldsystems.org,, conidia, Leslie and Summerell

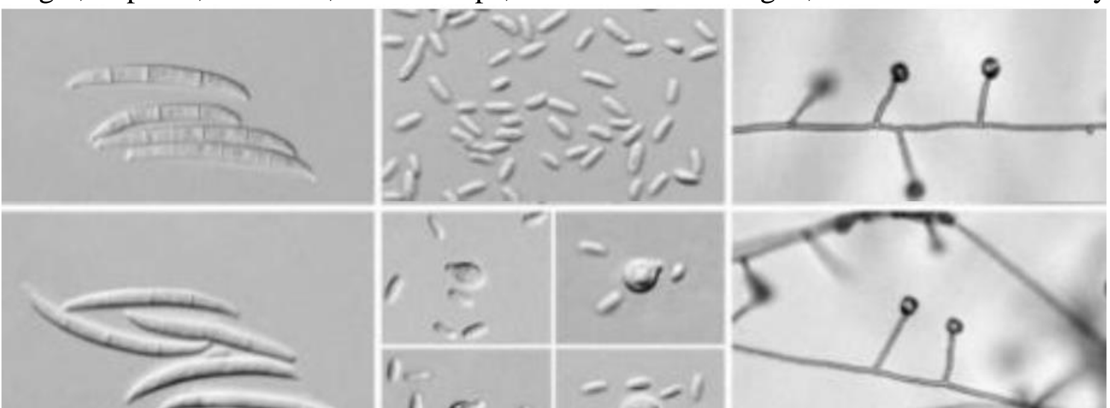
2. Fusarium brevicatenulatum Nirenberg & O'Donnell, Mycologia 90: 446 (1998)

Colony margin entire. Aerial mycelium whitish; lanose to fluffy. Pigmentation in reverse greyish orange, becoming dark bluish-gray. Sporodochia formed after 10 days. Conidiophores on the aerial mycelium prostrate, mostly identical with phialides, occasionally with one lateral branch. Phialides of conidiophores on the aerial mycelium cylindrical, mostly monophialidic, occasionally polyphialidic. Microonidia borne on the aerial mycelium long-oval to obovoid, mostly O-septate, sometimes 1- and 2-septate. Macroconidia borne in sporodochia rare, falcate, slender, straight, up to 3-4 septate, apical cell bent, basal cell foot-like. Chlamydospores absent.


John F. Leslie and Brett A. Summerell

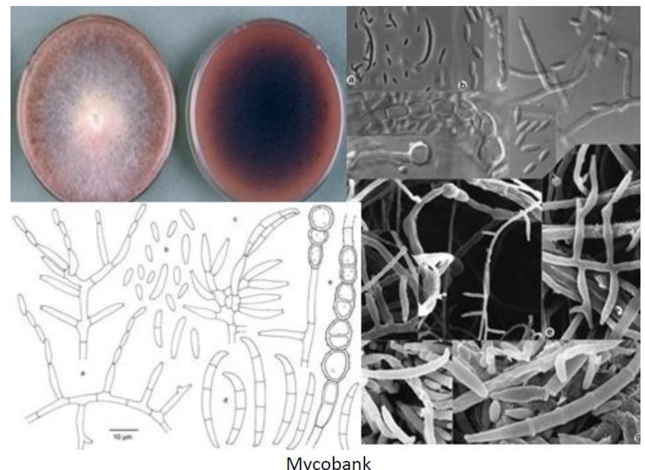
3. Fusarium dlaminii Marasas, P.E. Nelson & Toussoun, Mycologia 77: 971 (1986)

Macroconidia: abundant in sporodochia , moderately long , thin-walled, falcate or straight, 3-5 septa, apical cell curved and tapering, basal cell foot-shaped. Sporodochia: orange. Microconidia: abundant on aerial myceliamostly fusiform non-septate and some are napiform , 0-1 septa. Chlamydospores abundant in 4-6 weeks, single, in pairs , in chains, or in clumps, in aerial or submerged, terminal or intercalary


John F. Leslie and Brett A. Summerell

4. Fusarium nygamai L.W. Burgess & Trimboli, Mycologia 78: 223 (1986)

Macroconidia: abundant in sporodochia, 5-septa, moderately long, straight to slightly curved, apical cell short and tapering, basal cell notched or foot-shaped. Sporodochia: abundant, orange. Microconidia: small, oval or club-shaped, 0-1 septa. Chlamydospores: rare to abundant

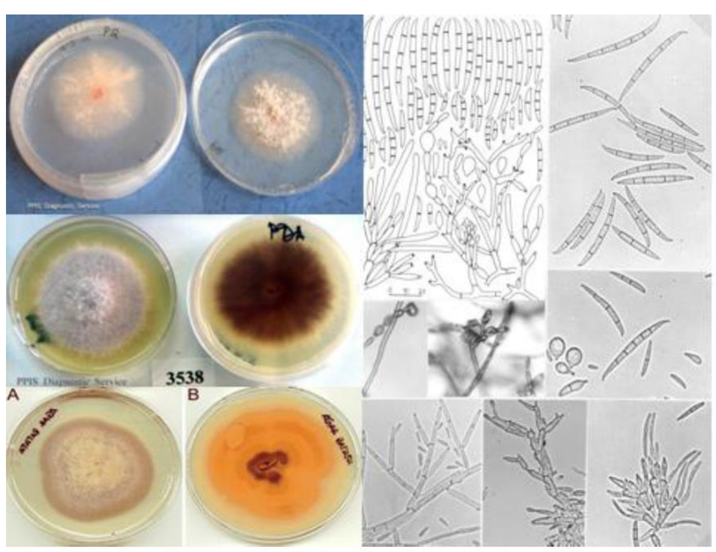


Mycobank

5. Fusarium proliferatum (Matsush.) Nirenberg, Biologischen Bundesanstalt für Land- und Forstwirtschaft 169: 38 (1976)

≡Cephalosporium proliferatum Matsush., Microfungi of the Solomon Islands and Papua-New Guinea: 11 (1971)
≡Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach & Nirenberg, Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 209: 309 (1982)

Macroconidia: in chains of moderate length, thin-walled, straight, 3-5 septa, apical cell curved, basal cell poorly developed,. Sporodochia: pale orange. Microconidia, club-shaped to pyriform, 0-septa, may be in chains. Chlamydospores: absent



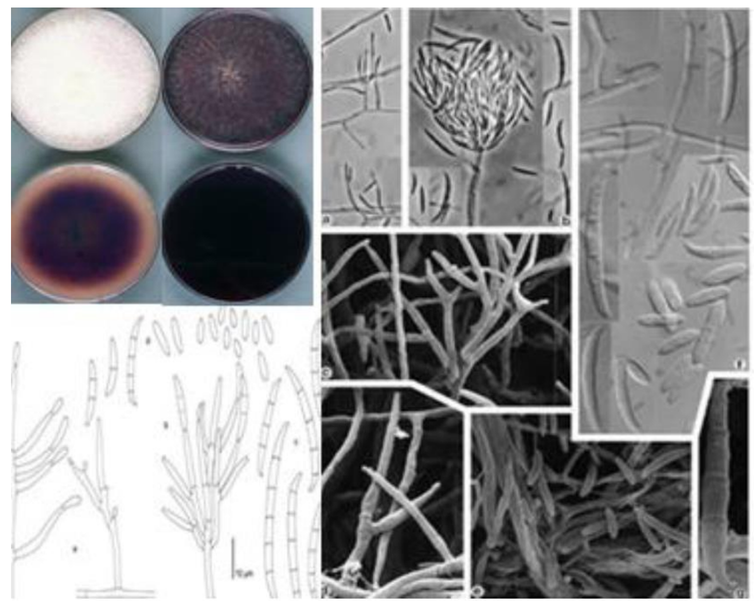
www.ppis.moag.gov.il www.ppis.moag.gov. jcm.asm.org G. Hagedorn, M. Burhenne & H. I. Nirenberg, Ferrer et al., 2005

6. Fusarium subglutinans (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas, Fusarium species, an illustrated manual for identification: 135 (1983)

≡Fusarium moniliforme var. subglutinans Wollenw. & Reinking, Phytopathol 15 (3): 163 (1925)

≡Gibberella fujikuroi var. subglutinans (Wollenw. & Reinking) E.T. Edwards, Agri. Gazette of New South Wales 44 (12): 896 (1933)
≡Fusarium neoceras var. subglutinans (Wollenw. & Reinking) Raillo, Fungi of the genus Fusarium: 263 (1950)
≡Fusarium sacchari var. subglutinans (Wollenw. & Reinking) Nirenberg, Mitteil. Biolog. Bund.. Land- un. Forstwirt. 169: 53 (1976)
≡Gibberella subglutinans (E.T. Edwards) P.E. Nelson, Toussoun & Marasas, Fusarium species, an illustrated manual for identification: 135 (1983)

Colonies produce white mycelium, becomes violate in old cultures. Macroconidia sparse, in tan-orange sporodochia, slender, slighltly falcate, thin-walled, apical cell curves, basal cell poorly developed. Microconidia in false heads from mon- and polyphialides, oval 0- septate or fusiform 2-3 septate. Chlamydospores absent

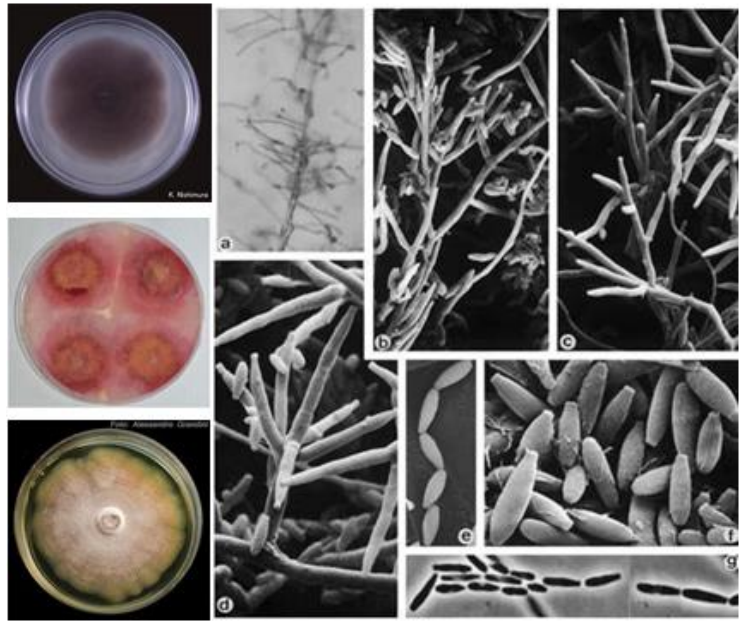


Nelson et al., 1983, Mycobank

7. Fusarium verticillioides (Sacc.) Nirenberg, Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 169: 26 (1976)

≡Oospora verticillioides Sacc., Fung. Ital.: fig. 789 (1881)
≡Alysidium verticillioides (Sacc.) Kuntze, Revisio generum plantarum 3: 442 (1898) ≡Alysidium verticilliodes (Sacc.) Kuntze (1898)
=Fusarium moniliforme J. Sheld., Annual Report of the Nebraska Agricultural Experimental Station 17: 23 (1904)
=Fusarium celosiae Abe, Mem. Coll. Agric. Kyoto Univ.: 51-64 (1928)
=Oospora cephalosporioides Luchetti & Favilli, Ann. Fac. Agrar. R. Univ. Pisa N.S.: 399 (1938)

Colonies produce white mycelium, violete pigmenta with age. Macroconidia rare, in pale orange sporodochia, long. Slender,thinwalled, 3-5-septate, apical cell curved and pointed, basal cell notched to foot-shaped. Microconidia, monophilides abundant on the aerial mycelium, club-shaped, 0-septate. Chlamydospores absent



***Fusarium verticillioides* Mycobank**

Reports:

Ledoux *et al.* (1992) evaluated the effects of dietary **fumonisin B1** in young broiler chicks. The experimental design consisted of 5 treatments each with 9 randomly allotted male broiler chicks. Day-old chicks were fed diets containing 0 (feed control), 100, 200, 300, or 400 mg fumonisin B1/kg feed for 21 days. Response variables measured were chick performance, organ weights, serum biochemistry, and histologic parameters. Body weights and average daily gain dramatically decreased with increasing dietary fumonisin B1, and liver, proventriculus, and gizzard weights increased. Diarrhea, thymic cortical atrophy, multifocal hepatic necrosis, biliary hyperplasia, and rickets were present in chicks fed diets containing fumonisin B,. Serum calcium, cholesterol, and aspartate aminotransferase levels all increased at higher fumonisin dietary levels. Results indicate that fumonisin, from Fusarium moniliforme culture material, is toxic in young chicks.

Nelson *et al.* (1992) tested strains of Fusarium proliferatum, F. subglutinans, F. anthophilum, F. annulatum, F. succisae, F. beomiforme, F. dlamini, F. napiforme, and F. nygamai from a variety of substrates and geographic areas were tested for the production of fumonisin B1 in culture. None of the cultures of F. subglutinans (0 of 23), F. annulatum (0 of 1), F. succisae (0 of 2), or F. beomiforme (0 of 15) produced fumonisin B1 in culture. Strains of F. proliferatum (19 of 31; 61%) produced fumonisin B1 in amounts ranging from 155 to 2,936 ppm, strains of F. anthophilum (3 of 17; 18%) produced fumonisin B1 in amounts ranging from 58 to 613 ppm, strains of F. dlamini (5 of 9; 56%) produced fumonisin B1 in amounts ranging from 42 to 82 ppm, strains of F. napiforme (5 of 33; 15%) produced fumonisin B1 in amounts ranging from 16 to 479 ppm, and strains of F. nygamai (10 of 27; 37%) produced fumonisin B1 in amounts ranging from 17 to 7,162 ppm. Of the species tested, F. proliferatum is the most important producer of fumonisin B1 because of its association with corn and animal mycotoxicoses such as porcine pulmonary edema. F. napiforme and F. nygamai also may be important because of their association with the food grains millet and sorghum. At present, F. anthophilum and F. dlamini are of minor importance because they are not associated with corn or other major food

grains and have only a limited geographic range. This is the first report of the production of fumonisins by F. anthophilum, F. dlamini, and F. napiforme.

Weibking *et al*. (1993) used one hundred ninety-two day-old female Arbor Acres X Peterson broiler chicks in a 21-day dietary study. The experimental design consisted of eight dietary treatments with four pen replicates of six birds allotted randomly to each dietary treatment. The day-old chicks were fed experimental diets from hatching to 21 days of age. Dietary treatments were prepared by substituting ground *F. moniliforme* M-1325 culture material for ground corn in a typical corn-soybean meal basal diet. Fumonisin culture material contained 7,800 mg FB_1/kg by analysis (FB_2 and FB_3 levels not provided) and made up 0, 1.02, 2.04, 3.06, 4.08, 5.10, 6.12, and 7.14% of the respective diets. Thus, the diets were calculated to contain 0, 75, 150, 225, 300, 375, 450, and 525 mg of FB_1/kg. The total dietary fumonisin levels (FB_1 + FB_2 + FB_3) were reported as 0, 89, 190, 283, 389, 481, 592 and 681 ppm; however, the method used to analyze the diets was not provided. Broilers fed diets containing 89 and 190 ppm of FB_1 + FB_2 + FB_3 showed no statistical differences from controls in feed intake, weight gain, feed conversion, liver weight (wt), kidney wt, heart wt, gizzard wt, proventriculus wt, bursa of Fabricius wt, hemoglobin, erythrocytes, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, glucose, total protein, albumin, AST, or GGT. Broilers fed diets containing 89 ppm of FB_1 + FB_2 + FB_3 showed statistical increases in mean corpuscular hemoglobin concentration (MCHC) (26.3 g/dl vs. 24.1 g/dl in controls) and a statistically significant decrease in cholesterol (107 mg/dl vs. 134 mg/dl in controls). Both of these changes appeared to be statistical happenstance as the MCHC values in the 190, 283, 389, and 481 ppm groups and the cholesterol values in the 190, 283, 481, 592, and 681 ppm groups were not statistically different than controls. Compared with controls, all chicks fed diets containing fumonisins had statistically increased ($P<.05$) serum sphinganine/sphingosine ratios [approx. values for the control, 89 and 190 ppm groups were 0.11, 0.27 and 0.31, respectively]. Histopathology of brain, kidney, proventriculus, duodenum, pancreas, jejunum, ileum, cecum, lung, bursa, thymus, spleen, proximal tibiotarsus, heart and skeletal muscle was unremarkable (no lesions or incidental findings) in all treatment groups. Isolated foci of hepatic necrosis with a mild heterophil and macrophage infiltration, moderate diffuse hepatocellular hyperplasia, mild biliary hyperplasia, and moderate to severe periportal granulocytic cell proliferation were noted only in broilers fed >283 ppm total fumonisins. No gross or histologic liver lesions were mentioned in the 89 and 190 ppm groups

Espada *et al.* (1994) evaluated the effects of fumonisin B1 (FB1) intoxication in chickens in three experiments. Two-day-old broiler chicks were fed a diet containing 10 mg pure FB1/kg feed for 6 days; some chicks were necropsied at this time, and others were allowed to recover for 5 weeks before necropsy. In two other experiments, 2-day-old chicks were fed a broiler starter ration prepared with Fusarium moniliforme culture material containing FB1; one group received 30 mg/kg for 2 weeks, and another received 300 mg FB1/kg for 8 days. Compared with controls, intoxicated chicks exhibited diarrhea; decreases in body weight and in liver, spleen, and bursa absolute weights; a hepatic relative weight increase; and spleen relative weight decrease. Triglycerides, uric acid levels, and alkaline phosphatase activity decreased, and gamma glutamyl transferase, aspartate aminotransferase, lactic dehydrogenase, creatine kinase, and cholesterol increased. The results indicate that

low doses of pure FB1 (10 mg/kg) and FB1 from Fusarium moniliforme culture material (30 mg/kg) are toxic to young chicks.

Nagaraj *et al.* (1994) investigated the toxicity of Fusarium proliferatum M-7176 cultured on corn (FPC) and nutritional intervention in baby chicks (New Hampshire x Single Comb White Leghorn) in three 2-wk feeding experiments. In Experiment 1, 30% FPC decreased weight gain ($P < .05$) and increased relative heart weight (RHW) ($P < .01$). Experiment 2 included a 2 x 2 factorial arrangement of FPC (0 or 30%) and Se (0 or 5 mg/kg) and two detached treatments of Se (2.5 mg/kg) or thiamin (B1, 25 mg/kg) supplementations to 30% FPC. Only B1 was inhibitory to the toxic effects of FPC on weight gain, feed efficiency, and RHW ($P < .05$). Experiment 3 included 2 x 2 factorial arrangement between FPC (0 or 30%) and Se (0 or 4 mg/kg), or B1 (0 or 50 mg/kg), or vitamin E (0 or 50 IU/kg) and additional supplementations of Se (2 mg/kg), B1 (10 or 25 mg/kg), or E (10 IU/kg) to 30% FPC. A new batch of FPC was used and it caused 36% mortality. Vitamin E did not interact with FPC, but SE interacted with FPC only on RHW ($P < .01$). Thiamin interacted with FPC on all measured variables with significance ranging from $P < .1$ to $P < .01$. Supplementation of B1 as low as 10 mg/kg was inhibitory to some toxic effects of FPC. However, B1 as high as 50 mg/kg did not completely negate the cardiotoxicity. Water-extractable B1 in FPC diets was 13 to 27% of the control diets. Water extract of FPC reduced B1 recovery from a standard solution by 40%. The anti-thiamin factor was heat-sensitive. Both fumonisins and moniliformin were present in FPC. However, the results indicate that the anti-thiamin factor is also a major toxic factor of F. proliferatum M-7176.

Weibking *et al.* (1994) evaluated the individual and combined effects of fumonisin B1 (FB1) and aflatoxin B1 (AF) using a 2 x 2 factorial with treatments of 0 and 75 mg FB1/kg feed and 0 and 200 micrograms AF/kg feed. Each of the four diets was fed to eight pen replicates of six poults from Day 1 to 21. Body weight gain was reduced ($P < .05$) by AF and the FB1-AF combination. Poults fed AF or the FB1-AF combination were less efficient ($P < .05$) in converting feed to gain. Fumonisin B1 increased ($P < .05$) liver weights whereas AF and the FB1-AF combination increased ($P < .05$) spleen weights. The AF and the FB1-AF combination decreased ($P < .05$) serum concentrations of albumin, total protein, and cholesterol. Fumonisin B1 and the FB1-AF combination increased ($P < .05$) serum sphinganine:sphingosine (SA:SO) ratios. Treatment-associated lesions were observed only in the liver. Hepatocellular hyperplasia and biliary hyperplasia were seen in poults fed 75 mg FB1/kg and 200 micrograms AF/kg, respectively. The combination of FB1 and AF caused an increased primary immune response to sheep red blood cells. However, the phytohemagglutinin delayed hypersensitivity response was not affected by dietary treatment. These data indicate that FB1 and AF, alone and in combination, can adversely affect poult performance and health at these dietary concentrations.

Bermudez *et al.* (1995) fed Fusarium moniliforme culture material containing fumonisin B1 (FB1) to white Pekin ducklings from 1 to 21 days of age. Four dietary treatments were prepared with 0, 100, 200, and 400 mg FB1/kg ration. Ducklings fed rations containing FB1 had a dose-dependent decrease in feed intake and weight gain. Increasing levels of FB1 in the ration were associated with increasing absolute organ weights of liver, heart, kidney, pancreas, and proventriculus. Liver sphinganine to sphingosine ratios increased significantly in ducklings fed FB1. Two of eight ducklings fed a ration containing 400 mg FB1/kg died prior to the termination of the

experiment. Mild to moderate hepatocellular hyperplasia was evident in all ducklings fed FB1. Mild to moderate biliary hyperplasia was also noted in the liver sections of ducklings fed 400 mg FB1/kg in the ration. Ducklings, like other poultry, are relatively resistant to the toxic effects of FB1.

Kubena *et al.* (1995a) fed diets containing 200 mg fumonisin B1/kg of feed and .75 mg aflatoxin/kg of feed singly or in combination to female turkey poults (Nicholas Large White) from day of hatch to 21 d of age. When compared with controls, 21-d body weight gains were reduced 10% by fumonisin B1, 39% by aflatoxins, and 47% by the combination. Relative weights (grams/100 g body weight) of the kidney and pancreas increased in poults fed the diet containing aflatoxins alone, whereas the relative weight of the liver decreased. Relative weights of the liver and pancreas increased in the poults fed the fumonisin diet. Relative weights of the kidney, pancreas, and gizzard increased in the poults fed the combination diet, whereas the relative weight of the liver decreased. Most serum constituents, hematology values, and activities of enzymes measured were altered in poults receiving the diets containing aflatoxins with or without fumonisin B1. No major histological lesions were observed in tissues from control poults or poults fed the diet containing fumonisin alone. Lesions associated with aflatoxins were only observed in the liver and occasionally in the kidney of poults fed the diets containing aflatoxins with or without fumonisin B1. The primary hepatic change was bile duct hyperplasia with some hepatocellular degeneration and necrosis and megalocytosis. Occasional necrotic and degenerating tubular epithelial cells were observed in the kidneys. The increased toxicity in poults fed the combination diet for most variables can best be described as additive, although some variables showed less than additive toxicity.

Kubena *et al.* (1995b) fed diets containing 300 mg fumonisin B1 (FB1)/kg of feed and 5 mg T-2 toxin/kg of feed singly or in combination to female turkey poults (Nicholas Large White) from day of hatch to 21 d of age. When compared with controls, 21-d body weight gains were reduced 21% by FB1, 26% by T-2, and 47% by the combination. the efficiency of feed utilization was adversely affected by FB1 and the combination of FB1 and T-2. Relative weights (grams/100 g BW) of the liver and gizzard were increased in poults fed the FB1 and the combination diets; whereas, the relative weight of the pancreas was increased in all treated groups. All poults were scored for oral lesions using a scale of 1 to 4 (1 = no visible lesions, 4 = severe lesions). Oral lesions were present in all poults fed the T-2 diet (average score of 3.29) or the combination diet (average score of 3.54). Serum concentration of cholesterol was decreased and lactate dehydrogenase activity was increased in poults fed the FB1 and combination diets. The activity of aspartate aminotransferase and the values for red blood cells, hemoglobin, and hematocrit were increased only in poults fed the combination diet. Inorganic phosphorus concentration was decreased only in poults fed the combination diet. The increased toxicity in poults fed the combination diet for most variables can best be described as additive, although some variables not altered by FB1 or T-2 singly were significantly affected by the combination, indicating that the combination may pose a potentially greater problem to the turkey industry than either of the mycotoxins individually.

Qureshi *et al.* (1995) fed White Leghorn Cornell K-strain chicks (3 replicates of 16 per pen) at Day 7 a feed amended with Fusarium proliferatum culture material containing fumonisin B1, fumonisin B2, and moniliformin at 61, 10.5, and 42.7 ppm, respectively. Observed effects on performance of treated birds included reduced feed conversion at 2 wk, and reduced body weight of males and females up to 6 wk (P < or

= .05). Splenic, thymic, and liver weights, normalized for body weight, were reduced (P < or = .05) with no change in bursa of Fabricius. No significant changes were observed histologically in the spleen, bursa, kidney, heart, liver, cecal tonsils, colon, or tibia. Significant suppression in total Ig and IgG levels occurred. Macrophages from treated chicks exhibited a 34% reduction in phagocytic activity. Natural killer cell activity was not affected. These findings, which showed that Fusarium toxins alter performance and immune end points in chickens, imply that chickens exposed to mycotoxins may be more susceptible to infectious diseases.

Vudathala *et al.* (1995) studied the pharmacokinetics of FB1 in laying hens following oral and intravenous administration of 14C-labelled FB1. After iv dosing (2.0 mg = 23.68 kBq/kg bw) plasma radioactivity underwent a very rapid bi-exponential decline (t1/2 alpha = 2.5 +/- 0.3 min; t1/2 beta = 48.8 +/- 11.2 min) with negligible levels measured after 4-6 hr. Mean value for the apparent volume of distribution at steady state (Vdss) was 18.27 ml/kg, apparent volume of central compartment (Vd beta) was 82.20 ml/kg and plasma clearance was 1.18 ml/min/kg. At 24 hr post-dosing only trace residues were present in liver, kidney, and cecum. When dosed by the oral route (2.0 mg = 47.36 kBq/kg bw), systemic absorption of fumonisin appeared to be poor (F = 0.71 +/- 0.5%) with peak plasma concentrations of only 40-145 dpm/ml (equivalent to 28-103 ng FB1 and/or metabolites per ml) between 1.5 and 2.5 hr. At 24 hr post-dosing only trace amounts were present in crop, liver, kidney, small intestine, and cecum. In both orally and iv dosed birds almost all (97.7 +/- 3.73%) of the radioactivity was recovered in excreta by the end of the 24 hr experiment period and no residues were found in eggs laid during the 24 hr post-dosing period.

Wu *et al.* (1995) compared two water-soluble **Fusarium** metabolites, fumonisin B1 (FB1) and moniliformin (MN) for their cytotoxicity in a variety of chicken primary cell cultures. Cardiac and skeletal myocytes and hepatocytes derived from embryos, and splenocytes, macrophages, and chondrocytes derived from 3- to 4-week old chickens were cultured in media containing either FB1 or MN (0 to 1 mM) for 48 hr. The colorimetric tetrazolium cleavage assay was then used for measuring cell survival. FB1 was not toxic to macrophages, hepatocytes, cardiac and skeletal myocytes but toxic to splenocytes and chondrocytes. MN was not toxic to chondrocytes and macrophages, but toxic to splenocytes, cardiac and skeletal myocytes. Median effective concentration (EC50) of MN in skeletal myocytes was 42 mu M (fiducial limits: 33 to 50 mu M) and in cardiac myocytes was 95 mu M (fiducial limits: 84 to 122 mu M). Estimated EC50 of FB1 in chondrocytes and splenocytes and EC50 of MN in splenocytes were all greater than 200 mu M.

Bermudez *et al.* (1996) fed fourteen 1-day-old male turkeys balanced rations containing 0 and 75 mg fumonisin B1 (FB1)/kg for 18 weeks. Inclusion of FB1 in the ration caused decreased body weight gain on weeks 4, 10, and 12 during the trial. Turkeys fed 75 mg FB1/kg had significantly heavier livers after treatment for 18 weeks. Chronic FB1 exposure resulted in an increased total white blood cell count, absolute heterophil count, absolute lymphocyte count, and heterophil-to-lymphocyte ratio. No mortality was noted in turkeys in either treatment group. Turkeys are relatively resistant to chronic FB1 exposure.

Ledoux *et al.* (1996) studied the effects of feeding Fusarium moniliforme culture material, containing known concentrations of fumonisin B1 (FB1), in turkey poults.

Day-old poults were allotted randomly to dietary treatments containing 0, 0.41, 0.82, 1.23, 1.64. 2.87, 4.10, 5.33, 6.56, and 7.79% fumonisin culture material (FCM). These levels of FCM supplied 0, 25, 50, 75, 100, 175, 250, 325, 400, and 475 mg FB1/kg of feed. Each dietary treatment was fed to six pen replicates of six poults each for 21 d. Poults fed FCM that supplied 325 to 475 mg FB1/kg diet had lower ($P < 0.05$) feed intakes and BW gains. Increased ($P < 0.05$) liver and pancreas weights were observed in poults fed FCM that supplied > or = to 175 mg FB1/kg. Poults fed FCM that supplied 400 and 475 mg FB1/kg diet had increased ($P < 0.05$) red blood cell counts and increased ($P < 0.05$) serum concentrations of gamma glutamyl transferase and aspartate aminotransferase. Compared with controls, poults fed FCM that supplied 25, and 75 to 475 mg FB1/kg had increased ($P < 0.05$) liver sphinganine:sphingosine ratios. Hepatocellular hyperplasia was mild at 75 and 100 mg FB1/kg diet, moderate to severe at 250 mg/kg FB1, and severe at 325 to 475 mg FB1/kg. Multifocal to generalized loss of cross striations and thinning of cardiomyocytes was observed in poults fed FCM that supplied 475 mg FB1/kg diet. Results indicated that diets containing < or = to 1.23% FCM that supplied > or = to 75 mg FB1 /kg are toxic to young turkeys.

Leslie *et al.* (1996) tested Fusarium strains for the ability to produce fumonisins B1, B2, and B3 and moniliformin and for toxicity to 1-day-old ducklings. Most of the members of the A mating population (19 of 20 strains) produced more than 60 micrograms of total fumonisins per g, whereas only 3 of 20 members of the F mating population produced more than trace levels of these toxins and none produced more than 40 micrograms of total fumonisins per g. In addition, only 3 of 20 members of the A mating population produced more than 1 microgram of moniliformin per g (and none produced more than 175 micrograms/g), while all 20 strains of the F mating population produced more than 85 micrograms of this toxin per g and 1 strain produced 10,345 micrograms/g. The duckling toxicity profiles of the strains of the two mating populations were similar, however, and the level of either toxin by itself was not strongly correlated with duckling toxicity. On the basis of our data we think that it is likely that the members of both of these mating populations produce additional toxins that have yet to be chemically identified. These toxins may act singly or synergistically with other compounds to induce the observed duckling toxicity.

Bermudez *et al.* (1997) fed turkeys a control ration, or rations containing 200 mg FB1/kg, 100 mg M/kg, or a combination of both 200 mg FB1/kg and 100 mg M/kg feed from 1 to 21 days of age. These rations contained 0, 3.8, 1.0, and 4.8% culture material, respectively. In comparison to controls, turkeys fed FB1 had increased relative liver weights. Both aspartate aminotransferase and lactate dehydrogenase were increased in poults fed FB1. Turkeys fed M had decreased feed intake and body weight gains and increased relative heart weights in comparison to controls. Poults fed FB1 had moderate diffuse hepatocellular hyperplasia and poults fed moniliformin had a loss of cardiomyocyte cross striations. Turkeys fed the ration containing both M and FB1 had all the above changes; however, no additive or synergistic effects were evident for any single parameter measured. No treatment-related morbidity or mortality was observed in the study.

Espada *et al.* (1997) evaluated the effects of fumonisin B1 (FB1) intoxication in chickens in three experiments. Two-day-old broiler chicks were fed a diet containing 10 mg pure FB1/kg feed for 6 days; some chicks were necropsied at this time, and

others were allowed to recover for 5 wk before necropsy. In two other experiments, 2-day-old chicks were fed a broiler starter ration prepared with Fusarium moniliforme culture material containing FB1; one group received 30 mg/kg for 2 wk, and another received 300 mg FB1/kg for 8 days. Compared with controls, intoxicated chicks exhibited decreased prothrombin time, increased plasma fibrinogen (not included for the group receiving 30 mg/kg of culture material), and increased antithrombin III activity. Simultaneously decreased serum albumin concentration and increased serum globulins could be observed in groups intoxicated with F. moniliforme culture material containing FB1. The group allowed to recover for 5 wk did not exhibit modifications in hemostasis or serum proteins compared with controls. The results indicate that low doses of pure FB1 (10 mg/kg) and FB1 from F. moniliforme culture material (30 mg/kg) may alter hemostasis and serum proteins in young chicks.

Kubena *et al.* (1997) evaluated the individual and combined effects of feeding diets containing 300 mg fumonisin B1 (FB1), and 5 mg T-2 toxin (T-2)/kg of diet, or 15 mg/kg deoxynivalenol (DON, vomitoxin) from naturally contaminated wheat in two studies in male broiler chicks from day of hatch to 19 or 21 d of age in Experiments 1 and 2, respectively. When compared with controls, body weight gains were reduced 18 to 20% by FB1, 18% by T-2, 2% by DON, 32% by the FB1 and T-2 combination, and 19% by the FB1 and DON combination. The efficiency of feed utilization was adversely affected by FB1 with or without T-2 or DON. Mortality ranged from none for the controls to 15% for the FB1 and T-2 combination. Relative weights of the liver and kidney were significantly increased by FB1 with or without T-2 or DON. Serum concentrations of cholesterol were increased in chicks fed FB1 with or without T-2 or DON. Activities of aspartate aminotransferase, lactate dehydrogenase, and gamma glutamyltransferase were increased in chicks fed FB1 at 300 mg/kg alone and in combination with T-2 or DON, indicating possible tissue damage and leakage of the enzymes into the blood. Results indicate additive toxicity when chicks were fed diets containing 300 mg FB1 and 5 mg T-2/kg of diet and less than additive toxicity when chicks were fed 300 mg FB1 and 15 mg DON/kg of diet. Of importance to the poultry industry is the fact that toxic synergy was not observed for either of these toxin combinations and the likelihood of encountering FB1 at this concentration in finished feed is small. However, under field conditions with additional stress factors, the toxicity of these mycotoxins could be altered to adversely affect the health and performance of poultry.

Vesonder and Wu (1998) fermented 5 isolates of Fusarium moniliforme and two isolates Fusarium proliferatum of the Section Liseola on rice for 21 d at 25 C. Each Fusarium-fermented rice, when dried and mixed into a poultry diet (10% by weight), caused a varied degree of acute mortality in baby Pekin ducklings. The acute (death in less than 48 h) mortality correlated significantly only to the amount of moniliformin in fermented rice, thus in the diet, but not to the amount of fumonisin B1 in fermented rice. This correlation of moniliformin concentration and noncorrelation of fumonisin B1 concentrations to acute toxicity were confirmed by duckling assay using diets containing these purified mycotoxins.

Buim *et al.* (1999) used monoclonal anti-fumonisin B1 antibody (anti-FB1) and avidin-biotin-peroxidase system for the detection and distribution of fumonisins (FBs) in liver and kidneys of broiler chicks. One hundred and fifty micrograms of FB1 or culture extract of Fusarium moniliforme str. 113F containing 150 microg of FB1 and 4 microg of FB2 were administered into the vitelline sac of 1-day old, specific pathogen-free chicks. The animals were killed 24 h after injection, and renal and

hepatic tissues submitted for immunohistochemical analysis. FBs were detected in the epithelial cells of convoluted distal and proximal tubules of the kidneys, as well as in the cytoplasm of hepatocytes. This novel immunohistochemical method developed is expected to be an efficient way for monitoring the target of the FB toxins in tissues.

Kubena *et al.* (1999) fed, beginning at 24 wk of age, control diets or diets containing 50 or 100 mg/kg moniliformin (M), 100 or 200 mg/kg fumonisin B1 (FB1), or a combination of 50 mg M and 100 mg FB1/kg of diet to White Leghorn laying hens for 420 d. The hens were then fed the control diet for an additional 60 d. At the beginning of the experiment, each treatment consisted of four replicates of six hens. Egg production was reduced by approximately 50% by the end of the second 28-d laying period and remained at approximately this level for the 420 d in only the hens fed the diet containing 100 mg M/kg feed. Production returned to control levels or above within 60 d after hens were fed the control diet. Egg weights were reduced by the 100-mg M diet during the first three 28-d laying periods before returning to weights comparable with controls. The hens in this group also had significantly lower body weights than the other treatments. Mortality was minimal except in hens fed the 100 mg M/kg diet and the 100 mg FB1/kg diet, on which approximately 20% of the hens died. The hens were artificially inseminated with semen from males fed control diets, and fertility was not affected by the dietary treatments. Importantly, toxic synergy between M and FB1 was not observed for any of the parameters measured. Results indicate that laying hens may be able to tolerate relatively high concentrations of M and FB1 for long periods of time without adversely affecting health and performance. Interestingly, hens fed the 100-mg M/kg diet were able to recover when returned to control diets. The likelihood of encountering M or FB1 at these concentrations in finished feed is small.

Li et *al.* (1999) conducted three experiments to evaluate immune responses in chicks fed fumonisin B1 (FB1). Day-old male chicks were randomly allotted to dietary treatments: 0, 50, 100, or 200 mg FB1/kg diet. In Experiment 1, chicks were fed diets for 3 wk and were injected intravenously with 4.6 × 106 Escherichia coli on Day 21. Blood samples were collected at 60, 120, and 180 min post injection, and liver, spleen, and lung were collected after 180 min. Chicks fed 200 mg FB1/kg diet had significantly higher numbers of bacterial colonies in blood, spleen, and liver ($P < 0.05$) than control chicks. In Experiment 2, chicks were placed on the diets for 4 wk and were injected with 0.5 mL inactivated Newcastle Disease virus vaccine on Weeks 2 and 3 of the experiment, and primary and secondary antibody titers were measured 7 d after each injection. The secondary antibody response in chicks fed 200 mg FB1/kg diet was significantly lower ($P < 0.05$) than that of control chicks. In Experiment 3, lymphocyte proliferation in chicks exposed to FB1 in vivo or in vitro was determined. Results of the in vivo study showed that cell proliferation in response to mitogens was lower ($P < 0.05$) in chicks fed 200 mg FB1/kg diet than in control chicks. For the in vitro study, cell proliferation was lower ($P < 0.05$) when cells were exposed to ≥ 2.5 µg FB1/ml. Data of the current study suggested that FB1 is immunosuppressive in chicks when present in the ration at 200 mg FB1/kg diet.

Li et *al.* (2000) evaluated effects of feeding diets containing fumonisin B1 (FB1) and moniliformin (M), singly or in combination, on performance and immune response in poults. Day-old poults were randomly assigned to one of four dietary treatments with four replicates of four poults each. Dietary treatments were 1) control; 2) 200 mg FB1, 0 mg M/kg diet; 3) 0 mg FB1, 100 mg M/kg diet; and 4) 200 mg FB1, 100 mg M/kg diet. In Experiment 1, poults were injected with 0.25 mL Newcastle disease

virus (NDV) vaccine on Weeks 2 and 3 of the experiment, and anti-NDV antibody titers were measured 7 d after each injection. Compared with controls, poults fed FB1 had significantly lower ($P < 0.05$) secondary antibody response. Poults fed M and the combination of FB1 and M had significantly lower ($P < 0.05$) primary and secondary antibody response. Lower relative thymus weights were observed in poults fed diets containing FB1 or M. De-creased relative bursa and spleen weights were observed in poults fed M. In Experiment 2, poults were placed on dietary treatments for 3 wk. On Day 21, 2 × 106 peripheral lymphocytes were incubated with mitogens. Poults fed diets containing FB1 had a significantly lower ($P < 0.05$) proliferative response to mitogens in comparison to controls. In Experiment 3, poults were placed on the diets for 3 wk and were injected with 4.4 × 107 E. coli/kg body weight on Day 21. Significantly higher ($P < 0.05$) numbers of E. coli colonies were observed in the blood and tissue homogenates of poults fed M. In all three experiments, feed intake and body weight gains were significantly lower ($P < 0.05$) in turkeys fed diets containing M. Data from the present study suggest that FB1 and M are immunosuppressive in poults and that M not only suppresses immune response but also performance. However, neither synergistic nor additive effects between FB1 and M were observed for any of the parameters measured.

Magnoli *et al.* (1999) investigated Fusarium species and fumonisin production by toxigenic strains. During 1996-1998, 158 samples of poultry feeds were collected from a factory located in the department of Rio Cuarto Córdoba province, Argentina. The most common species of Fusarium were F. moniliforme (60.7%) and F. nygamai (35.4%) followed by F. semitectum, F. subglutinans, F. proliferatum, F. dlamini, F. solani, F. oxysporum and F. napiforme. Fungal counts ranged from 1 x 10(3) to 8 x 10(5) CFU/g with mean values from 1.5 x 10(3) to 2.3 x 10(5) CFU/g. The highest counts were for F. dlamini, F. subglutinans, F. moniliforme and F. nygamai. Strains of F. moniliforme, F. nygamai, and E. proliferatum were screened for their potential to produce fumonisin B1 (FB1), fumonisin B2 (FB2) and fumonisin B3 (FB3) in corn grain. The samples were analysed using a modified high performance liquid chromatography method. The strains assayed, 43 strains, produced three fumonisins. There was a high degree of variability in the quantities of FB1, FB2, and FB3 produced. The toxin produced in highest levels by the majority of the strains was FB1. The range of concentration varied from 5.4 to 3,991, 1.01 to 189 and 0.4 to 765 ppm per gram of corn for FB1, FB2 and FB3 respectively. The toxigenic pattern of strains was normal, although two strains of F. moniliforme produced exceptionally high concentrations of FB3 and minor concentrations of FB2 and FB1. This is the first report from Argentina on Fusarium species in poultry feeds and fumonisin production by these strains.

Henry *et al.* (2000) conducted an investigation of the toxicity of fumonisin B1 (FB1), a toxic metabolite of Fusarium moniliforme, in broiler chicks. Purified FB1 (98.1% pure) was incorporated into the diets of broiler chicks at 0, 20, 40, and 80 mg/kg, and fed to chicks from 0 to 21 d of age. Dietary FB1, at concentrations of 80 mg/kg or less, did not adversely affect body weight, feed efficiency, or water consumption of broiler chicks. The relative weights of the liver, spleen, kidney, proventriculus, and bursa of Fabricius were also unaffected ($P < 0.05$) by any dietary concentration of FB1 compared with the control (0 mg/kg) group. Total liver lipids of chicks fed 40 or 80 mg FB1/kg were significantly lower than those of the chicks fed either 0 or 20 mg FB1/kg of feed. Liver sphinganine concentration and the sphinganine sphingosine

ratio were increased significantly in all treated groups. Chicks fed dietary FB1 at 80 mg/kg had significantly higher serum glutamate oxaloacetate aminotransaminase: aspartate aminotransferase ratios and levels of free sphinganine in the serum. The results of this investigation agree with the results previously described, in which FB1 was supplied to diets from the use of F. moniliforme-contaminated grain; therefore, the use of such material as the source of the mycotoxin in animal feeding studies is appropriate.

Bailly *et al*. (2001) investigated Fusarium moniliforme culture material toxicity containing fumonisin B1 (FB1) into four groups of five growing ducks, each receiving 0,5,15 or 45 mg/kg FB1 by daily oral administration over 12 days. Treatments did not lead to lethality, but the average body weight gain was slightly retarded in treated versus control animals, without apparent dose relation. A dose-dependent increase of the liver weight with a disorganization of the span and implementation of a microglandular structure in both periportal and centrolobular areas was obtained. In the plasma, together protein, cholesterol, alanine aminotransferase, lactate dehydrogenase, gammaglutamyl transferase and sphinganine to sphingosine ratio (SA/SO) were increased. No sign of apoptosis was present neither in the liver nor in peripheral blood lymphocytes and only moderate oxidative damages were obtained. These results are of interest, because although FB1 increases SA/SO and is hepatotoxic in all investigated species, liver hyperplasia with increased liver weight were obtained in ducks, whereas decreased liver weight and apoptosis are observed in rats. Finally, although ducks appeared resistant to FB1 toxicity in terms of mortality, liver alterations were obtained with only 5 mg/kg per day of FB1 for 12 days. Considering the fact that high levels of FB1 may occur in corn (100-300 mg/kg), liver pathology could have an impact in farming conditions.

Henry and Wyatt (2001) evaluated the toxicity of purified FB1, FB2, and FB3, individually and in combination (3:1:1 ratio) with regard to their embryo toxicity by injection of the toxins into the air cell of chicken eggs at 72 h of incubation. Under these conditions, FB1 at doses of 0, 2, 4, 8, 16, 32, and 64 microg per egg resulted in embryonic mortality of 5, 12.5, 17.5, 20.0, 52.5, 77.5, and 100%, respectively. The 50% lethal dose for FB1, when injected into the air cell of embryonating chicken eggs, was determined to be 18.73 microg per egg. A comparison of the toxicity of FB1, FB2, and FB3, individually and in combination (3:1:1 ratio), at doses of 16 microg of total fumonisin per egg, indicated that the toxicity of the fumonisins differed, FB1 being the most toxic. Microscopic examination of chicken embryos exposed to fumonisin did not reveal any gross developmental abnormalities; however, severe hemorrhages of the head, neck, and thoracic area of the dead embryos were evident.

Broomhead *et al.* (2002) conducted floor pen studies with 270 broiler chicks and 144 turkey poults, all 1 wk old, to evaluate the chronic effects of fumonisin B1 (FB1). A completely randomized design was used in both studies with six pen replicates of 15 chicks or eight pen replicates of six poults assigned to each of three dietary treatments from Weeks 1 to 7 (broilers) or to Week 14 (turkeys). Fusarium moniliforme (M-1325) culture material was added to a typical corn-soybean basal diet to supply 0, 25, or 50 mg FB1/kg diet. Feed intake, body weight gain, and feed conversion of chicks were not affected ($P > 0.05$) by FB1. Turkeys fed 50 mg FB1/kg had significantly ($P < 0.05$) lower feed intake than the controls. Compared with controls, chicks and turkeys fed FB1 diets had significantly higher liver sphinganine to sphingosine ratios ($P < 0.05$). Relative organ weights of chicks were not affected ($P > 0.05$) by FB1,

other than those chicks fed 25 mg FB1/kg, which had lower ($P < 0.05$) relative proventriculus weights than the chicks fed 0 or 50 mg FB1/kg. Broilers fed 50 mg FB1/kg had decreased serum calcium and increased serum chloride when compared to broilers fed 0 or 25 mg FB1/kg. Hematology was not affected ($P > 0.05$) by dietary FB1. No lesions were present in any organ examined microscopically. Results indicate that 50 mg FB1/kg diet is detrimental to turkeys but is not toxic to broilers fed to market age.

Dombrink-Kurtzman (2003) exposed turkey peripheral blood lymphocytes in vitro for 72 hours to fumonisin B1 (FB1), fumonisin B2 (FB2), hydrolyzed fumonisin B1 (HFB1), moniliformin and tricarballylic acid (TCA) (0.01-25 microg/ml). A decrease in cell proliferation, as determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] bioassay, occurred in the order: FB2 > FB1 > HFB1, with IC50 = 0.6 microM, 1 microM and 10 microM, respectively. Internucleosomal DNA fragmentation and morphological features characteristic of apoptosis were observed following exposure to fumonisin B1 and beauvericin; cytoplasmic condensation and membrane blebbing were seen by light microscopy. Tricarballylic acid and moniliformin did not interfere with cell proliferation. Results suggested that fumonisin B1 and beauvericin may affect immune functions by suppressing proliferation and inducing apoptosis of lymphocytes.

Bouhet *et al*. (2004) performed a study to investigate the effects of FB1 on IPEC-1, a porcine intestinal epithelial cell line. They first verified that low concentrations of FB1 did not exert any cytotoxic effect on IPEC-1. Indeed, significant LDH release was only observed for FB1 concentrations greater than 50 and 700 microM on proliferating and non-proliferating cells, respectively. They then demonstrated that FB1 inhibits proliferation of IPEC-1. Fluorescence-activated cell sorting (FACS) analysis of the cell cycle indicated that FB1 blocks the proliferation of intestinal cells in the G0/G1 phase. Similar results were obtained with LLC-PK1, a renal porcine epithelial cell line, which is considered to be a good model for studying FB1 in vitro effects. They have also assessed the effects of FB1 on the integrity of the barrier formed by the intestinal epithelium. They demonstrated that FB1 decreased the transepithelial electrical resistance (TEER) of IPEC-1 in a time- and dose-dependent manner. This effect was only noticed after a long exposure (8-12 days of treatment). FB1 induced the TEER decrease independently of the cell differentiation stage, and this effect was partially reversible. Taken together, the data indicated that FB1 alters the proliferation and the barrier function of intestinal cells.

Ogido *et al.* (2004) studied the effect of prolonged administration of fumonisin B1 and aflatoxin B1 in laying Japanese quails. In this study, 288 8-wk-old Japanese quail were randomly distributed into 6 experimental groups (48 birds per group) and fed the following diets for 140 d: 1) 0 (control); 2) 10 mg of fumonisin B1 (FB1); 3) 50 µg of aflatoxin B1 (AFB1); 4) 50 µg of AFB1 + 10 mg of FB1; 5) 200 µg of AFB1; and 6) 200 µg of AFB1 + 10 mg of FB1/kg of feed. Each treatment consisted of 4 replicates of 12 quail. Egg production and individual egg weight were checked daily. Feed intake and feed conversion were determined weekly. Results showed that by the end of the fifth cycle, average egg weight was lower ($P < 0.05$) in groups fed 10 mg of FB1/kg, 50 µg of AFB1/kg, 200 µg of AFB1/kg, and 10 mg of FB1 + 50 µg of AFB1/kg of feed. Egg production decreased ($P < 0.05$) in birds fed 10 mg of FB1/kg by the third, fourth, and fifth cycles. Feed intake was lower ($P < 0.05$) in birds fed 10 mg of FB1/kg by the fourth and fifth cycles, and in birds fed 50 and 200 µg of

AFB1/kg in the fifth cycle. Birds fed 10 mg of FB1 + 50 µg of AFB1/kg consumed less feed ($P < 0.05$) in the first, second, and fifth cycles. Results indicated that prolonged administration of FB1 and AFB1, singly or in combination at the levels evaluated, may cause economic losses to quail egg producers

Tardieu *et al.* (2004) investigated the toxicity of maize containing known doses of fumonisin B1 (FB1) in mallard ducks during force-feeding. Seventy-five ducks at 12 wk of age were randomly divided into 3 groups of 25, and received control maize, naturally contaminated maize containing 20 mg/kg of FB1, or a mixture of control and contaminated maize (50/50, vol/vol). Force-feeding was performed during 12 d that correspond to a final average feed intake of approximately 10 kg of maize per duck. At the end of the study, 8% mortality was observed in ducks fed 20 mg of FB1/kg of feed, whereas no mortality occurred in the other groups. Liver weight, and plasma concentrations of protein, cholesterol, alanine aminotransferase (ALAT), and lactate dehydrogenase (LDH) were increased by force-feeding, whereas feed conversion ratio appeared decreased by the toxin. Microscopic examination of the liver showed that steatosis was mostly macrovacuolar in control ducks, whereas it was microvacuolar in ducks fed 20 mg of FB1/kg of feed. Free sphingolipid concentrations were measured in liver and plasma. Sphinganine (Sa) and sphinganine to sphingosine (Sa/So) ratio were increased in all treatment groups. These parameters were not affected by force-feeding and all individual values obtained in the treated ducks were higher than those obtained in control ducks. Our results suggest that free Sa level and Sa/So ratio can be used to reveal exposure of ducks to FB1 at doses of 10 mg/kg or greater in feed.

Bouhet *et al*. (2005) performed a study to investigate the effects of FB1 on IPEC-1, a porcine intestinal epithelial cell line. They first verified that low concentrations of FB1 did not exert any cytotoxic effect on IPEC-1. Indeed, significant LDH release was only observed for FB1 concentrations greater than 50 and 700 microM on proliferating and nonproliferating cells, respectively. They then demonstrated that FB1 inhibits proliferation of IPEC-1. Fluorescence-activated cell sorting (FACS) analysis of the cell cycle indicated that FB1 blocks the proliferation of intestinal cells in the G0/G1 phase. Similar results were obtained with LLC-PK1, a renal porcine epithelial cell line, which is considered to be a good model for studying FB1 in vitro effects. They have also assessed the effects of FB1 on the integrity of the barrier formed by the intestinal epithelium. They demonstrated that FB1 decreased the transepithelial electrical resistance (TEER) of IPEC-1 in a time- and dose-dependent manner. Thiseffect was only noticed after a long exposure (8-12 days of treatment). FB1 induced the TEER decrease independently of the cell differentiation stage, and this effect was partially reversible. Taken together, our data indicate that FB1 alters the proliferation and the barrier function of intestinal cells. These results may have implications for humans and animals consuming FB1-contaminated food or feed.

Del Bianchi *et al.* (2005) evaluated the effects of prolonged oral administration of aflatoxin B1 (AFB1) and fumonisin B1 (FB1) mycotoxins in broiler chickens from 21 to 42 d of age. A total of 192 birds were housed in experimental batteries and assigned to 32 cages, 6 birds per cage. The following treatments were applied: 1) 0 mycotoxins (control), 2) 10 mg of FB1, 3) 50 microg of AFB1, 4) 50 microg of AFB1 + 10 mg of FB1, 5) 350 microg of AFB1, 6) 350 microg of AFB1 + 10 mg of FB1, 7) 2,450 microg of AFB1, 8) 2,450 microg of AFB1 + 10 mg of FB1/kg of feed. Each treatment consisted of 4 replicates of 6 birds each. At the end of the trial, blood

samples from 12 birds per treatment were collected, and the birds were necropsied. Compared with controls, the percentage of heterophils was lower (P < 0.05) in birds from groups receiving 50 microg of AFB1/kg + 10 mg of FB1/ kg and 2450 microg of AFB1/kg alone or in combination with FB1. A higher percentage of lymphocytes (P < 0.05) was observed in birds fed 50 microg of AFB1/kg + 10 mg of FB1/ kg, 350 microg of AFB1/kg, and 2,450 microg of AFB1/kg. A decrease in plasma albumin was observed only in birds fed 2,450 microg of AFB1/kg + 10 mg of FB1/kg. The liver of AFB1-treated birds had focal areas of necrosis and inflammatory infiltrates. In birds fed rations containing only 10 mg of FB1/kg, bile duct hyperplasia with fibrosis and a mononuclear infiltrate accompanied by trabecular derangement were observed. In contrast, in treatments in which FB1 was administered in combination, hepatic vacuolar degeneration was observed, and renal tissue presented corpuscles with increased cellular agglomeration, characterizing glomerulonephritis, and a clearly visible tubular epithelium with areas of degeneration and necrosis. The FB1 residues were detected in liver and in excreta of all FB1-treated groups, at levels that ranged from 0.013 to 0.051 mg/kg and from 1.19 to 2.79 mg/kg, respectively. Results indicated that FB1 and AFB1, singly or in combination at the levels evaluated, do not change markedly the hematological and serological parameters of broiler chickens, but may cause relevant lesions in liver and in kidneys.

Deshmukh *et al.* (2005a) divided three hundred day-old Japanese quail (Coturnix coturnix japonica) into two groups with 150 quail in each group. One group was maintained on quail mash alone, while Fusarium moniliforme culture material was added to quail mash in the second group from day 5 of age and was supplied at a rate of 150 ppm fumonisin B1 (FB1)/kg mash. At day 21, each group was further subdivided into two groups, yielding four groups with 75 birds apiece, which served as the control (group CX), the *Salmonella* Gallinarum alone group (group CS), the FB1 alone group (group FX), and the group fed FB1 and infected with *Salmonella Gallinarum* (group FS). An oral challenge with *Salmonella* Gallinarum organisms (2 x 10(4) colony-forming units/ml) was given to groups CS and FS at 21 days of age. Three quail each were necropsied on day 21 (0 day interval) from groups CX and FX only. At subsequent intervals (i.e., 1, 2, 3, 5, 7, 10, 14, and 21 days post infection [DPI]), three quail were euthanatized from all four groups (CX, CS, FX, and FS). The gross and microscopic lesions were recorded in both mortality and euthanatized birds at the above intervals. The ultrastructural studies were done at 5 DPI. Mild to moderate hepatomegaly and pale discoloration of liver were observed in group FX, while congestion, hemorrhages, necrosis, and mild to severe hepatomegaly were the predominant gross lesions in both infected groups (CS and FS). The gross lesions in quail inoculated with *Salmonella* Gallinarum alone (group CS) generally developed slowly, appeared more widely scattered, and involved comparatively less surface area in contrast to the rapidly progressive and frequently confluent lesions in the combination group (FS), especially in the first 5 days of infection. Mild to marked hepatocellular swelling, multifocal hepatic necrosis, and hepatocellular and bile duct hyperplasia were the characteristic microscopic changes in the FX group. Microscopic lesions in quail of group CS comprised congestion, vacuolar changes, and focal necrosis in early stages, followed by granulomatous lesions at later intervals. Similar but more severe lesions were observed in the combination group (FS). Based on transmission electron microscopy, the maximum effect of FB1 toxicity was observed on mitochondria and endoplasmic reticulum. In general, the mitochondriae showed diverse form and structure, some of which appeared to lose their intact outer

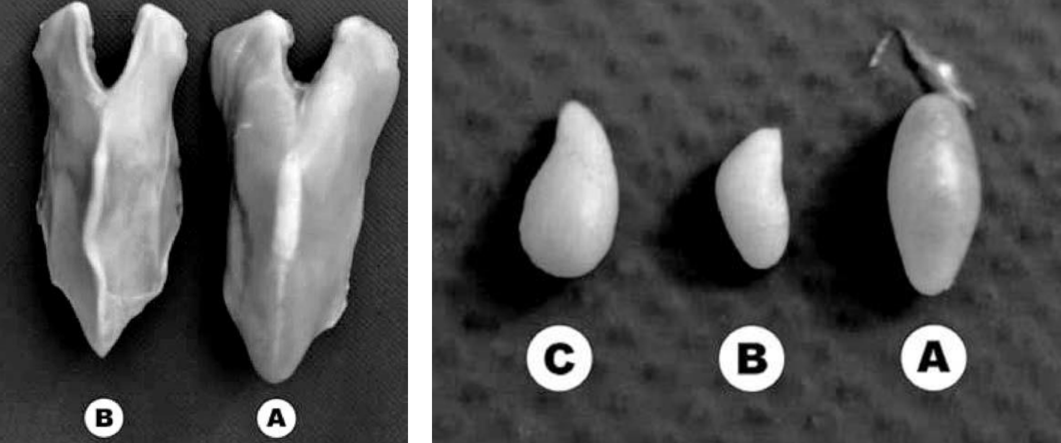
membrane, and the mitochondrial cristae were disoriented. The deformity in the cisternae structure of rough endoplasmic reticulum, with their rearrangement into round or tubular forms either bearing granular surface or leading to accumulation of smooth endoplasmic reticulum, was evident only in groups FX and FS. We conclude that the continuous presence of fumonisins in the diets of young quail might increase their susceptibility to or the severity of *Salmonella* Gallinarum infection.

Deshmukh *et al.* (2005b) fed Japanese quail (Coturnix coturnix japonica) Fusarium moniliforme culture material (2.5%), 150 mg FB1/kg ration to study the individual and combined effects of fumonisin B1 (FB1) toxicity and Salmonella serotype Gallinarum infection. The birds were subsequently challenged orally with Salmonella Gallinarum organisms (2 x 10(4) colony-forming units) at 21 days of age. The chicks were fed culture material containing FB1 from day 5 till the end of the experiment. After being infected with Salmonella Gallinarum, observations were made 1, 2, 3, 5, 7, 10, 14, and 21 days postinfection. The clinical signs of diarrhea with bloody discharges were more pronounced in the Salmonella-infected birds on the FB1 diet. Mortality caused by Salmonella Gallinarum increased by 12% in the presence of FB1. Mean body weights in both the Salmonella-infected and FB1-fed groups were significantly lower than those of the controls at almost all intervals. Mean values of hemoglobin, packed cell volume, and total erythrocyte count were slightly higher in birds fed FB1 but were lower in the Salmonella Gallinarum groups fed FB1 and plain chick mash. Anemia was evident, between 5 and 10 days postinfection, in quail chicks infected with Salmonella Gallinarum alone. Total leukocyte counts were higher in Salmonella-infected and FB1-fed groups because of an increase in the number of heterophils and lymphocytes. However, the increase in lymphocyte response to infection was lower by 4.27%-30.09% between 3 and 21 days postinfection in the FB1-fed chicks compared with chicks infected with Salmonella Gallinarum. Alanine transaminase and total serum protein were slightly higher in both the infected and FB1-fed groups. This study revealed that the continuous presence of fumonisins in the diets of quail chicks might increase the susceptibility to or the severity of Salmonella Gallinarum infection.

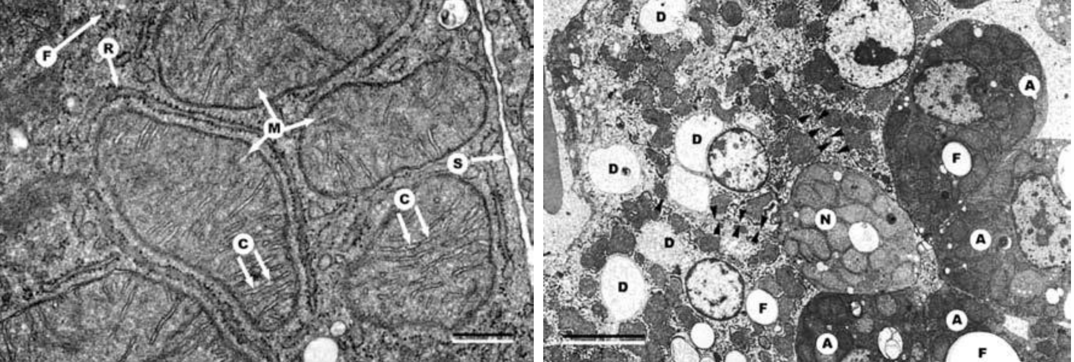
Javed *et al.* (2005) gave feed amended with autoclaved culture material (CM) of Fusarium proliferatum containing fumonisin B1 (FB1) (61-546 ppm), fumonisin B2 (FB2) (14-98 ppm) and moniliformin (66-367 ppm) to 228 male chicks in three separate feeding trials. In a fourth feeding trial, purified FB1 (125 and 274 ppm) and moniliformin (27 and 154 ppm) were given separately and in combination (137 and 77 ppm, respectively). Chicks that died during the trial periods, survivors and controls were subjected to postmortem examination. Specimens (liver, kidney, pancreas, lung, brain, intestine, testis, bursa of Fabricius, heart and skeletal muscle) were examined grossly and preserved for subsequent histopathologic and ultrastructural examination. Prominent gross lesions in affected birds fed diets amended with CM or purified FB1 and moniliformin included ascites, hydropericardium, hepatopathy, nephropathy, cardiomyopathy, pneumonitis, gizzard ulceration, and enlarged bursa of Fabricius filled with caseous material. The various concentrations of FB1 and moniliformin in the amended rations produced well-defined dose-response lesions in all groups in all four trials. Histopathologic changes included hemorrhage, leucocytic infiltration, fatty change or infiltration, individual cell necrosis and fibrosis in liver, kidneys, lungs, heart, intestines, gizzard, bursa of Fabricius and pancreas. Edema and hemorrhage were prominent in brains of treated birds. Ultrastructural changes included cytoplasmic and nuclear enlargement of cells in affected liver, lungs, kidneys, heart

and pancreas. There were thickened membranes of the smooth endoplasmic reticulum, dilation of the rough endoplasmic reticulum with loss of ribosomes and vacuolated or deformed mitochondria.

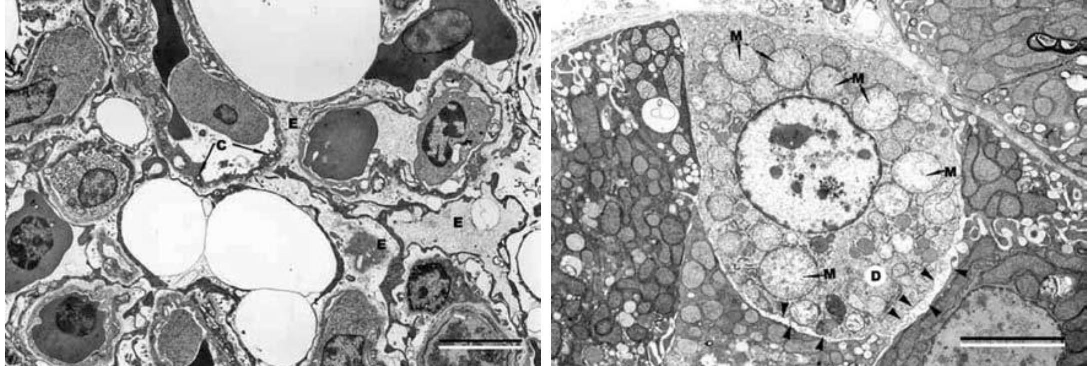
Lung from toxin-fed bird (treatment PF 4 at 54 h) has congestion and edema; note prominent rib impressions ("). Gizzard from toxin-fed bird (treatment T4 at 60 h) **Javed *et al.* (2005)**



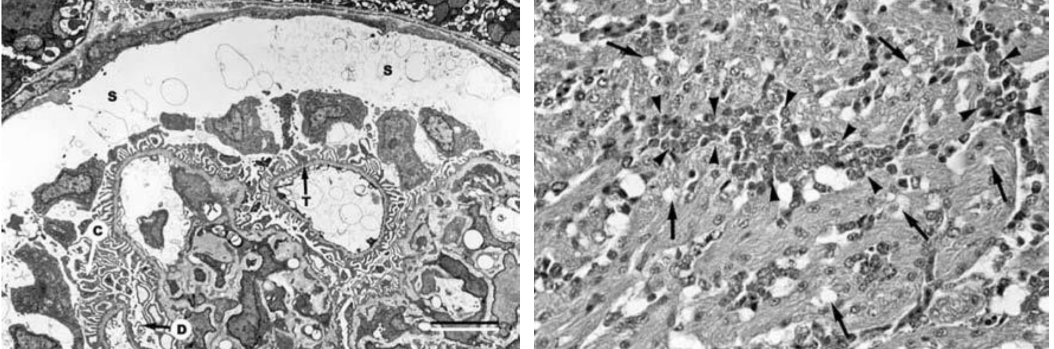
Breast of control bird (A) has straight keel bone and well-developed muscles. Breast of toxin-fed bird (B) (treatment T2 at 60 h) has markedly wavy keel bone and underdeveloped muscles., Normal testis from control bird (A) is compared to small testis (B) and misshapen, pedunculated testis (C) from toxin-fed birds (treatment T3 at 60 h). **Javed *et al.* (2005)**



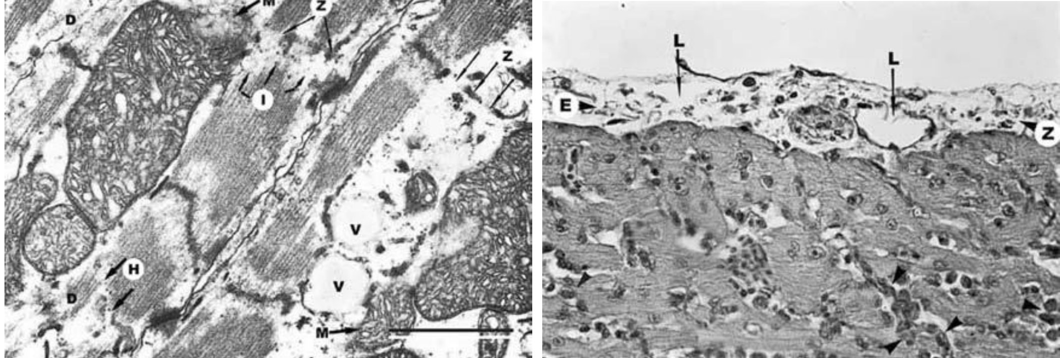
Electron micrograph of hepatocyte from toxin-fed bird (treatment T4 at 77 h) bordered by variably widened intercellular space (S) and containing mitochondria (M) with cristae (C) of slightly variable width, RER (R) with fewer attached ribosomes and increased number of free cytosolic ribosomes (F). Bar = 0.5 lm. **Javed *et al.* (2005)**



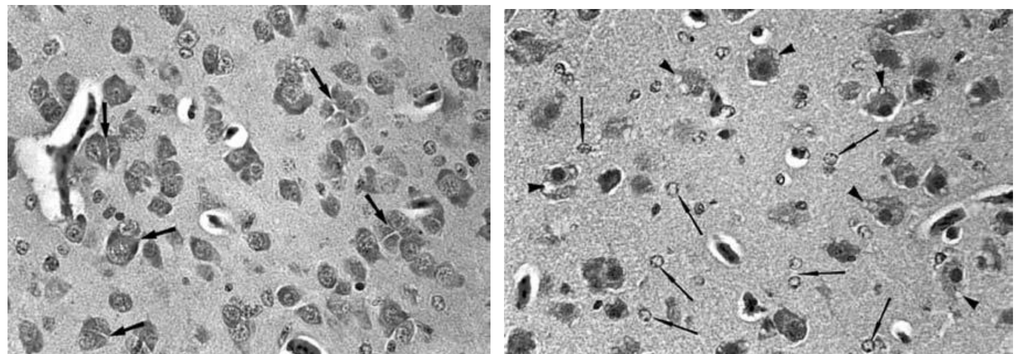
Electron micrograph of lung from toxin-fed bird (treatment T2 at 138 h) showing cytovacuolation of endothelial cells (C) and marked intercellular edema (E). Bar = 5 lm. Electron micrograph of tubular epithelial cells from toxin-fed bird (treatment T2 at 173 h) showing degenerative cell (D) with swollen rounded mitochondria (M), containing electron lucent matrix, fewer cristae, and irregular, widened intercellular spaces (m). Bar = 4 lm. **Javed *et al.* (2005)**



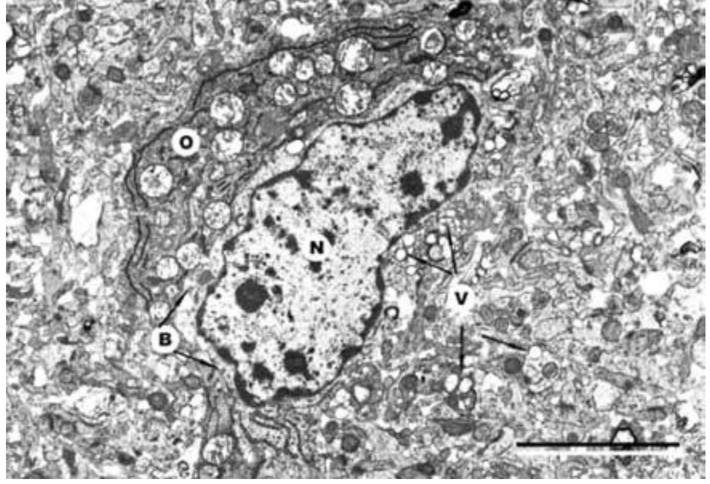
Electron micrograph of glomerular tuft from toxinfed bird (treatment T2 at 173 h) showing widened Bowman's space (S), and detached (D), thickened (T), elongated (C) and misshapen podocyte foot processes. Bar = 5 lm Heart from toxin-fed bird (treatment T2 at 3 weeks) has myofiber vacuolation ("), disorientation, fragmentation and infiltrate of heterophils, macrophages and lymphocytes (m). H&E stain, 400·.**Javed *et al.* (2005)**.



Electron micrograph of heart from toxin-fed bird (treatment T2 at 173 h) showing detachment and dissolution of myofibrils (D) with vacuolation of sarcoplasm (V), destruction of Z (Z), I (I) and H (H) bands, and mitochondrial swelling and membrane dissolution (M). Bar = 1 lm. Heart from toxin-fed bird (treatment T4 at 77 h) shows epicardium thickened by edema fluid (E) and containing dilated lymphatic channels (L), macrophages and lymphocytes and destruction of Z (Z) bands; the myocardium contains foci of heterophils and macrophages (m). H&E stain, 400·.**Javed *et al.* (2005)**



Brain from toxin-fed bird (treatment T2 at 1 week shows satellitosis ("). H&E stain, 400·.Brain from toxin-fed bird (treatment T2 at 1 week) has neuronal degeneration, reduced cellularity, astrocyte proliferation (") and cytovacuolation (m). H&E stain, 400·.**Javed *et al.* (2005)**



Electron micrograph of brain from toxin-fed bird (treatment T2 at 3 weeks) showing satellitosis with oligodendrocyte (O) adjacent to neuronal cell; note cytovacuolation (V), mitochrondrial degeneration, cytoplasm (B) and nucleus (N).mBar = 4 lm. **Javed *et al.* (2005)**

Labuda *et al.* (2005) analyzed a total of 50 samples of poultry feed mixtures of Slovak origin for fumonisin B(1) and B(2) (FB(1), FB(2)) and moniliformin (MON) using SAX-clean up procedure being detected by high pressure liquid chromatography with mass spectrometry (HPLC-MS) and diode array detection (HPLC-DAD), respectively. The samples were also simultaneously investigated for Fusarium species occurrence, and for the capability of Fusarium isolates recovered to produce FB(1) and MON in vitro. FB1 was detected in 49 samples (98 %) in concentrations ranging from 43 to 798 microg x kg(-1), and FB(2) in 42 samples (84 %) in concentrations ranging from 26 to 362 microg x kg(-1). MON was detected in 26 samples (52 %) in concentrations that ranged from 42 to 1,214 microg x kg(-1). Only two Fusarium populations were encountered, namely F. proliferatum and F. subglutinans, of which the former was the most dominant and frequent. All 86 F. proliferatum isolates tested for FB1-production ability proved to be producers of the toxin although none of them produced MON. On the contrary, MON production was observed in a half out of 16 F. subglutinans isolates tested, yet no FB1 production was detected in this case. Despite the limited number of samples investigated during this study, it is obvious that poultry feed mixtures may represent a risk from a toxicological point of view and should be regarded as a potential source of the Fusarium mycotoxins in central Europe.

Tran *et al.* (2005) administered partially purified fumonisin B1 (FB1) orally for 77 d to 5 groups of 8 mule ducks starting at 7 d of age; the concentrations corresponded to 5 diets containing 0, 2, 8, 32, and 128 mg of FB1/kg of feed. No mortality was observed, and no effects on feed consumption and body weight gain were observed at the end of the treatment period. But, surprisingly, FB1 ingested at 32 and 128 mg/kg led to decreased body weight from d 28 to 63 and from d 7 to 63, respectively. FB1 had no effect on the relative weight of heart and breast muscle, whereas a significant increases in the relative weights of gizzard, spleen, and liver were measured in ducks receiving 32 and 128 mg of FB1/kg of feed without evidence of detectable microscopic modification of these organs. FB1 had no significant effect of the serum aspartate aminotransferase and gamma-glutamyltransferase levels but increased serum total protein, cholesterol, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase levels when 128 mg of FB1/kg of feed was given. Serum, liver, and kidney sphinganine to sphingosine ratio was significantly increased in ducks fed 8 to 128 mg of FB1/kg of feed. The biggest increase was observed in kidneys, suggesting that this organ is the most sensitive to detect FB1-induced disruption of sphingolipid metabolism.

Effect of fumonisin B1 (FB1) on BW of mule ducks receiving 0 (on the left) or 128 mg of FB1/kg diet (on the right). A) after 14 d of treatment, B) after 35 d of treatment **Tran *et al.* (2005)**

Asrani *et al.* (2006) divided one hundred fifty 1-d-old quail chicks (Coturnix coturnix japonica) into 2 groups. The 2 groups were designated as controls (CX) and fumonisin-fed birds (FX) with each containing 50 and 100 chicks, respectively. The birds in group CX were maintained on quail mash alone, whereas the birds in group FX were maintained on diets supplemented with 300 ppm of fumonisin B1 from Fusarium verticillioides (formerly Fusarium moniliforme) culture material from 1 d. Quail chicks in both groups were examined daily for clinical signs and mortality. Five randomly selected quail from each group were individually weighed on 0, 7, 14, 21, and 28 d post-feeding (DPF). After weighing, blood was collected from these birds at 7, 14, 21, and 28 DPF for hematological studies and at 14, 21, and 28 DPF for biochemical studies. Fumonisin B1-fed birds (FX) had ruffled feathers, reduced feed and water intake, poor body growth, and greenish mucus diarrhea with 59% mortality. Nearly 30% of the fumonisin B1-fed birds showed nervous signs during the 4-wk experimental period. From 7 DPF onward, BW in group FX were significantly lower than those in group CX. Fumonisin feeding significantly increased hemoglobin, packed cell volume, total erythrocyte count, and total leukocyte count. There was also a significant increase in aspartate transaminase and alanine transaminase in the fumonisin-fed group. Fumonisins significantly increased concentrations of total serum protein and albumin on 14 and 21 DPF, serum calcium and cholesterol levels from 14

DPF onward, and creatinine from 21 DPF onward. This study revealed that the addition of F. verticillioides culture material supplying a level of 300 ppm of FB1/kg of diet is highly toxic to quail chicks, resulting in heavy mortality, decreased growth rate, and significant alterations in hemato-biochemical parameters.

Cheng *et al.* (2006) conducted a study on the effect of fumonisins on macrophage immune functions and gene expression of cytokines in broilers. Ninety-six birds were allotted into four treatments fed with diets containing 0 (control), 5, 10, or 15 mg/kg of FB_1 for three weeks. The results showed that the growth performance was not influenced by the FB_1 challenge, but relative bursa weight was significantly decreased. The activity of serum aspartate aminotransferase, and the serum levels of albumin and cholesterol were significantly elevated by the FB_1 challenges. When broilers were stimulated with injection of lipopolysaccharides, mRNA abundance (determined by semi-quantitative RT-PCR) interleukin-1β (IL-1β), IL-2, interferon-α (IFN-α), IFN-γ, and inducible nitric oxide synthase (iNOS) reached a plateau at 3 h, and declined at 6 h. A FB_1challenge for three weeks increased cytokine mRNA abundance in broilers. The results also showed that 15 mg FB_1 per kg feed significantly inhibited the expression of IL-1β, IL-2, IFN-α, IFN-γ, but had no effect on iNOS. The macrophage functional profile was significantly changed under an exposure of 15 mg FB_1 per kg for three weeks. Taken together, our results suggest that FB_1 up to 15 mg/kg does not affect growth performance, but impairs some parameters of blood biochemistry and the immunocompetence in broilers.

Keck *et al.* (2006) carried out a study to determine whether FB1 altered immunological responses in various cell populations of Single Comb White Leghorn chicks. Cells collected for this study were obtained from those immunological organs with well-defined responses (i.e., spleen, thymus, and blood). Cell populations were exposed to 5 to 50 microg/mL FB1 in vitro for 24 to 72 h, and viability and mitogenic response were evaluated. The effects of FB1 on the mitogenic response were evaluated in cell populations from the spleen and blood stimulated with the mitogens, lipopolysaccharide, concanavalin A, and pokeweed mitogen and in thymocytes stimulated with concanavalin A. The 3-(4,5-dimethylthazol-2-yl)-diphenyl-2H-tetrazolium bromide (MTT) reduction assay was used to assess viability and mitogenic response. Fumonisin B1 decreased spleen cell viability and mitogenic response, albeit the degree of decrease varied with mitogen and time of exposure. Fumonisin B1 increased number of viable thymic cells at 50 microg/mL but had no effect on the mitogenic response of thymocytes. Fumonisin B1 had no effect on blood lymphocyte viability or mitogenic response.

Oliveira *et al.* (2006) evaluated the natural occurrence of aflatoxin B(1), fumonisin B(1) and zearalenone in poultry feed samples. Fungal counts were similar between all culture media tested (10(3)CFU g(-1)). The most frequent genus isolated was Penicillium spp. (41.26%) followed by Aspergillus spp. (33.33%) and Fusarium spp. (20.63%). High precision liquid chromatography was applied to quantify aflatoxin B(1) and fumonisin B(1). Thin layer chromatography was used to determine zearalenone levels. Aflatoxin B(1)values ranged between 1.2 and 17.5 microg kg(-1). Fumonisin B(1) levels ranged between 1.5 and 5.5 microg g(-1). Zearalenone levels ranged between 0.1 and 7 microg g(-1). The present study shows the simultaneous occurrence of two carcinogenic mycotoxins, aflatoxin B(1) and fumonisin B(1), together with another Fusarium mycotoxin (zearalenone) in feed intended

for poultry consumption. Many samples contained AFB(1)levels near the permissible maximum and it could affect young animals. A synergistic toxic response is possible in animals under simultaneous exposure.

Tardieu *et al*. (2006) performed a study to investigate the kinetics of Sa and of the Sa/So in both liver and kidney of ducks. Analysis were performed on treatment days 0, 7, 14, 28 and 77 in five groups of ducks fed fumonisins obtained from an extract of Fusarium verticillioides culture material by daily gavage to obtain an exposure equal to 0, 2, 8, 32 and 128 mg FB1/kg feed. Sa and the Sa/So ratio in tissues were then correlated with Sa and the Sa/So ratio previously obtained in serum. The amounts on sphinganine 1-phosphate (Sa1P) and sphingosine1-phosphate (So1P) in the liver were also investigated. On day 7 of treatment, 2mg/kg FB1 in the feed were sufficient to increase Sa and the Sa/So ratio in liver (by 165 and 148%, respectively) and kidney (by 193 and 104%, respectively). At a rate of 128 mg/kg FB1 in the feed, a very high increase in Sa concentration was observed in both liver and kidney without mortality and/or signs of necrosis (respective increase of 2034 and 3768%). Although the precise mechanism of the resistance of ducks to fumonisin-induced hepatotoxicity is still uncertain, it might be linked to the rate at which the sphingoid bases sphinganine and sphingosine are converted to their 1-phosphate or other metabolite and eliminated from target tissues.

Tessari *et al.* (2006) evaluated the toxic effects of aflatoxin B1 (AFB1) and fumonisin B1 (FB1), administered singly or in combination to broilers. Feeds were prepared with concentrations equal to 0, 50 and 200 microg AFB1/kg, and/or 0, 50 and 200 mg FB1/kg, and offered to broiler chicks from 8 to 41 d of age. The experimental design was totally randomized, in a 3 x 3 factorial arrangement with 9 treatments and 12 birds per treatment. Animals were vaccinated against Newcastle disease on d 14 of life and killed at 41 d. 3. Compared with controls, all mycotoxin-treated groups at 41 d had lower body weight and weight gain, and higher relative heart weight. The relative weight of the liver increased only in birds fed diets containing 200 mg FB1, singly or in combination with AFB1. 4. At 35 d, all groups receiving mycotoxin-treated rations had reduced geometrical mean antibody titres, with birds from groups fed combinations of AFB1 and FB1/kg having even lower values, when compared to the other groups. 5. Histological changes were observed only in liver from birds fed mycotoxin-contaminated rations, and in kidneys of birds fed the diet containing 200 microg AFB1 and 200 mg FB1/kg. Main alterations included vacuolar degeneration and cell proliferation of bile ducts in the liver, and hydropic degeneration in renal tubules in the kidneys. 6. We concluded that AFB1 and FB1 in combination have primarily additive effects on body weight, liver structure and immunological response of broilers at the concentrations used.

Tran *et al.* (2006) carried out a study to investigate the kinetics of Sa and Sa/So in the serum of ducks over a 77-day exposure to 0, 2, 8, 32 and 128 mg FB1/kg feeds. Serum biochemistry was also investigated to reveal hepatotoxicity. The results obtained indicate that the kinetics of sphingolipids and serum biochemistry are closely linked with the duration of the exposure. After a strong and rapid increase Sa and Sa/So decrease then stabilize. The lowest investigated dose able to determine a detectable effect is 2 mg/kg feeds, the Sa/So ratio being the most sensitive biomarker of FB1 exposure.

Deshmukh *et al*. (2007) divided three hundred 1-day-old Japanese quail (Coturnix coturnix japonica) into two groups of 150 each. One group was maintained on quail

mash alone, whereas Fusarium verticillioides culture material (FCM) was added to quail mash in the second group from 5 days of age and supplied 150 mg FB1/kg mash. At day 21, each group was further subdivided into two groups, yielding four groups with 75 birds apiece, which served as the control (group CX), the Salmonella Gallinarum alone group (group CS), the FB1 alone group (group FX), and the group fed FB1 and infected with Salmonella Gallinarum (group FS). An oral challenge with Salmonella Gallinarum organisms (2 x 10(4) colony-forming units [cfu]/ml) was given to groups CS and FS at 21 days of age. Three quail each, were necropsied on day 21 (0 day interval) from groups CX and FX, whereas at subsequent intervals, i.e., 1, 2, 3, 5, 7, 10, 14, and 21 days postinfection (DPI), they were sacrificed from all four groups (CX, CS, FX, and FS) to study the agglutinin response to Salmonella Gallinarum and pathologic changes. The agglutinin titers to Salmonella Gallinarum in the combination group (FS) were generally lower when compared with those in group CS. A reduction in the size of spleen along with depletion of white pulp, thinning of cardiomyocytes, lymphoid cell depletion from bursal follicles, and renal tubular nephrosis were characteristic pathologic changes in group FX. In contrast, there was mild to severe enlargement of spleen accompanied by necrosis and reticuloendothelial cell hyperplasia, pericarditis, myocarditis, and focal interstitial nephritis in groups CS. Similar but more severe lesions were observed in the combination group (FS). In addition, the flabby texture of heart, hydropericardium, and ascites were mainly observed in group FS. It is concluded that continuous presence of fumonisins at 150 mg/kg diet increases the severity of Salmonella Gallinarum infection in young Japanese quail.

Tardieu *et al.* (2007a) characterized the effects of exposure to fumonisins (concentrations of 0, 5, 10, and 20 mg of fumonisin B1 + fumonisin B2/kg of feed) on feed consumption and growth in turkeys over a period of 9 wk. Main biochemical parameters of the liver and alteration of sphingolipid metabolism were investigated in plasma, liver, and kidney. The main results showed no effect on feed consumption and growth in exposed turkeys. Moreover, no effect was observed on the weight of tissues and markers of liver injury. By contrast, a disruption of sphingolipid metabolism was clear at a level of exposure of 10 and 20 mg of fumonisin B1 + fumonisin B2 mg/kg of feed. Both hepatic and kidney concentrations of sphinganine increased gradually throughout the exposure period. These results reveal that disruption of sphingolipid metabolism is an early and sensitive biomarker of fumonisins exposure in turkeys; the consequences on these alterations remain to be established.

Tardieu *et al*. (2007b) investigated the kinetic of fumonisin B1 (FB1) after a single IV and oral dose, and FB1 persistence in tissue in turkey poults by HPLC after purification of samples on columns. After IV administration 4 (single-dose: 10 mg FB1/kg bw), serum concentration–time curves were best described by a three- 5 compartment open model. Elimination half-life and mean residence time of FB1 were 85 and 52 min, 6 respectively. After oral administration (single-dose: 100 mg FB1/kg bw) bioavailability was 3.2%; 7 elimination half-life and mean residence time were 214 and 408 min, respectively. Clearance of FB1 8 was 7.6 and 7.5 ml/min/kg for IV and oral administration respectively. Twenty four hours after the 9 administration of FB1 by the intravenous route, liver and kidney contained the highest levels of FB1 in 10 tissues, level in muscle was low or below the limit of detection (LD, 13 µg/kg). The persistence of FB1 11 in tissue was also studied after administration for nine weeks of a feed that contained 5, 10 and 20 mg 12 FB1+FB2/kg diet. Eight hours

after the last intake of 20 mg FB1+FB2/kg feed (maximum 13 recommended concentration of fumonisins established by the EU for avian feed), hepatic and renal 14 FB1 concentrations were 119 and 22 µg/kg, level in muscles was below the LD. Seven-day old male

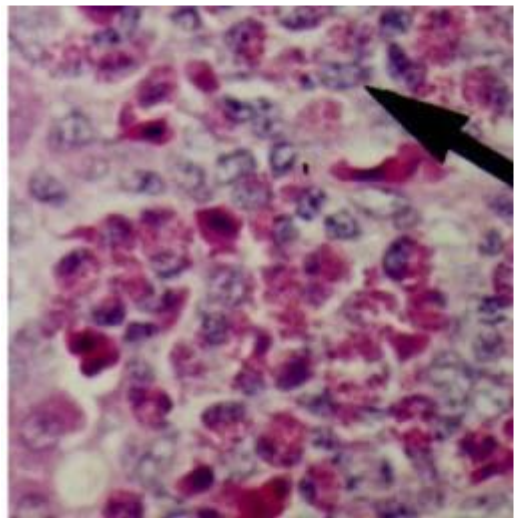
Sharma *et a*l. (2008) studied the individual and combined effects of fumonisin B_1 and moniliformin on clinicopathological and cell-mediated immune response in Japanese quail. A total of 390 one-day-old quail chicks (Coturnix coturnix japonica) were divided into 4 groups (3 replicates per treatment), viz. CX, FX, MX, and FM, containing 75, 105, 105, and 105 birds, respectively. Birds in the control group (CX) were fed quail mash alone, whereas birds in group FX were fed 200 ppm of fumonisin B(1) (FB(1)) from Fusarium verticillioides culture material; group MX was fed 100 ppm of moniliformin (M) from Fusarium fujikuroi culture material; and group FM was fed a combination of 200 ppm of FB(1) and 100 ppm of M. Diets were fed from d 1 to 35 to study clinical signs, growth response, serum biochemical changes, and cell-mediated immune response. Birds fed FB(1) (FX) showed ruffled feathers and poor growth. Birds in group MX appeared more stunted than those in group FX and exhibited signs of poor feathering and decreased feed and water intake. Clinical signs observed in group FM were more or less similar to those observed in groups FX and MX. Total mortality was 12.38, 7.62, and 20.95% for groups FX, MX, and FM, respectively. Mean BW in groups FX, MX, and FM were significantly lower than those in the control group (CX) at almost all intervals. Total serum proteins, albumin, cholesterol, aspartate transaminase, lactate dehydrogenase, and creatine kinase values were higher in all treatment groups compared with the control group. Cell-mediated immune response was more or less comparable in groups CX and MX, whereas the presence of FB(1) in the diet of groups FX and FM was found to be associated with a gradual increase in skin thickness, and the mononuclear inflammatory cell response was poor as compared with groups CX and MX throughout the study. Except for mortality (additive effect) and serum aspartate transaminase values (less than an additive effect up to 14 DPF), no additive or synergistic effects were observed for any of the other response variables measured in the current study, where all statistical differences were attributed to either one mycotoxin or the other.

Tardieu *et al.* (2008) investigated the kinetic of fumonisin B1 (FB1) after a single IV and oral dose, and FB1 persistence in tissue administration (single-dose: 10mg FB1/kg bw), serum concentration-time curves were best described by a three-compartment open model. Elimination half-life and mean residence time of FB1 were 85 and 52min, respectively. After oral administration (single-dose: 100mg FB1/kg bw) bioavailability was 3.2%; elimination half-life and mean residence time were 214 and 408min, respectively. Clearance of FB1 was 7.6 and 7.5ml/min/kg for IV and oral administration, respectively. Twenty-four hours after the administration of FB1 by the intravenous route, liver and kidney contained the highest levels of FB1 in tissues, level in muscle was low or below the limit of detection (LD, 13microg/kg). The persistence of FB1 in tissue was also studied after administration for 9 weeks of a feed that contained 5, 10 and 20mg FB1+FB2/kg diet. Eight hours after the last intake of 20mg FB1+FB2/kg feed (maximum recommended concentration of fumonisins established by the EU for avian feed), hepatic and renal FB1 concentrations were 119 and 22microg/kg, level in muscles was below the LD.

Tardieu *et al.* (2009) investigated the toxicity and persistence of fumonisin B1 (FB1) in liver, kidney and muscle in ducks fed 5, 10 and 20mg FB1+FB2/kg feed during

force-feeding. Mortality and signs of toxicity were only obtained with 20mg/kg, whereas an increased Sa/So ratio was observed from 5mg/kg on. Persistence of FB1 was only found in liver (16 and 20 microg FB1/kg liver in ducks fed 10 and 20 mg FB1+FB2/kg feed, respectively). Toxicokinetic studies were conducted by the intravenous route (IV, single dose: 10mg FB1/kg body weight) and the oral route (single dose: 100mg FB1/kg body weight), in growing ducks and in ducks during force-feeding. After IV administration, serum concentration-time curves were described by a two-compartment open model. Elimination half-life and mean residence time of FB1 were 26 and 24 min, respectively, clearance was 19.3 ml/min/kg. After oral administration, bioavailability, elimination half-life, mean residence time and clearance varied during force-feeding and growth from 2-2.3%, 71-80 min, 200-188 min, 16.7-17 ml/min/kg, respectively. Taken together these results demonstrate that the risk of persistence of FB1 in ducks after force-feeding is very low, Sa/So being a good biomarker which increases before signs of toxicity and risk of persistence of FB1 in tissue (limit of detection 13 microg/kg).

Tessari *et al.* (2010) evaluated the individual and combined effects of dietary aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) on liver pathology, serum levels of aspartate amino-transferase (AST) and plasma total protein (TP) of broilers from 8 to 41 days of age. Dietary treatments included a 3 × 3 factorial arrangement with three levels of AFB_1 (0, 50 and 200 µg AFB_1/kg), and three levels of FB_1 (0, 50 and 200 mg FB_1/kg). At 33 days post feeding, with the exception of birds fed 50 mg FB_1 only, concentrations of AST were higher ($p < 0.05$) in all other treatment groups when compared with controls. Plasma TP was lower ($p < 0.05$) at six days post feeding in groups fed 200 µg AFB_1/kg alone or in combination with FB_1. At day 33 days post feeding, with the exception of birds fed the highest combination of AFB_1 and FB_1 which had higher plasma TP than control birds, plasma TP of birds fed other dietary treatments were similar to controls. Broilers receiving the highest levels of AFB_1 and FB_1 had bile duct proliferation and trabecular disorder in liver samples. AFB_1singly or in combination with FB at the levels studied, caused liver damage and an increase in serum levels of AST.



Liver of broilers fed rations containing 200 µg/kg AFB_1 and 200 mg/kg FB_1 for 33 days. Note the hyperplasia of bile ducts (curved arrow) and heterophilic infiltration (straight arrow). Haematoxylin and eosin, magnification = 400×. **Tessari *et al.* (2010)**

Benlashehr *et al.* (2011) developed two extraction steps combined with HPLC with fluorescence detection to determine the toxicokinetics of fumonisin B(2) (FB(2)) in ducks and turkeys. The limit of quantification of the method was 25 ng of FB(2)/mL. The mean extraction was 63%. After intravenous administration (single dose: 1 mg of FB(2)/kg of BW), plasma concentration time curves were best described by a 2-compartment open model. In ducks, elimination half-life, mean residence time, and clearance of FB(2) were 32 min, 12.9 min, and 9.3 mL/min per kilogram, respectively. In turkeys, these toxicokinetics parameters were 12.4 min, 5 min, and 8.7 mL/min per kilogram, respectively. Only a small amount of FB(2) was detected in plasma after oral dosing of 10 mg of FB(2)/kg of BW.

Stępień *et al.* (2011) mentioned that Fumonisins are polyketide-derived mycotoxins, produced by several Fusarium species, and its biosynthetic pathway is controlled by the FUM cluster--a group of genes exhibiting a common expression pattern during fumonisin biosynthesis. The most common are the B analogues with fumonisin B(1) (FB(1)) being the most prevalent. At least a part of the inter- and intraspecific variation in FBs synthesis level can be explained by the sequence differences inside FUM cluster. The aim of our study was to evaluate the toxin production and sequence variability in FUM genes and intergenic regions among thirty isolates of seven species reported as potential fumonisins producers: Fusarium anthophilum, Fusarium fujikuroi, Fusarium nygamai, Fusarium oxysporum, Fusarium proliferatum, Fusarium subglutinans and Fusarium verticillioides, particularly with respect to FBs synthesis. Fumonisins were produced in high amounts (over 1mg g(-1)) by one isolate of F. subglutinans, three of F. verticillioides and all F. proliferatum isolates except one, regardless of the host organism. The remaining isolates produced low amounts of FBs and two F. verticillioides isolates didn't produce it at all. The lowest variation in amount of toxin produced was found among F. proliferatum isolates. Based on the translation elongation factor 1α (tef-1α) sequence of F. fujikuroi, a species-specific marker was developed. The intergenic region presents similar opportunity for F. nygamai identification. The phylogenetic reconstruction based on FUM1 gene generally reflects the scenario presented by tef-1α sequences. Although the sequence similarities for intergenic regions were lower than in coding regions, there are clearly conserved patterns enabling separation of different subsets of species, including the non-producer species.

Rauber *et al.* (2012) evaluated the individual and combined effects of *Salmonella Typhimurium* lipopolysaccharide (sLPS) and fumonisin B_1 (FB) on performance, relative weight of liver, biological parameters, and histological evaluation of several tissues from four hundred thirty-two 1-d-old male broiler chickens divided into 9 treatments according to the dose of FB (0, 100, or 200 mg/kg, from d 1 to d 28) and sLPS (0, 250, or 500 µg/application per bird, every other day, from d 15 to 27) administered. At the end of the experiment (28 d), significant effects caused by sLPS, FB, and the interaction of sLPS × FB were observed on several parameters. Histopathological evaluations showed significant lesions in liver and kidney caused by sLPS, FB, and their association. According to these results, both sLPS and FB (isolated or in association) cause significant effects on performance and biological parameters of broilers at 28 d of age.

Sharma *et al.* (2012) examined the effects of fumonisin B_1 (FB_1) and moniliformin (M) on the heart of Japanese quail (*Coturnix coturnix japonica*). Three hundred and ninety day-old Japanese quail were randomly divided into four groups: 1) FB_1 alone

(FX), 2) M alone (MX), 3) FB_1 and M (FM), and 4) chick mash alone (CX). We used three pen replicates of 35 quail per pen in groups FX, MX, and FM and three pen replicates of 25 quail per pen in group CX. Gross and microscopic changes in the heart were studied in nine birds (three birds per replicate) from each group at weekly intervals up to 28 days postfeeding (DPF). Ultrastructural changes were studied in the heart of three birds (one bird per replicate) from each group at 21 DPF. Thinning of the heart was the only significant gross lesion in group FX. In contrast, mild-to-severe cardiomegaly was a significant finding in groups MX and FM throughout the study. Microscopically, thinning of cardiomyocytes was evident at 7 DPF in group FX. In addition to the hypertrophy of cardiomyocytes evident as early as 7 DPF, myocardial karyomegaly, nuclear hyperchromasia, and myofibril disarray exhibiting a wavy pattern were more pronounced at 28 DPF in group MX. Similar but more severe lesions were observed in the FM combination group that included myocardial hemorrhages, vacuolar changes, hypertrophy of cardiomyocytes, focal myocarditis, and loss of myofibrils cross-striations. Via transmission electron microscopy, the maximum effect of FB_1 toxicity was observed on mitochondria. In addition to an increase in the number of mitochondria, the mitochondria seemed invariably swollen and pleomorphic, although the outer membrane was intact, and the membrane cristae were usually distinct. Myofibrils seemed thinner, without much disruption in their architecture. Large numbers of vacuolar bodies of irregular size, both in the sarcoplasm and in between the myofibrils, were conspicuous in group FX. In contrast to group FX, the increase in number of mitochondria resulted in widespread separation of muscle fibers in group MX. In addition, the mitochondria were swollen and varied from round to oval to slightly elongated and occasionally forked, and vacuolation was rarely noticed in group MX. In the FM combination group, a significant increase in the number of mitochondria caused muscle fibers to look much thinner and assume a wavy pattern. We conclude that the effect of M on the heart is exaggerated in the presence of FB_1. Although the overall interactive effect of FB_1 and M was less than additive, the interactive effects between the two toxins for cardiac lesions were greater than additive to synergistic up to the second week, raising serious concerns on early age exposure to a combination of these two mycotoxins.

Scott (2012) mentioned in his review that fumonisins are well known mycotoxins produced by Fusarium verticillioides, F. proliferatum and other Fusarium species. Many new fumonisins and fumonisin-like compounds have been detected by mass spectrometry in cultures of F. verticillioides. Recently, fumonisins B2 and B4 were produced by Aspergillus niger isolated from coffee and fumonisin B2 in A. niger from grapes. Fumonisin B2 was itself detected in coffee beans, wine and beer, adding to the list of foodstuffs and feedstuffs other than corn (maize) and sorghum in which fumonisins have been found in recent years. Fumonisin B1 (FB1) can bind to proteins (PB FB1) and to other matrix components during food processing involving heat. The occurrence of bound fumonisins in processed corn foods is common. Another type of binding (or association) relates to observed instability of fumonisins in rice flour, corn starch and corn meal at room temperature; this can affect the immunoaffinity column clean-up procedure in analysis of naturally contaminated starch-containing corn foods for fumonisins. The occurrence of N-fatty acylated fumonisin derivatives in retail

fried corn foods has also been demonstrated. Bioaccessibility of free FB1 and total bound FB1 (TB FB1) present in corn flakes has been estimated by in vitro digestion experiments. Intentional binding of fumonisins to cholestyramine has been demonstrated in vivo and is a potential means of detoxification of animal feed

BENLASHEHR (2013) mentioned that Fumonisins (FBs) are the major mycotoxins produced by Fusarium verticillioides and Fusarium proliferatum, which are found worldwide in maize and maize products. FBs toxic dose and clinical signs of toxicity vary from one species to another. FBs toxicity is commonly linked to their ability on blocking sphingolipids metabolism in all animal species, including avian species. Previous studies have demonstrated that ducks exhibit higher sensitivity to FBs toxicity than turkeys, whereas, the accumulation of sphinganine (Sa) in tissues is more pronounced in turkeys than in ducks. The objectives of our works were to investigate the causes which lead to different toxicity between ducks and turkeys to FBs exposure. The following three hypotheses were investigated: i) Toxicokinetics of fumonisin B2 in ducks and turkeys. ii) Ability of bird cells to protect themselves against high accumulation of free sphingolipids by increasing their catabolism (phosphorylation). iii) Other toxicity mechanisms of FBs rather than their alteration of sphingolipids metabolism (oxidative stress damage and inflammatory responses). The analysis of toxicokinetic parameters of fumonisin B2 did not provide a significant difference between ducks and turkeys. The measurement of simultaneous toxicity of FBs in ducks and turkeys confirmed higher sensibility of ducks. Also the accumulation of Sphingasine-1-Phosphate (Sa1P) in the liver correlated with the amount of Sa but not parameters of hepatic toxicity. Moreover, this study revealed that the amount of Sa in the liver was strongly dependent on the amount of FBs. On the other hand, FBs had no effect on oxidative damages parameters in both species. Interestingly, FBs had mild inflammatory response effect in ducks but not in turkeys. Further investigation on the effects of FBs on ceramide metabolism and inflammatory processes would be necessary to understand the different toxicity between ducks and turkeys to FBs exposure.

Rauber *et al.* (2013) performed a study to determine the effects of three doses of fumonisin B_1 (0, 100, and 200mg/kg of feed) on biological variables (relative weight of liver [RWL], total plasma protein [TPP], albumin [Alb], calcium [Ca], phosphorus [P], uric acid [UA], alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma glutamyltransferase [GGT], alkaline phosphatase [AP], total cholesterol [Chol], triglycerides [Tri], sphinganine-to-sphingosine ratio [SA:SO], and C-reactive protein [CRP]), morphological evaluation of the small intestine (villus height [VH], crypt depth [CD], and villus-to-crypt ratio [V:C]), histological evaluation, and on performance (body weight [BW], feed intake [FI], and feed conversion rate [FCR]) of broiler chickens. Significant effects of FB were observed on BW and FI (reduced), on RWL, TPP, Ca, ALT, AST, GGT, Chol, and Tri (increased) at both 14 and 28 days evaluations. In addition, significant increase was observed on FCR, Alb, P, SA:SO, and CRP and significant reduction in UA, VH, and V:C only at the 28 days evaluation. Significant histological lesions were observed on liver and kidney of FB inoculated broilers at 14 and 28 days. Those results show that FB has a significant effect on biological and histological variables and on performance of broiler chickens.

Machado *et al.* (2013) used ninety six one-day-old broiler chickens to evaluate the effect of feeding naturally contaminated rations with low levels of fumonisins (FBs) and the protective effect of a commercial anti-mycotoxin additive (AMA) on circulating and intestinal immune cells, blood biochemistry, hematological variables and biomarkers of FBs exposure. Birds were assigned in three groups: Negative control (NC), positive control (PC) containing low level (17 ppm) of FBs (FB1 + FB2) in feed, and PC with AMA at 0,2% (AMA + PC). Blood was collected and used to quantify circulating leucocytes through flow cytometry, activity of aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and levels of uric acid (UA), total protein (TP), albumin (Alb), globulin (Glb), Alb:Glb ratio, total leucocytes count (TLC) and hematocrit (Ht), as well as free esphinganine to esphinogosine ratio (SA:SO). On day 3, FBs reduced circulating $CD4^{+}TCRV\beta1^{-}$ and $CD8^{+}CD28^{-}$ lymphocytes in PC; reduced B ~~L~~lymphocytes and increased $Kul^{-}MHCII^{+}$ in both PC and PC+AMA, and increased $Kul^{+}MHCII^{+}$ cells in PC+AMA birds. On day 7, circulating $CD4^{+}TCRV\beta1^{+}$ and $CD8^{-}CD28^{+}$ and $CD3^{+}$ in jejunum were increased only in PC, while $CD4^{-}TCRV\beta1^{+}$ were increased in both PC and PC+AMA birds. FBs reduced TLC and Alb:Glb in both PC and PC+AMA birds after 14 days and only in PC after 28 days, while increased Glb after 14 days in both PC and PC+AMA. On day 28, FBs increased Alb:Glb and reduced Ht only in PC birds, increasing Alb levels and GGT activity in both PC and PC+AMA birds. Serum SA:SO was increased only in PC birds on day 28. These results showed that low levels of naturally occurring FBs could induce rapid immune alterations and impaired liver function and blood homeostasis, which may reflect in a reduction in the overall birds' competence to respond to challenges. Therefore, even if the regulatory standards of FBs are met, toxicity may occur and can be detected by sensitive markers. The use of an AMA was able to alleviate most of these effects.

Antonissen *et al.* (2015a) performed a toxicokinetic study with two groups of 6 broiler chickens to investigate whether chronic exposure to DON could influence the intestinal absorption of FBs leading to an altered exposure and increased toxic effects of this mycotoxin in broiler chickens. All broiler chickens were administered an oral bolus of 2.5 mg FBs/kg BW after three-week exposure to either uncontaminated feed (group 1) or feed contaminated with 3.12 mg DON/kg feed (group 2). No significant differences in toxicokinetic parameters of FB1 could be demonstrated between the groups. Also, no increased or decreased body exposure to FB1 was observed, since the relative oral bioavailability of FB1 after chronic DON exposure was 92.2%. The plasma concentration-time profile revealed that FB1 reached the maximum plasma concentration (Tmax) at 20 min after oral dosing in both control and DON contaminated group. This rapid appearance of FB1 in the systemic circulation indicated that the ingested toxin is absorbed mainly in the proximal part of the intestinal tract.

Antonissen *et al.* (2015b) evaluated whether FBs predispose broilers to necrotic enteritis. One-day-old broiler chicks were divided into a group fed a control diet, and a group fed a FBs contaminated diet (18.6 mg FB1+FB2/kg feed). A significant increase in the plasma sphinganine/sphingosine ratio in the FBs-treated group (0.21 ± 0.016) compared to the control (0.14 ± 0.014) indicated disturbance of the sphingolipid biosynthesis. Furthermore, villus height and crypt depth of the ileum was significantly reduced by FBs. Denaturing gradient gel electrophoresis showed a shift

in the microbiota composition in the ileum in the FBs group compared to the control. A reduced presence of low-GC containing operational taxonomic units in ileal digesta of birds exposed to FBs was demonstrated, and identified as a reduced abundance of *Candidatus* Savagella and *Lactobaccilus* spp. Quantification of total *Clostridium perfringens* in these ileal samples, previous to experimental infection, using *cpa* gene (alpha toxin) quantification by qPCR showed an increase in *C. perfringens* in chickens fed a FBs contaminated diet compared to control (7.5 ± 0.30 versus 6.3 ± 0.24 log10 copies/g intestinal content). After *C. perfringens* challenge, a higher percentage of birds developed subclinical necrotic enteritis in the group fed a FBs contaminated diet as compared to the control (44.9 ± 2.22% versus 29.8 ± 5.46%).

Grenier *et al.* (2015) determined the effects in chickens consuming diets prepared with ***Fusarium verticillioides*** culture material containing FB on intestinal gene expression and on the sphinganine (Sa)/sphingosine (So) ratio (Sa/So; a biomarker of FB effect due to disruption of sphingolipid metabolism). Male broilers were assigned to 6 diets (6 cages/diet; 6 birds/cage) from hatch to 20 days containing 0.4, 5.6, 11.3, 17.5, 47.8, or 104.8 mg FB/kg diet. Exposure to FB altered the Sa/So ratio in all tissues analyzed, albeit to varying extents. Linear dose-responses were observed in the kidney, jejunum and cecum. The liver and the ileum were very sensitive and data fit a cubic and quadratic polynomial model, respectively. Gene expression in the small intestine revealed low but significant upregulations of cytokines involved in the pro-inflammatory, Th1/Th17 and Treg responses, especially at 10 days of age. Interestingly, the cecal tonsils exhibited a biphasic response. Unlike the sphingolipid analysis, the effects seen on gene expression were not dose dependent, even showing more effects when birds were exposed to 11.3 mg FB/kg. In conclusion, this is the first report on the disruption of the sphingolipid metabolism by FB in the GIT of poultry. Further studies are needed to reach conclusions on the biological meaning of the immunomodulation observed in the GIT, but the susceptibility of chickens to intestinal pathogens when exposed to FB, at doses lower than those that would cause overt clinical symptoms, should be addressed.

Guerre (2015) mentioned that fusariotoxins are mycotoxins produced by different species of the genus *Fusarium* whose occurrence and toxicity vary considerably. Despite the fact avian species are highly exposed to fusariotoxins, the avian species are considered as resistant to their toxic effects, partly because of low absorption and rapid elimination, thereby reducing the risk of persistence of residues in tissues destined for human consumption. This review focused on the main fusariotoxins deoxynivalenol, T-2 and HT-2 toxins, zearalenone and fumonisin B1 and B2. The key parameters used in the toxicokinetic studies are presented along with the factors responsible for their variations. Then, each toxin is analyzed separately. Results of studies conducted with radiolabelled toxins are compared with the more recent data obtained with HPLC/MS-MS detection. The metabolic pathways of deoxynivalenol, T-2 toxin, and zearalenone are described, with attention paid to the differences among the avian species.

Oliveira *et al.* (2015) conducted an experiment to evaluate the performance and nutrient metabolizability of broilers fed diets containing fumonisin B1 (FB1) and an esterified glucomannan (EGM). In total, 420 male broilers were distributed according to a 3 x 2 + 1 factorial arrangement, corresponding to three FB1 exposure times (seven, 21, or 35 days), two dietary glucomannan addition levels (0 or 0.1% EGM),

and control diet, totaling seven treatments. The following diets were fed: 1) Control diet, 2) pre-starter diet containing FB1, 3) pre-starter diet containing FB1 and 0.1% EGM, 4) starter diet containing FB1, 5) starter diet containing FB1 and 0.1% EGM, 6) grower diet containing FB1, and 7) grower diet containing FB1 and 0.1% EGM. On d 7, broilers fed FB1 presented lower body weight gain and feed intake ($p<0.05$) compared with control treatment. On d 21, no significant performance differences were detected among treatment groups ($p>0.05$). At 35 days of exposure to FB1 body weight gain was reduced ($p<0.05$) compared with broilers fed fumonisin B1 for seven days. From 4 to 7 days and 18 to 21 days of age, FB1 reduced nutrient metabolizability ($p<0.05$). From 36 to 39 days of age, the EGM allowed maintaining apparent metabolizability for ether extract. It was concluded that the EGM did not reduce FB1 effects on performance or nutrient metabolizability in broilers, except for apparent metabolizability of ether extract.

Liu *et al.* (2016) conducted a survey to determine whether mycotoxins present in the foods consumed by red-crowned cranes (*Grus japonensis*) in the Yancheng Biosphere Reserve, China., collected in the reserve's core, buffer, and experimental zones during overwintering periods of 2013 to 2015, a total of 113 food samples were analyzed for aflatoxin B_1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A using high performance liquid chromatography (HPLC). The contamination incidences vary among different zones and the mycotoxins levels of different food samples also presented disparity. Average mycotoxin concentration from rice grain was greater than that from other food types. Among mycotoxin-positive samples, 59.3% were simultaneously contaminated with more than one toxin. This study demonstrated for the first time that red-crowned cranes were exposed to mycotoxins in the Yancheng Biosphere Reserve and suggested that artificial wetlands could not be considered good habitats for the birds in this reserve, especially rice fields.

Masching *et al.* (2016) evaluated the capability of the fumonisin carboxylesterase FumD to degrade FB_1 to its less toxic metabolite hydrolyzed FB_1 (HFB_1) in the gastrointestinal tract of turkeys and pigs. First, an *ex vivo* pig model was used to examine the activity of FumD under digestive conditions. Within 2 h of incubation with FumD, FB_1 was completely degraded to HFB_1 in the duodenum and jejunum, respectively. To test the efficacy of the commercial application of FumD (FUM*zyme*) *in vivo*, female turkeys (n = 5) received either basal feed (CON), fumonisin-contaminated feed (15 mg/kg FB_1+FB_2; FB) or fumonisin-contaminated feed supplemented with FUM*zyme* (15 U/kg; FB+FUM*zyme*) for 14 days *ad libitum*. Addition of FUM*zyme* resulted in significantly decreased levels of FB_1 in excreta, whereas HFB_1 concentrations were significantly increased. Compared to the FB group (0.24 ± 0.02), the mean serum sphinganine-to-sphingosine (Sa/So) ratio was significantly reduced in the FB+FUM*zyme* group (0.19 ± 0.02), thus resembling values of the CON group (0.16 ± 0.02). Similarly, exposure of piglets (n = 10) to 2 mg/kg FB_1+FB_2 for 42 days caused significantly elevated serum Sa/So ratios (0.39 ± 0.15) compared to the CON group (0.14 ± 0.01). Supplementation with FUM*zyme* (60 U/kg) resulted in gastrointestinal degradation of FB_1 and unaffected Sa/So ratios (0.16 ± 0.02). Thus, the carboxylesterase FumD represents an effective strategy to detoxify FB_1 in the digestive tract of turkeys and pigs.

References

1. Antonissen, Gunther, Mathias Devreese, Filip Van Immerseel, Siegrid De Baere, Sabine Hessenberger, An Martel, and Siska Croubels. Chronic Exposure to Deoxynivalenol Has No Influence on the Oral Bioavailability of Fumonisin B_1 in Broiler Chickens. Toxins (Basel). 2015a Feb; 7(2): 560–571.
2. Antonissen, G., Siska Croubels, Frank Pasmans, Richard Ducatelle, Venessa Eeckhaut, Mathias Devreese, Marc Verlinden, Freddy Haesebrouck, Mia Eeckhout, Sarah De Saeger, Birgit Antlinger, Barbara Novak, An Martel, and Filip Van Immerseel. Fumonisins affect the intestinal microbial homeostasis in broiler chickens, predisposing to necrotic enteritis. Vet Res. 2015b; 46(1): 98.
3. Bailly JD, Benard G, Jouglar JY, Durand S, Guerre P. Toxicity of Fusarium moniliforme culture material containing known levels of fumonisin B1 in ducks. Toxicology. 2001 May 28;163(1):11-22
4. Bailly D., JD, Skiba F, Métayer JP, Grosjean F, Guerre P. Chronic toxicity of fumonisins in turkeys. Poult Sci. 2007 Sep;86(9):1887-93.
5. Benlashehr I., Repussard C., Jouglar J.Y., Tardieu D., Guerre P. Toxicokinetics of fumonisin B2 in ducks and turkeys. Poult. Sci. 2011;90:1671–1675.
6. BENLASHEHR, M. IMAD. FUMONISIN TOXICITY IN DUCKS AND TURKEYS Thessis, Ecole Nationale Vétérinaire de Toulouse, 2013
7. Bermudez AJ, Ledoux DR, Rottinghaus GE, Bennett GA. The individual and combined effects of the Fusarium mycotoxins moniliformin and fumonisin B1 in turkeys. Avian Dis. 1997 Apr-Jun;41(2):304-11.
8. Bermudez AJ, Ledoux DR, Turk JR, Rottinghaus GE. The chronic effects of Fusarium moniliforme culture material, containing known levels of fumonisin B1, in turkeys. Avian Dis. 1996 Jan-Mar;40(1):231-5.
9. Bermudez AJ, Ledoux DR, Rottinghaus GE. Effects of Fusarium moniliforme culture material containing known levels of fumonisin B1 in ducklings. Avian Dis. 1995 Oct-Dec;39(4):879-86.
10. Bouhet, S.; Oswald, I.P. The intestine as a possible target for fumonisin toxicity. *Mol. Nutr. Food Res.*2007, *51*, 925–931.
11. Broomhead JN, Ledoux DR, Bermudez AJ, Rottinghaus GE. Chronic effects of fumonisin B1 in broilers and turkeys fed dietary treatments to market age. Poult Sci. 2002 Jan;81(1):56-61.
12. BRUCKNER B., BLECHSCHMIDT D., SCHUBERT B. (1989): Fusarium moniliformea fungus producing a broad spectrum of bioactive metabolites. Zentralbl. Mikrobiol. 144: 3-12.
13. BUCCI T. J., HOWARD P. C., TOLLESON W. H., LABORDE J. B., HANSEN D. K. (1998):Renal effects of fumonisin mycotoxins in animals. Toxic. Pathol. 26: 160-164.
14. BUCK W.B. (1979): Amer. Assn. Veterinary Laboratory Diagnosticians. 22nd Annual Proceedings: 239-258
15. Buim MR, Bracarense AP, Guimarães IG, Kawamura O, Ueno Y, Hirooka EY. Immunohistochemistry of fumonisin in poultry using avidin-biotin-peroxidase system. Nat Toxins. 1999;7(6):279-82.
16. BUTLER T. (1902): Notes on a feeding experiment to produce leucoencephalitis in a horse with positive result. Am. Vet. Rev. 26: 748-751.
17. Chen, J. P., C. J. Mirocha, W. Xie, L. Hogge, and D. Olson. 1992. Production of the mycotoxin fumonisin B_1 by *Alternaria alternata* f. sp. lycopersici. Appl. Environ. Microbiol. 58:3928-3931.
18. Cheng, Yeong-Hsiang , Shih-Torng Ding & Dr Ming-Huang Chang. Effect of fumonisins on macrophage immune functions and gene expression of cytokines in broilers. Archives of Animal NutritionVolume 60, Issue 4, 2006

19. Dänicke S., Ueberschär K.H., Halle I., Valenta H., Flachowsky G. Excretion kinetics and metabolism of zearalenone in broilers in dependence on a detoxifying agent. Arch. Tierernahr. 2001;55:299–313.
20. Dänicke S., Ueberschär K.H., Halle I., Matthes S., Valenta H., Flachowsky G. Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or Fusarium toxin-contaminated maize on performance of hens and on carryover of zearalenone. Poult. Sci. 2002;81:1671–1680.
21. Dänicke S., Matthes S., Halle I., Ueberschär K.H., Döll S., Valenta H. Effects of graded levels of Fusarium toxin-contaminated wheat and of a detoxifying agent in broiler diets on performance, nutrient digestibility and blood chemical parameters. Br. Poult. Sci. 2003;44:113–126.
22. Dänicke S., Ueberschär K.H., Valenta H., Matthes S., Matthäus K., Halle I. Effects of graded levels of Fusarium-toxin-contaminated wheat in Pekin duck diets on performance, health and metabolism of deoxynivalenol and zearalenone. Br. Poult. Sci. 2004;45:264–272.
23. Del Bianchi M, Oliveira CA, Albuquerque R, Guerra JL, Correa B (2005). Effects of prolonged oral administration of aflatoxin B1 and fumonisin B1 in broiler chickens. Poult. Sci. 84:1835-1840.
24. Deshmukh S, Asrani RK, Ledoux DR, Jindal N, Bermudez AJ, Rottinghaus GE, Sharma M, Singh SP. Individual and combined effects of Fusarium moniliforme culture material, containing known levels of fumonisin B1, and Salmonella gallinarum infection on liver of Japanese quail. Avian Dis. 2005a Dec;49 (4):592-600.
25. Deshmukh S, Asrani RK, Jindal N, Ledoux DR, Rottinghaus GE, Sharma M, Singh SP. Effects of Fusarium moniliforme culture material containing known levels of fumonisin B1 on progress of Salmonella Gallinarum infection in Japanese quail: clinical signs and hematologic studies. Avian Dis. 2005b Jun;49(2):274-80.
26. Deshmukh S, Asrani RK, Ledoux DR, Rottinghaus GE, Bermudez AJ, Gupta VK. Pathologic changes in extrahepatic organs and agglutinin response to Salmonella Gallinarum infection in Japanese quail fed Fusarium verticillioides culture material containing known levels of fumonisin B1. Avian Dis. 2007 Sep;51(3):705-12.
27. Devreese, M., P. De Backer, S. Croubels. Overview of the most important mycotoxins for the pig and poultry husbandry. Vlaams Diergeneeskundig Tijdschrift, 2013, 82 . 171-180
28. Diaz G.J., Boermans H.J. (1994). Fumonisin toxicosis in domestic animals: a review. Veterinary and Human Toxicology 36, 548-555.
29. Dombrink-Kurtzman MA. Fumonisin and beauvericin induce apoptosis in turkey peripheral blood lymphocytes. Mycopathologia. 2003;156(4):357-64.
30. Espada Y, Ruiz de Gopegui R, Cuadradas C, Cabañes FJ. Fumonisin mycotoxicosis in broilers. Weights and serum chemistry modifications. Avian Dis. 1994 Jul-Sep;38(3):454-60.
31. Espada Y, Ruiz de Gopegui R, Cuadradas C, Cabañes FJ. Fumonisin mycotoxicosis in broilers: plasma proteins and coagulation modifications. Avian Dis. 1997 Jan-Mar;41(1):73-9.
32. FDA (2001): Background Paper in Support of Fumonisin Levels in Animal Feed: Executive Summary of this Scientific Support Document http://www.fda.gov/Food/FoodborneIllnessContaminants/NaturalToxins/ucm212900.htm
33. Frisvad JC[1], Smedsgaard J, Samson RA, Larsen TO, Thrane U. Fumonisin B2 production by Aspergillus niger. J Agric Food Chem. 2007 Nov 14;55(23):9727-32
34. Grenier B., Bracarense A.P., Schwartz H.E., Trumel C., Cossalter A.M., Schatzmayr G., Kolf-Clauw M., Moll W.D., Oswald I.P. The low intestinal and hepatic toxicity of hydrolyzed fumonisin B_1 correlates with its inability to alter the metabolism of sphingolipids. Biochem. Pharmacol. 2012;83:1465–1473

35. Grenier, Bertrand Heidi E. Schwartz-Zimmermann , Sylvia Caha , Wulf Dieter Moll, Gerd Schatzmayr and Todd J. Applegate. Dose-Dependent Effects on Sphingoid Bases and Cytokines in Chickens Fed Diets Prepared with Fusarium Verticillioides Culture Material Containing Fumonisins, Toxins 2015, 7, 1253-1272; doi:10.3390/toxins7041253
36. Guerre.Ph. Fusariotoxins in Avian Species: Toxicokinetics, Metabolism and Persistence in Tissues. Toxins (Basel). 2015 Jun; 7(6): 2289–2305.
37. Haschek W.M., Gumprecht L.A., Smith G., Tumbleson M.E., Constable P.D. (2001). Fumonisin toxicosis in swine: an overview of porcine pulmonary edema and current perspectives. Environmental Health Perspectives 109, 251-257
38. Henry MH, Wyatt RD. The toxicity of fumonisin B1, B2, and B3, individually and in combination, in chicken embryos. Poult Sci. 2001 Apr;80(4):401-7.
39. Henry MH, Wyatt RD, Fletchert OJ. The toxicity of purified fumonisin B1 in broiler chicks. Poult Sci. 2000 Oct;79(10):1378-84.
40. Javed T, Bunte RM, Dombrink-Kurtzman MA, Richard JL, Bennett GA, Côté LM, Buck WB. Comparative pathologic changes in broiler chicks on feed amended with Fusarium proliferatum culture material or purified fumonisin B1 and moniliformin. Mycopathologia. 2005 Jun;159(4):553-64.
41. Kubena LF, Edrington TS, Kamps-Holtzapple C, Harvey RB, Elissalde MH, Rottinghaus GE. Influence of fumonisin B1, present in Fusarium moniliforme culture material, and T-2 toxin on turkey poults. Poult Sci. 1995a Feb;74(2):306-13.
42. Kubena LF, Edrington TS, Kamps-Holtzapple C, Harvey RB, Elissalde MH, Rottinghaus GE. Effects of feeding fumonisin B1 present in Fusarium moniliforme culture material and aflatoxin singly and in combination to turkey poults. Poult Sci. 1995b Aug;74(8):1295-303.
43. Kubena LF, Edrington TS, Harvey RB, Buckley SA, Phillips TD, Rottinghaus GE, Casper HH. Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and T-2 toxin or deoxynivalenol in broiler chicks. Poult Sci. 1997 Sep;76(9):1239-47.
44. Kubena LF, Harvey RB, Buckley SA, Bailey RH, Rottinghaus GE. Effects of long-term feeding of diets containing moniliformin, supplied by Fusarium fujikuroi culture material, and fumonisin, supplied by Fusarium moniliforme culture material, to laying hens. Poult Sci. 1999 Nov;78(11):1499-505.
45. Ledoux D.R., Brown T.P., Weibking T.S., Rottinghaus G.E. (1992). Fumonisin toxicity in broiler chicks. Journal of Veterinary Diagnostic Investigation 4, 330-333
46. Ledoux, D.R.; Bermudez, A.J.; Rottinghaus, G.E. Effects of feeding *Fusarium moniliforme* culture material, containing known levels of fumonisin B1, in the young turkey poult. *Poult. Sci.*1996, *75*, 1472–1478.
47. Leslie, J F , W F Marasas, G S Shephard, E W Sydenham, S Stockenström, and P G Thiel. Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and E mating populations of Gibberella fujikuroi (Fusarium moniliforme). Appl Environ Microbiol. 1996 Apr; 62(4): 1182–1187.
48. LI, Y. C., D. R. LEDOUX, ,1 A. J. BERMUDEZ,† K. L. FRITSCHE,* and G. E. ROTTINGHAUS. Effects of Fumonisin B1 on Selected Immune Responses in Broiler Chicks. 1999 Poultry Science 78:1275–1282
49. Liu , Da-wei , Hong-yi Liu, Hai-bin Zhang, Ming-chang Cao, Yong Sun, Wen-da Wuand Chang-hu Lu. Potential natural exposure of endangered red-crowned crane (*Grus japonensis*) to mycotoxins aflatoxin B_1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A[*] J Zhejiang Univ Sci B. 2016 Feb; 17(2): 158–168.
50. Li,Y. C., D. R. Ledoux, A. J. Bermudez, K. L. Fritsche, and G. E. Rottinghaus. The Individual and Combined Effects of Fumonisin B1 and Moniliformin on Performance and Selected Immune Parameters in Turkey Poults. Poultry Science 79:871–878, 2000

51. Magnoli CE, Saenz MA, Chiacchiera SM, Dalcero AM. Natural occurrence of Fusarium species and fumonisin-production by toxigenic strains isolated from poultryfeeds in Argentina. Mycopathologia. 1999;145(1):35-41.
52. Machado Júnior, P. C., Beirão, B. C. B., Filho, T. F., Ingberman, M., Fávaro Júnior, C., & Caron*, L. F. (2014). Naturally contaminated feed with low levels of fumonisins with anti-mycotoxin additive and its impact in the immune cells and blood variables in broiler chickens. Journal of Toxicology and Environmental Health Sciences, 6(10), 203-211.
53. MAJA S. (2001): Fumonisins and their effects on animal health a brief review. Veterinarski Arhiv. 71:299-32 22.
54. MARASAS W. F. O., KRIEK N. P. J., FINCHAM J. E. AND VAN RENSBURG S. J. (1984): Primary liver cancer and esophageal basal cell hyperplasia in rats caused by Fusurium moniliforme. Int. J. Cancer 34:383-387.
55. MARIJANOVIC C.D.R., HOLT P., NORRED W.P., BACON C.W., VOSS K.A., STANCELP.C., RAGLAND W.L. (1991): Immunosuppressive effects of *Fusarium moniliforme* corn culture in chickens. Poultr Sci. 70: 1895- 1901.
56. Martinez-Larranaga, M.R.; Anadon, A.; Diaz, M.J.; Fernandez-Cruz, M.L.; Martinez, M.A.; Frejo, M.T.; Martinez, M.; Fernandez, R.; Anton, R.M.; Morales, M.E.; *et al.* Toxicokinetics and oral bioavailability of fumonisin B1. *Vet. Hum. Toxicol.* 1999, *41*, 357–362.
57. Masching, Sabine , Karin Naehrer, Heidi-Elisabeth Schwartz-Zimmermann, Mihai Sărăndan, Simone Schaumberger, Ilse Dohnal, Veronika Nagl, and Dian Schatzmayr. Gastrointestinal Degradation of Fumonisin B_1 by Carboxylesterase FumD Prevents Fumonisin Induced Alteration of Sphingolipid Metabolism in Turkey and Swine. Toxins (Basel). 2016 Mar; 8(3): 84.
58. Merrill, A.H. Effects of feeding *Fusarium moniliforme* culture material, containing known levels of fumonisin B1, on the young broiler chick. *Poult. Sci.* 1993, *72*, 456–466.
59. Merrill A.H., Sullards M.C., Wang E., Voss K.A., Riley Weibking T.S., Ledoux D.R., Bermudez A.J., Rottinghaus G.E. (1994). Individual and combined effects of feeding Fusarium moniliforme culture material, containing known levels of fumonisin B1, and afl atoxin B1 in the young turkey poult. Poultry Science 73, 1517-1525.
60. Merrill A.H., Sullards M.C., Wang E., Voss K.A., Riley R.T. (2001). Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. Environmental Health Perspectives 109, 283-289
61. Nagaraj RY, Wu WD, Vesonder RF. Toxicity of corn culture material of Fusarium proliferatum M-7176 and nutritional intervention in chicks. Poult Sci. 1994 May;73(5):617-26.
62. Nelson, P. E., Plattner, R. D., Shackelford, D. D., & Desjardins, A. E. (1992). Fumonisin B1 production by Fusarium species other than F. moniliforme in section Liseola and by some related species. *Applied and Environmental Microbiology*, *58*(3), 984–989.
63. Ogido, R., C. A. F. Oliveira,†,1 D. R. Ledoux,‡ G. E. Rottinghaus,‡ B. Correˆa,§ P. Butkeraitis, T. A. Reis,§ E. Gonc¸ales,# and R. Albuquerque. Effects of Prolonged Administration of Aflatoxin B1 and Fumonisin B1 in Laying Japanese Quail. 2004 Poultry Science 83:1953–1958
64. Oliveira, EM, Tanure, CBGS, Castejon, FV, Castro, RMAD, Rocha, FRT, Carvalho, FB, Andrade, MA, & Stringhini, JH. (2015). PERFORMANCE AND NUTRIENT METABOLIZABILITY IN BROILERS FED DIETS CONTAINING CORN CONTAMINATED WITH FUMONISIN B1 AND ESTERIFIED GLUCOMANNAN. *Revista Brasileira de Ciência Avícola*, *17*(3), 313-318.
65. Prelusky, D.B.; Trenholm, H.L.; Savard, M.E. Pharmacokinetic fate of 14C-labelled fumonisin B1 in swine. *Nat. Toxins* 1994, *2*, 73–80.

66. Qureshi MA, Garlich JD, Hagler WM Jr, Weinstock D. Fusarium proliferatum culture material alters several production and immune performance parameters in White Leghorn chickens. Immunopharmacol Immunotoxicol. 1995 Nov;17(4):791-804.
67. Rauber R. H., P. Dilkin, A. O. Mallmann, A. Marchioro, C. A. Mallmann, A. Borsoi, and V. P. Nascimento Individual and combined effects of *Salmonella typhimurium*lipopolysaccharide and fumonisin B_1 in broiler chickensPoultry Science (2012) 91 (11): 2785-2791
68. Rauber, Ricardo H., Oliveira, Maurício S., Mallmann, Adriano O., Dilkin, Paulo, Mallmann, Carlos A., Giacomini, Leandro Z., & Nascimento, Vladimir P.. (2013). Effects of fumonisin B1 on selected biological responses and performance of broiler chickens.*Pesquisa Veterinária Brasileira*, *33*(9), 1081-1086. https://dx.doi.org/10.1590/S0100-736X2013000900006
69. Rheeder J.P., Marasas W.F.O., Vismer H.F. Production of fumonisin analogs by*Fusarium* species. Applied Environmental Microbiology 2002;68(5):2101-2105.
70. Riley, R.T.; Enongene, E.; Voss, K.A.; Norred, W.P.; Meredith, F.I.; Sharma, R.P.; Spitsbergen, J.; Williams, D.E.; Carlson, D.B.; Merrill, A.H. Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environ. Health Persp.* 2001, *109*, 301–308.
71. Sharma, D., R. K. Asrani, D. R. Ledoux, N. Jindal, G. E. Rottinghaus, and V. K. Gupta. 2008.Individual and combined effects of fumonisin B_1 and moniliformin on clinicopathological and cell-mediated immune response in Japanese quail. *Poult. Sci.* 87:1039–1051.
72. Sharma, R. K. Asrani, D. R. Ledoux, G. E. Rottinghaus, and V. K. Gupta Toxic Interaction Between Fumonisin B_1 and Moniliformin for Cardiac Lesions in Japanese Deepa Quail. Avian Diseases / Sep 2012 / pg(s) 545-554
73. SHEPHARD G.S. (1992): Fate of a single dose of the 14C-labelled mycotoxin, FB1, in rats. Toxicon, Pages. 768–770 23.
74. Scott. P.M. Recent research on fumonisins: a review . Food Additives and Contaminants Vol. 29, No. 2, February 2012, 242–248
75. Stępień L, Koczyk G, Waśkiewicz A. FUM cluster divergence in fumonisins-producing Fusarium species. Fungal Biol. 2011 Feb;115(2):112-23. Poult Sci. 2004 Aug;83(8):1287-93.
76. Tardieu, D.; Bailly, J.D.; Benard, G.; Tran, T.S.; Guerre, P. Toxicity of maize containing known levels of fumonisin B1 during force-feeding of ducks. *Poult. Sci.* 2004, *83*, 1287–1293.
77. TARDIEU D, Tran ST, Auvergne A, Babilé R, Benard G, Bailly JD, Guerre P. Effects of fumonisins on liver and kidney sphinganine and the sphinganine to sphingosine ratio during chronic exposure in ducks. Chem Biol Interact. 2006 Mar 10;160(1):51-60.
78. TARDIEU D., BAILLY J. D., SKIBA F, ME´TAYER J, GROSJEAN F, AND GUERRE P. (2007): Chronic toxicity of fumonisins in turkeys. Poultry Science. 86:1887–1893
79. Tardieu, D.; Bailly, J.D.; Skiba, F.; Grosjean, F.; Guerre, P. Toxicokinetics of fumonisin B1 in turkey poults and tissue persistence after exposure to a diet containing the maximum European tolerance for fumonisins in avian feeds. *Food Chem. Toxicol.* 2008, *46*, 3213–3218.*Toxins* 2015, *7* 570
80. Tardieu, D.; Bailly, J.D.; Benlashehr, I.; Auby, A.; Jouglar, J.Y.; Guerre, P. Tissue persistence of fumonisin B1 in ducks and after exposure to a diet containing the maximum European tolerance for fumonisins in avian feeds. *Chem. Biol. Interact.* 2009, *182*, 239–244.

81. Tessari EN[1], Oliveira CA, Cardoso AL, Ledoux DR, Rottinghaus GE. Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. Br Poult Sci. 2006 Jun;47(3):357-64.
82. Tessari, E. N. C., Kobashigawa, E., Cardoso, A. L. S. P., Ledoux, D. R., Rottinghaus, G. E., & Oliveira, C. A. F. (2010). Effects of Aflatoxin B_1 and Fumonisin B_1 on Blood Biochemical Parameters in Broilers. *Toxins*, *2*(4), 453–460. http://doi.org/10.3390/toxins2040453
83. Tran, S.T.; Auvergne, A.; Benard, G.; Bailly, J.D.; Tardieu, D.; Babile, R.; Guerre, P. Chronic effects of fumonisin B1 on ducks. *Poult. Sci.* 2005, *84*, 22–28.
84. Tran ST, Tardieu D, Auvergne A, Bailly JD, Babilé R, Durand S, Benard G, Guerre P. Serum sphinganine and the sphinganine to sphingosine ratio as a biomarker of dietary fumonisins during chronic exposure in ducks. Chem Biol Interact. 2006 Mar 10;160(1):41-50. Epub 2006 Jan 18
85. Vesonder RF, Wu W. Correlation of moniliformin, but not fumonisin B1 levels, in culture materials of Fusarium isolates to acute death in ducklings. Poult Sci. 1998 Jan;77(1):67-72.
86. Voss K.A., Smith G.W., Haschek W.M. (2007). Fumonisins: toxicokinetics, mechanism of action and toxicity. Animal Feed Science and Technology 137, 299-325
87. VUDATHALA D.K., PRELUSKY D.B., AYROUD M., TRENHOLM H.L., MILLER, J.D. (1994): Pharmacokinetic fate and pathological effects of 14C-FB1 in laying hens. Nat. Toxins 2: 81–88.
88. Vudathala, D.K.; Prelusky, D.B.; Ayroud, M.; Trenholm, H.L.; Miller, J.D. Pharmacokinetic fate and pathological effects of 14C-fumonisin B1 in laying hens. *Nat. Toxins* 1994, *2*, 81–88.
89. 30. Benlashehr, I.; Repussard, C.; Jouglar, J.Y.; Tardieu, D.; Guerre, P. Toxicokinetics of fumonisin B2 in ducks and turkeys. *Poult. Sci.* 2011, *90*, 1671–1675.
90. WALTER F.O. AND MARASAS W. F. O. (2001): Discovery and Occurrence of the Fumonisins. Historical Perspective Environ Health. 109: 239–243. 21.
91. WEIBKING T., LEDOUX D.R., BERMUDEZ A.J., TURK J.R. AND ROTTINGHAUS G.E. (1995): Effects on turkey poults of feeding fusarium-moniliforme m-1325 culture material grown under different environmental-conditions. Avian Dis. 39: 32-38
92. Weibking TS, Ledoux DR, Bermudez AJ, Rottinghaus GE. Individual and combined effects of feeding Fusarium moniliforme culture material, containing known levels of fumonisin B1, and aflatoxin B1 in the young turkey poult. Poult Sci. 1994 Oct;73(10):1517-25.
93. WHO Library (2005) Cataloguing in Publication Data, FB1.(Environmental health criteria ; 219) ISBN 92 4 157219 1 (NLM Classification: QD 341.P5) ISSN 0250-863X
94. WILSON B. J., MARONPOT R. R. (1971): Causative fungus agent of leucoencephalomalacia in equine animals. Vet. Rec. 88:484-486.
95. Wu W, Liu T, Vesonder RF. Comparative cytotoxicity of fumonisin B1 and moniliformin in chicken primary cell cultures. Mycopathologia. 1995 Nov;132(2):111-6.
96. Yoo H.S., Norred W.P., Showker J., Riley R.T. (1996). Elevated sphingoid bases and complex sphingolipid depletion as contributing factors in fumonisin-induced cytotoxicity. Toxicology and Applied Pharmacology 138, 211-218.

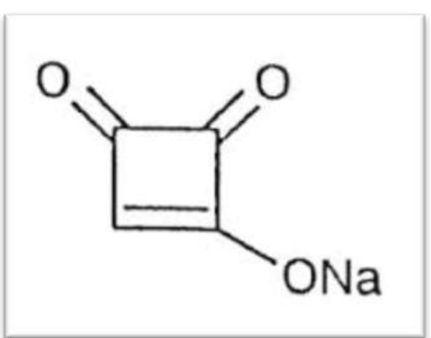
.

4.7. 3. Moniliformin

- **Moniliformin** is an unusual mycotoxin, a feed contaminant that is lethal to fowl, especially ducklings.
- **Moniliformin** is mainly cardiotoxic and causes ventricular hypertrophy.
- **Moniliformin** actually causes competitive inhibition of the activity of pyruvate dehydrogenase complex of respiratory reaction, which prevents pyruvic acid, product of glycolysis, to convert to acetyl CoA. .
- **Moniliformin** is a plant growth regulator and is phytotoxic as well.
- **Moniliformin** was first isolated from an isolate of Fusarium moniliforme, which was actually misidentified and should have been identified as F. proliferatum.
- **Moniliformin** is produced by several species of Fusarium., many of which are known plant pathogens in cereal grains.

Moniliformin chemical structure

- Moniliformin is an unusual mycotoxin, a feed contaminant that is lethal to
- Formula: C_4HNaO_3
- Molar mass: 120.04 g/mol
- Moniliformin is an ionic compound forming sodium and potassium salts
- Moniliformin is soluble in water and polar solvents.
- Moniliformin is light yellow crystals.
- Moniliformin decomposes at 150-153 C without melting. UV maxima are 229 nm and 260 nm in methanol.



Conditions favoring disease and toxin formation in the field

- Samples of oats, wheat, corn rye, and triticale have been shown to be contaminated with moniliformin. The exact conditions favoring production of moniliformin is unknown but one would suspect that conditions such as cool, wet weather may favor Fusarium contamination of grain in the field, especially if these conditions are persent at the time plants are flowering.

However, any condition that produces stress on the plant, such as corn, may be appropriate for the production of

- moniliformin often occurs in fumonisin-contaminated corn as both compounds are produced by isolates of F. proliferatum on this commodity.
- Insect damage may also provide for a portal of entry for the fungus to the host plant.
- corn kernels may or may not have visible evidence of fungus as the infection may be internal with no visible presence on the exterior.
- some grains will show a whitish to pink discoloration from the mould growth. Anything that disrupts the integrity of the seed coat should cause as awareness of the potential for the presence of fungi and mycotoxins.

Moniliformin (M) in poultry

- Initial studies of moniliformin toxicity in chicks involved the determination of the median lethal dose (LD50) (**Cole et al., 1973; Kriek et al., 1977; Burmeister et al., 1979).**
    - **Allen *et al.* (1981)** fed chicks moniliformin from culture material or in purified form at concentrations from 8 to 64 mg/kg of diet and observed reduced performance and mortality only in chicks fed 64 mg M/kg for 3 wk.
    - **Engelhardt *et al.* (1989)** fed chicks moniliformin from culture material at 0, 144, 288, or 576 mg/kg and found Day 10 mortality to be 80% in chicks fed 144 mg/ kg and 100% in chicks fed 288 or 576 mg/kg.
    - **Javed et al. (1993)** reported 40 and 70% mortality in chicks fed 27 and 154 mg of purified M/kg, respectively.
    - **Ledoux *et al.* (1995)** fed chicks concentrations ranging from 0 to 300 mg M from F. fujikuroi culture material and observed mortalities of 27, 57, and 83% at concentrations of 200, 250, and 300 mg M/kg of diet, respectively. Reduced performance was observed at concentrations of 100 mg M/kg or greater
    - **Nagaraj *et al.* (1996)** injected 3-week-old broiler chickens with moniliformin (40 mg/kg body weight, intramuscularly or 1 mg/kg body weight intravenously or an equal volume of normal saline (1 ml/kg body weight), and changes in electrocardiogram were monitored for 50 minutes.
        - Three of the seven birds injected with moniliformin died within 50 minutes post-injection.
        - Moniliformin caused a bradycardia, which became highly significant ($P < 0.05$) within 15 minutes post-injection.
        - The P-R, Q-T, and S-T intervals of moniliformin-injected birds were significantly lengthened throughout the 50-minute observation ($P < 0.05$).

- The results indicate that the moniliformin-induced mortality is due primarily to cardiac failure.

Reams et al. (1997) induceda sudden death syndrome in chicks and poults fed diets containing Fusarium fujikuroi, formulated to contain 0-330 mg/kg moniliformin (M) with or without the maximum recommended therapeutic concentration of monensin. Lesions of monensin toxicosis were not observed. Clinical signs were referable to cardiac dysfunction (sudden death, dyspnea, cyanosis, depression). Poults and chicks dying early in the study had no gross lesions or had lesions of right ventricular dilation.

Toxigenic Fusarium species

1. ***Fusarium acuminatum***
2. ***Fusarium andiyazi***
3. ***Fusarium anthophilum***
4. ***Fusarium avenaceum***
5. *Fusarium begonia*
6. *Fusarium beomiforme*
7. *Fusarium chlamydosporum*
8. *Fusarium culmorum*
9. *Fusarium denticulatum*
10. *Fusarium dlaminii*
11. ***Fusarium fujikuroi***
12. *Fusarium lactis*
13. *Fusarium musae*
14. *Fusarium napiforme*
15. *Fusarium nisikadoi*
16. *Fusarium nygamai*
17. *Fusarium oxysporum*
18. *Fusarium phyllophilum*
19. ***Fusarium proliferatum***
20. *Fusarium pseudocircinatum*
21. *Fusarium pseudonygamai*
22. *Fusarium ramigenum*
23. *Fusarium semitectum*
24. *Fusarium sporotrichioides*
25. *Fusarium sterilihyphosum*
26. ***Fusarium subglutinans***
27. *Fusarium temperatum*
28. *Fusarium thapsinum*
29. *Fusarium tricinctum*
30. ***Penicillium melanoconidium***

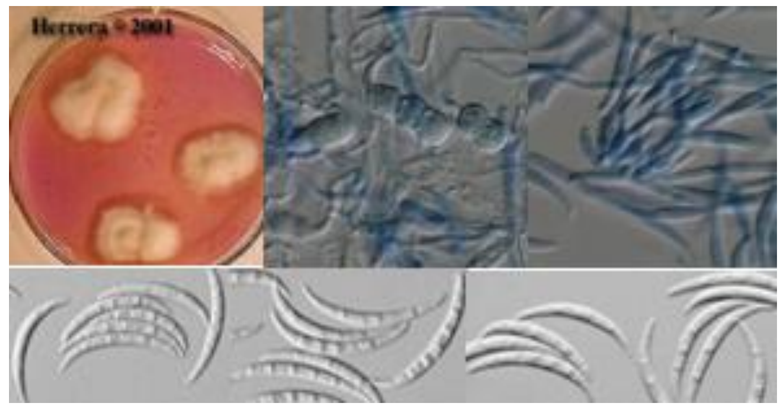
Description of some fungi producing moniliformin

1. Fusarium acuminatum Ellis & Everh., Proc. Acad.Nat. Sci.Philad. 47: 441 (1895)

≡Fusarium scirpi var. acuminatum (Ellis & Everh.) Wollenw., Fusaria Autographice Delineata 3: 930-933 (1930)
≡Fusarium scirpi subsp. Acuminatum (Ellis & Everh.) Raillo, Fungi of the genus Fusarium: 177 (1950)
≡Fusarium gibbosum var. acuminatum (Ellis & Everh.) Bilai, Mykrobiologichnyi Zhurnal Kiev 49 (6): 6 (1987)

Morphology

Colonies are slow-growing, with white aerial mycelium, developing brownish pigmentation in the center on PDA. The dorsal side of the colony has rose to burgundy pigmentation. Macroconidia are broadly falcate with 3-5 septa, apical cell long and tapered, basal cell foot- shaped. Microconidia are sparse, fusiform, 0-1 septa, conidiogenous cell monophialides and chlamydospores formed in chains.

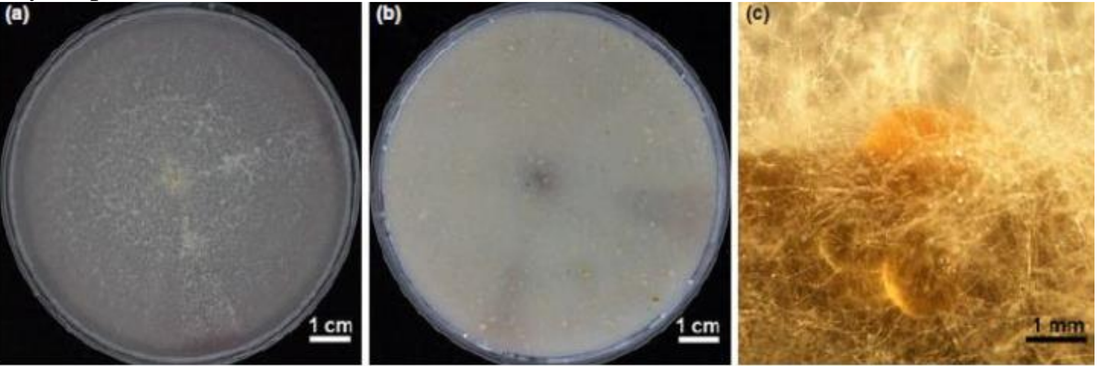


F. acuminatum colony, Paul Cannon Chlamydospores, conidiogenous cells, macroconidia, Leslie and Summerell

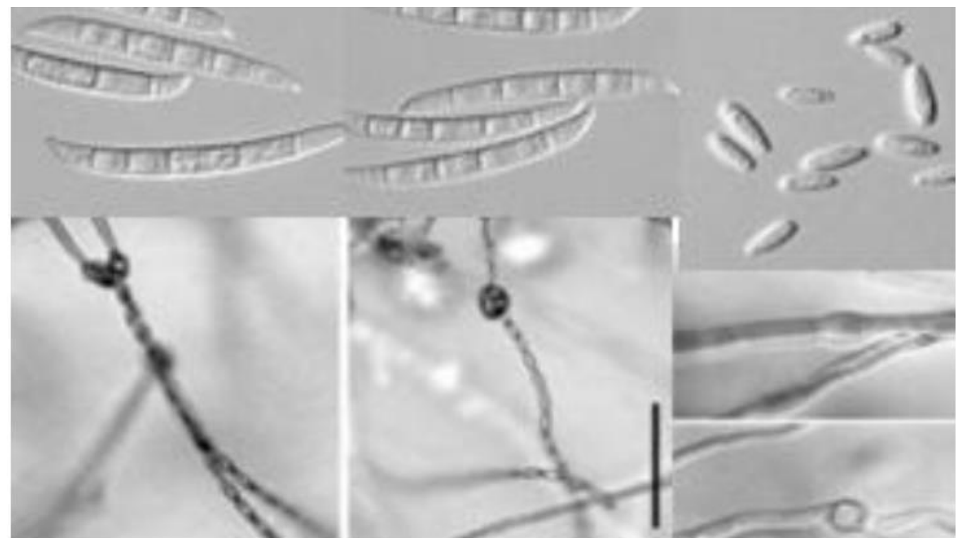
2. Fusarium andiyazi **Marasas, Rheeder, Lampr., K.A. Zeller & J.F. Leslie, Mycologia 93: 1205 (2001)**

= Fusarium moniliforme
= Fusarium verticillioides

Colonies on PDA produce white powdery to floccose mycelium and orange sporodochia, violet pigmentation is seen in the agar. Macroconidia are formed in sporodochia, on monophilides or on branched conidiophores, 3-6 septa, apical cell slightly curved, basal cell pedicillate. Microconida abundant, clavate to ovoid, in chains on monophilides, 0-septa. Chlamydospores absent, pseudochlamydospores may be present.



A,b 10-day old culture of Fusarium andiyazi on oatmeal agar, C sporodochia,. Kebabci et al., 2013

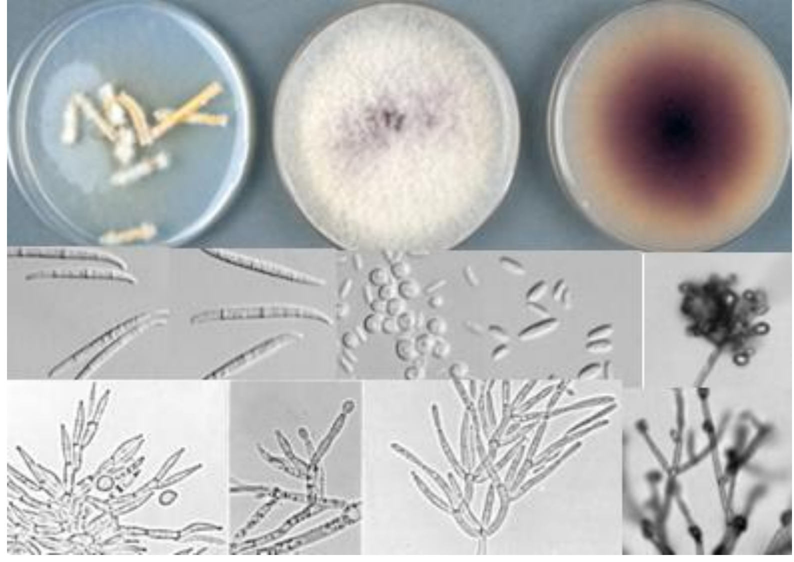


Macroconidia, microconidia,pseudochlamydospores, John F. Leslie and Brett A. Summerell

3. Fusarium anthophilum (A. Braun) Wollenw., Fusaria Autographice Delineata 1: 176 (1916)

≡Fusisporium anthophilum A. Braun, Fung. Europ.: no. 1964 (1875) ≡Fusarium moniliforme var. anthophilum (A. Braun) Wollenw., Fusaria Autographice Delineata 3: 975 (1930) ≡Fusarium wollenweberi Raillo, Fungi of the genus Fusarium: 189 (1950) ≡Fusarium tricinctum var. anthophilum (A. Braun) Bilai, Fusarii (Biologija i sistematika): 251 (1955) ≡Fusarium sporotrichiella var. anthophilum (A. Braun) Bilai, Mykrobiologichnyi Zhurnal Kiev 49 (6): 7 (1987)

Colonies on PDA form abundant white floccose mycelium turn to greyish violet in old cultures. Pigmentation in agar violet grey or dark. Sporodochia pale orange. Macroconidia are thin-walled, long, slender, almost straight, 3-5 septa,produced from monophilides on branched conidiophores in the sporodochia or on the hyphae, basal cell notched or foot-shaped, apical cell curved and tapered. Microconidia are abundant, from poly- or monophialides, globose, 1-2 celled, globose, or ovoid, in false heads. Chlamydospores absent.

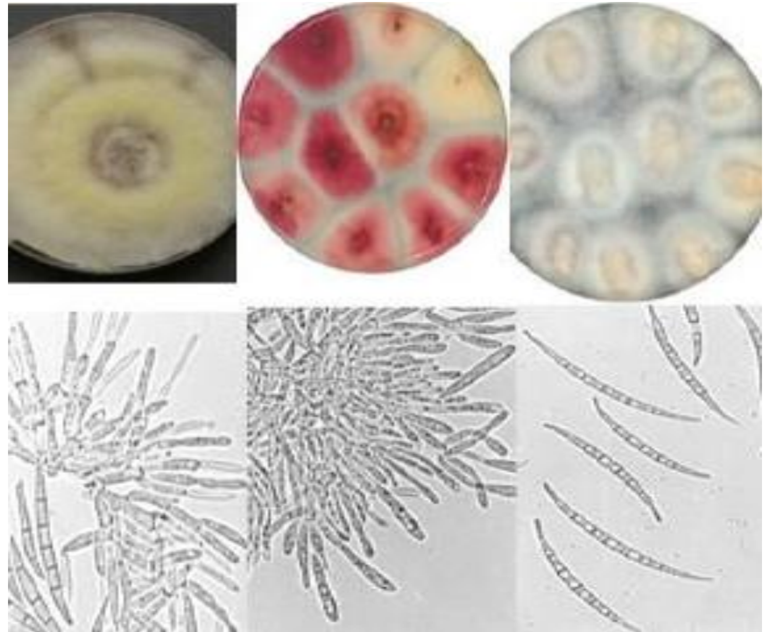


Leslie and Summerell , Hagedorn, Burhenne & Nirenberg

4. Fusarium avenaceum (Fr.) Sacc., Sylloge Fungorum 4: 713 (1886)

Fusisporium avenaceum Fries, Systema Mycologicum 3: 444 (1832)
≡Fusarium herbarum var. avenaceum (Fries) Wollenw., Fusaria Autographice Delineata 3: 899 (1930) [MB#252553]
=Selenosporium herbarum Corda, Icones fungorum hucusque cognitorum 3: 34, t. 6:88 (1839)

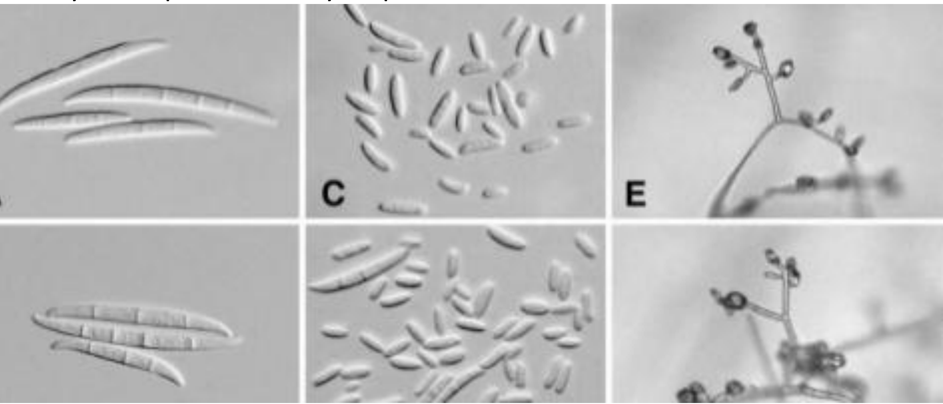
Colonies initially form abundant fluffy white mycelium and produce a golden orange pigment on PDA at 25°C. Sporodochia pale orange, Macroconidia are slightly falcate, thin-walled, usually 3 to 5 septate, with a tapering apical cell , basal cell notched. Microconidia are rare, fusoid, 1-2 septa, single. Chlamydospores are absent.



F. avenaceum colonies, www.grainscanada.gc.ca. Mycota, G. Hagedorn, M. Burhenne & H. I. Nirenberg

5. Fusarium begoniae Nirenberg & O'Donnell, Mycologia 90: 446 (1998)

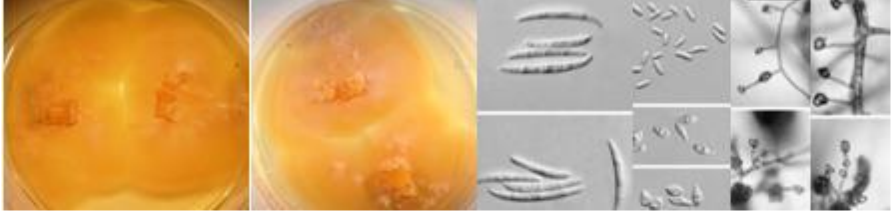
Colonies with entire margin. Aerial mycelium almost white, cottony. Pigmentation in reverse greyish-yellow. Microconidia borne in the aerial mycelium oval to allantoid and obovoid, 1-0 Septate, Macroconidia, abundant, borne in sporodochia slender, long falcate but almost straight, with a slightly beaked apical cell and a footlike basal cell, mostly 3-4 septate. Chlamydospores absent.



John F. Leslie and Brett A. Summerell

6. Fusarium beomiforme **P.E. Nelson, Toussoun & L.W. Burgess, Mycologia 79: 884-889 (1987)**

Colonies with floccose, white-pink aerial mycelium, developing a diffuse, orange-reddish-brown colouration in reverse. Microconidia of two forms: (a) abundant, ovoid to cylindrical; (b) less abundant, larger, globose to napiform, typically vacuolate; chains absent, spores collecting in slimy droplets. Conidiogenous cells monophialides, cylindrical, tapering slightly at the tip, with periclinal thickening. Macroconidia 3-4 (-5)-septate, falcate, apical cell slightly curved, tapering to a point, basal cell pedicellate. Chlamydospores hyaline, smooth, typically terminal, single or in pairs, not in intercalary chains.

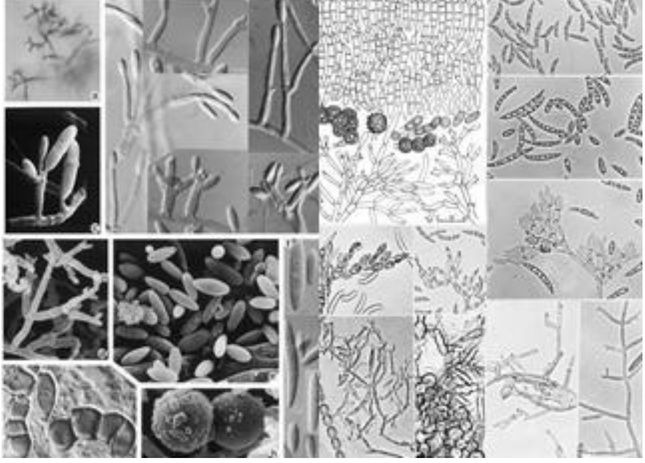


Fusarium beomiforme colonies,Truman State University
Macroconidia and microconidia of Fusarium beoforme, Leslie and Summerell

7. Fusarium chlamydosporum **Wollenw. & Reinking, Phytopathology 15 (3): 156 (1925)**

=Fusarium sporotrichioides var. chlamydosporum (Wollenw. & Reinking) Joffe, Mycopathologia et Mycologia Applicata 52 (1-4): 211 (1974)

Colonies produce white mycelium with grayish rose to burgundy or yellowish to pale brown pigmentation.Macroconidia: abundant, thick-walled, moderately curved, 3-5 septa, apicalcell short, curved and pointed, basal cell notched or foot-shaped. Sporodochia: rare. Microconidia: comma-shaped, 0-2 septe, single or in pairs fro, a phialide, abundant. Chlamydospores : abundant after 2-4 weeks, on aerial hyphae or submerged in agar, in pairs, chains or clusters, pale brown

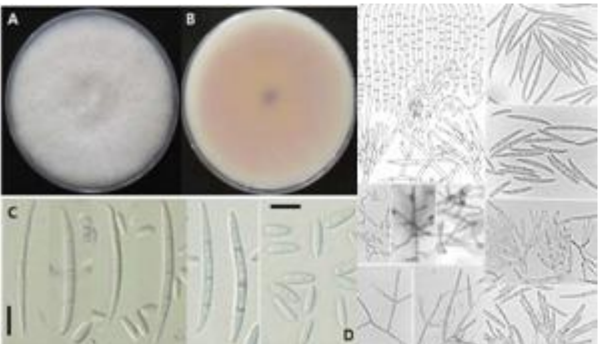


Mycobanc, G. Hagedorn, M. Burhenne & H. I. Nirenberg

8. Fusarium fujikuroi Nirenberg, **Mitteilungen der Biol. Bundesanstalt Land-** Forstwirtschaft 169: 32 (1976)

Macroconidia: abundant in sporodochia, slender, insign. Curved, medium length, 3-5 septa, apical cell tapered, basal cell poorly developed. Sporodochia: orange.

Microconidia: ovoid or club-shaped, 0-1 septa, abundant on the aerial mycelia. Chlamydospores : absent

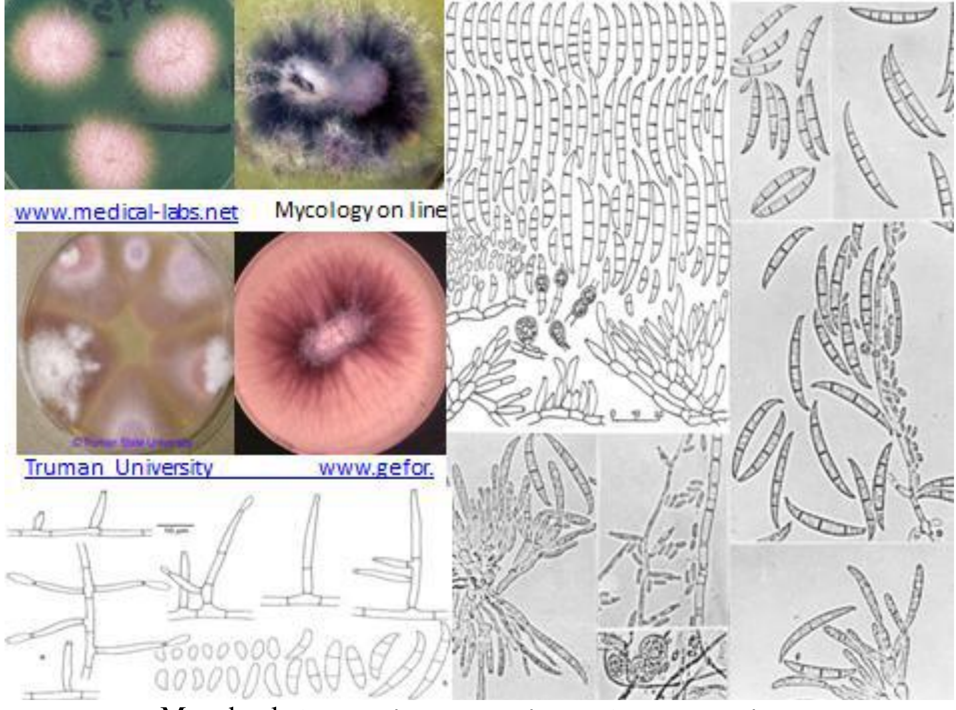


A, B; colony of F. fujikuroi, C; macroconidia, D; microconidia,Tae Jin An et al.,2013, G. Hagedorn, M. Burhenne & H. I. Nirenberg

9. Fusarium oxysporum **Schltdl., Flora Berolinensis, Pars secunda: Cryptogamia: 106 (1824)**

=Fusarium bulbigenum Cooke & Massee, Grevillea 16 (78): 49 (1887)
=Fusarium orthoceras Appel & Wollenw., Kaiser.Biol. Anstalt für Land- und Forstwirtschaft 8: 152 (1910)
=Fusarium citrinum Wollenw., Bull. Maine Agric. Exp. Sta.: 256 (1913)
=Fusarium angustum Sherb., Memoirs Cornell University Agricultural Experimental Station 6: 203 (1915)
=Fusarium oxysporum var. longius Sherb., Memoirs Cornell Unive Agric ExperiStation 6: 223 (1915)
=Fusarium lutulatum Sherb., Memoirs Cornell University Agricultural Experimental Station 6: 209 (1915)
=Fusarium lutulatum var. zonatum Sherb., Memoirs Cornell Univ Agricull ExperStation 6: 214 (1915)
=Fusarium bostrycoides Wollenw. & Reinking, Phytopathology 15 (3): 166 (1925)
=Diplosporium vaginae Nann., Atti Reale Accad. Fisiocrit. Siena: 491 (1926)

Macroconidia: abundant in sporodochia, 3- septa, thin-walled, short to moderately long, straight , apical cell short and slightly hooked, basal cell notched or foot-shaped. Sporodochia: abundant, pale orange . Microconidia: small, oval , elliptical or kidney-shaped, 0- septa. Chlamydospores: abundant

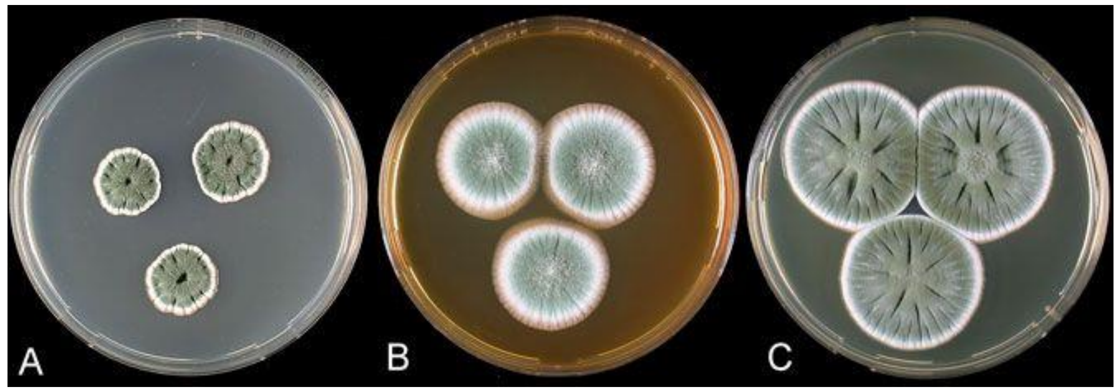


Mycobank G. Hagedorn, M. Burhenne & H. I. Nirenberg

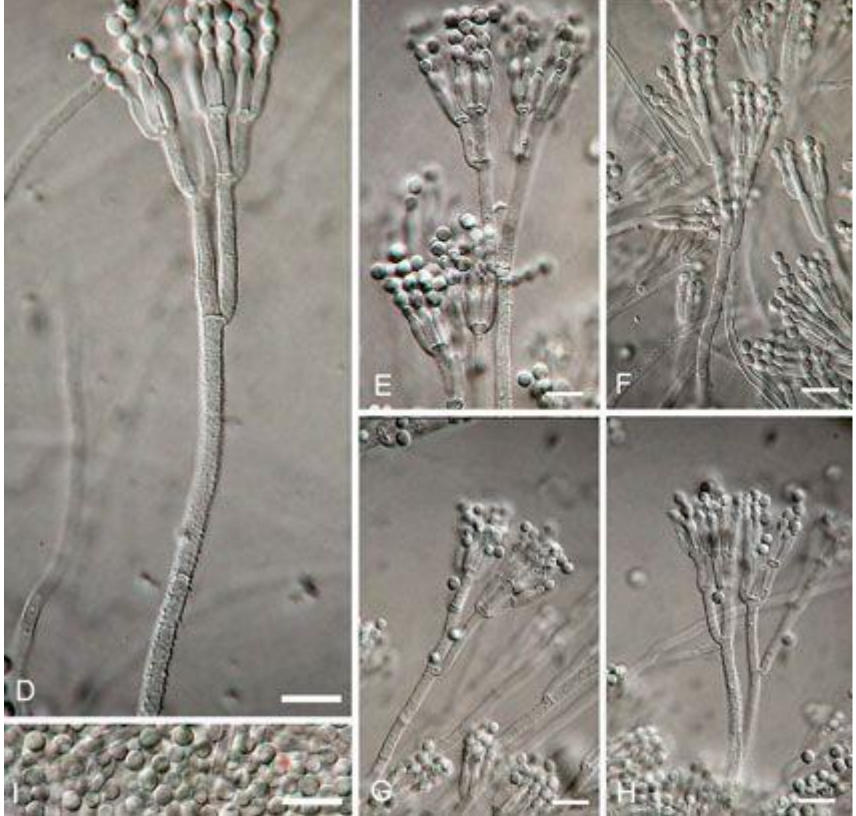
10. Penicillium melanoconidium (Frisvad) Frisvad & Samson, Studies in Mycology 49: 28 (2004)

≡Penicillium aurantiogriseum var. melanoconidium Frisvad, Mycologia 81: 849 (1990)
≡Penicillium melanoconidium (Frisvad) Frisvad & Samson, Mycological Research 98: 489 (1994)

Colonies on Czapek agar and CYA at 25°C growing restrictedly producing dark green conidia with a velvety to weakly granular colony surface, with clear exudate droplets. The colony reverse is cream yellow to yellow or curry. On MEA the conidia are green and colonies have a yellow reverse. On YES agar there is strong sporulation, reverse colour distinct yellow. On CREA weak growth but strong acid production. Conidiophores two-stage branched (terverticillate) with all elements adpressed, stipes rough-walled. Conidia smooth and globose to subglobose.



Penicillium melanoconidium ***colonies, Mycobank***



Penicillium melanoconidium ***Mycobank***

Reports:

Allen *et al.* (1981) fed to growing broiler chicks from 1 to 21 days of age. Up to 16 mg moniliformin/kg of diet from either source was without effect on chick weight gain, feed consumption, and mortality. Chicks fed 64 mg moniliformin/kg of diet from culture had reduced weight gain and feed consumption. Total daily moniliformin consumption by these chicks was nearly twice the reported single oral 50% lethal dose. Three of 10 chicks fed 64 mg/kg of moniliformin in the diet died. No lesions were found upon necropsy. The LD50 of purified moniliformin upon intravenous injection of 7-week-old female broiler chickens was 1.38 +/- .035 mg/kg body weight. Average time to death was 65 minutes. Progressive symptoms noted included lack of muscular coordination, tachypnea from moderate to severe followed by slow labored respiration, coma, terminal agonal struggle, and death.

Engelhardt *et al.* (1989) reported that corn-based diets contaminated with various concentrations of a moniliformin-producing isolate of Fusarium moniliforme var. subglutinans were found to be lethal for chicks, ducklings, and turkey poults. Ducklings appeared to be the most sensitive to the lethal effects of the toxic feed. Gross lesions were ascites, hydropericardium, and myocardial pallor. Microscopic lesions were limited to the heart and liver, and they consisted of degeneration and necrosis of the myocardium and degeneration of hepatocytes. Cardiotoxicosis was the apparent cause of death.

Dombrink-Kurtzman *et al.* (1993) isolated peripheral blood lymphocytes from broiler chicks that had ingested feed amended with autoclaved Fusarium proliferatum culture material containing fumonisin B1 (FB1), fumonisin B2 (FB2) and moniliformin. Lymphocyte viability was determined for birds that were placed on amended rations at day 1 or day 7 of age at three different levels of mycotoxins, ranging from 61-546 ppm FB1, 14-94 ppm FB2 and 66-367 ppm moniliformin. Reduction of the tetrazolium salt, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], to yield MTT formazan, based on mitochondrial metabolic activity, was used to assess cell viability. Lymphocyte cytotoxic effects were observed in all treatment groups on day 21; chicks that started on amended feed at day 1 of age were affected more than those that started at day 7. Abnormal erythrocytes resembling early stages of erythroblasts were observed in peripheral blood from test chicks. Abnormally shaped red cells (poikilocytes) having a spindle-shape with one or both ends pointed were present. Some red cells appeared to be undergoing mitosis. Both reduced lymphocyte viability and abnormal erythrogenesis occurred in chicks given feed amended with F. proliferatum culture material containing FB1, FB2 and moniliformin

Javed *et al.* (1993) gave two hundred twenty-eight male chicks (Columbia x New Hampshire) feed amended with autoclaved culture material (CM) of Fusarium proliferatum Containing fumonisin B1 (FB1), fumonisin B2 (FB2) and moniliformin in 3 separate feeding trials. Purified FB1 and moniliformin were given separately and in combination in a fourth feeding trial. Birds were given amended rations at day 1 (Trial 1 and 4), day 7 (Trial 2), and day 21 (Trial 3) and their respective ration was given for 28 days (Trial 1), 21 days (Trial 2), 7 days (Trial 3), and 14 days (Trial 4). FB1 concentrations were 546, 193, and 61 ppm; FB2 were 98, 38 and 14 ppm; and moniliformin were 367, 193, and 66 ppm in the first 3 feeding trial regimens. Chicks in Trial 4 were given dietary concentrations of purified FB1 at 274 and 125 ppm, and

moniliformin at 154 and 27 ppm. FB1 and moniliformin, both alone and in combination, produced dose-responsive clinical signs, reduced weight gains and mortality in chicks. Age of birds given amended feeds had little difference in the clinical response; however, those given the rations from days 7 or 21 were slightly less susceptible than those given rations beginning at 1 day of age. Additive effects were noted when the toxins were given in combination. When toxins were given separately, adverse effects took longer to occur. A system to monitor pattern and rate of defecation (RD) was developed for assessing the chicks' approach to feed, water and heat source as illness progressed. Our results indicate that chicks fed corn heavily infected with F. proliferatum under field conditions could suffer acute death similar to that described for 'spiking mortality syndrome' during the first 3 weeks of age.

Bermudez *et al.* (1995) fed Fusarium moniliforme culture material containing fumonisin B1 (FB1) to white Pekin ducklings from 1 to 21 days of age. Four dietary treatments were prepared with 0, 100, 200, and 400 mg FB1/kg ration. Ducklings fed rations containing FB1 had a dose-dependent decrease in feed intake and weight gain. Increasing levels of FB1 in the ration were associated with increasing absolute organ weights of liver, heart, kidney, pancreas, and proventriculus. Liver sphinganine to sphingosine ratios increased significantly in ducklings fed FB1. Two of eight ducklings fed a ration containing 400 mg FB1/kg died prior to the termination of the experiment. Mild to moderate hepatocellular hyperplasia was evident in all ducklings fed FB1. Mild to moderate biliary hyperplasia was also noted in the liver sections of ducklings fed 400 mg FB1/kg in the ration. Ducklings, like other poultry, are relatively resistant to the toxic effects of FB1.

Qureshi *et al.* (1995) fed White Leghorn Cornell K-strain chicks (3 replicates of 16 per pen) at Day 7 a feed amended with Fusarium proliferatum culture material containing fumonisin B1, fumonisin B2, and moniliformin at 61, 10.5, and 42.7 ppm, respectively. Observed effects on performance of treated birds included reduced feed conversion at 2 wk, and reduced body weight of males and females up to 6 wk (P < or = .05). Splenic, thymic, and liver weights, normalized for body weight, were reduced (P < or = .05) with no change in bursa of Fabricius. No significant changes were observed histologically in the spleen, bursa, kidney, heart, liver, cecal tonsils, colon, or tibia. Significant suppression in total Ig and IgG levels occurred. Macrophages from treated chicks exhibited a 34% reduction in phagocytic activity. Natural killer cell activity was not affected. These findings, which showed that Fusarium toxins alter performance and immune end points in chickens, imply that chickens exposed to mycotoxins may be more susceptible to infectious diseases.

Weibking *et al.* (1995) studied the effects of feeding Fusarium moniliforme M-1325 culture material (CM), grown under different environmental conditions, in turkey poults. Poults were fed a control diet or diets containing four levels of FB1 (75, 150, 225, or 300 mg/kg) prepared from F. moniliforme M-1325 cultures that produced 7800 (CM1) or 4000 mg FB1/kg (CM2). F. moniliforme M-1325 CM that produced a low concentration of FB1 (350 mg FB1/kg) was also used to prepare an additional diet containing 75 mg FB1/kg (CM3). Dose-dependent decreases in feed intake and body-weight gains and dose-dependent increases in liver weights and serum sphinganine (SA) to sphingosine (SO) ratios were observed in poults fed CM1 or CM2. Poults fed CM3 consumed more feed and had lower body-weight gains than controls or poults fed CM1 or CM2 (at 75 mg FB1/kg). Poults fed CM3 also had

increased liver weights and SA:SO ratios compared with control poults. Generalized hepatocellular hyperplasia was observed in all FB1 treatment groups. Biliary hyperplasia was evident in turkeys fed 150 to 300 mg FB1/kg. Results indicate that at equivalent dietary FB1 levels, F. moniliforme cultures producing different concentrations of FB1 differ in their effects on turkey poults.

Wu *et al.* (1995) compared two water-soluble **Fusarium** metabolites, fumonisin B1 (FB1) and moniliformin (MN) for their cytotoxicity in a variety of chicken primary cell cultures. Cardiac and skeletal myocytes and hepatocytes derived from embryos, and splenocytes, macrophages, and chondrocytes derived from 3- to 4-week old chickens were cultured in media containing either FB1 or MN (0 to 1 mM) for 48 hr. The colorimetric tetrazolium cleavage assay was then used for measuring cell survival. FB1 was not toxic to macrophages, hepatocytes, cardiac and skeletal myocytes but toxic to splenocytes and chondrocytes. MN was not toxic to chondrocytes and macrophages, but toxic to splenocytes, cardiac and skeletal myocytes. Median effective concentration (EC50) of MN in skeletal myocytes was 42 mu M (fiducial limits: 33 to 50 mu M) and in cardiac myocytes was 95 mu M (fiducial limits: 84 to 122 mu M). Estimated EC50 of FB1 in chondrocytes and splenocytes and EC50 of MN in splenocytes were all greater than 200 mu M.

Ledoux *et al.* (1996) studied the effects of feeding Fusarium moniliforme culture material, containing known concentrations of fumonisin B1 (FB1), in turkey poults. Day-old poults were allotted randomly to dietary treatments containing 0, 0.41, 0.82, 1.23, 1.64. 2.87, 4.10, 5.33, 6.56, and 7.79% fumonisin culture material (FCM). These levels of FCM supplied 0, 25, 50, 75, 100, 175, 250, 325, 400, and 475 mg FB1/kg of feed. Each dietary treatment was fed to six pen replicates of six poults each for 21 d. Poults fed FCM that supplied 325 to 475 mg FB1/kg diet had lower ($P < 0.05$) feed intakes and BW gains. Increased ($P < 0.05$) liver and pancreas weights were observed in poults fed FCM that supplied > or = to 175 mg FB1/kg. Poults fed FCM that supplied 400 and 475 mg FB1/kg diet had increased ($P < 0.05$) red blood cell counts and increased ($P < 0.05$) serum concentrations of gamma glutamyl transferase and aspartate aminotransferase. Compared with controls, poults fed FCM that supplied 25, and 75 to 475 mg FB1/kg had increased ($P < 0.05$) liver sphinganine:sphingosine ratios. Hepatocellular hyperplasia was mild at 75 and 100 mg FB1/kg diet, moderate to severe at 250 mg/kg FB1, and severe at 325 to 475 mg FB1/kg. Multifocal to generalized loss of cross striations and thinning of cardiomyocytes was observed in poults fed FCM that supplied 475 mg FB1/kg diet. Results indicated that diets containing < or = to 1.23% FCM that supplied > or = to 75 mg FB1 /kg are toxic to young turkeys.

Leslie *et al.* (1996) tested Fusarium strains for the ability to produce fumonisins B1, B2, and B3 and moniliformin and for toxicity to 1-day-old ducklings. Most of the members of the A mating population (19 of 20 strains) produced more than 60 micrograms of total fumonisins per g, whereas only 3 of 20 members of the F mating population produced more than trace levels of these toxins and none produced more than 40 micrograms of total fumonisins per g. In addition, only 3 of 20 members of the A mating population produced more than 1 microgram of moniliformin per g (and none produced more than 175 micrograms/g), while all 20 strains of the F mating population produced more than 85 micrograms of this toxin per g and 1 strain produced 10,345 micrograms/g. The duckling toxicity profiles of the strains of the two mating populations were similar, however, and the level of either toxin by itself

was not strongly correlated with duckling toxicity. On the basis of our data we think that it is likely that the members of both of these mating populations produce additional toxins that have yet to be chemically identified. These toxins may act singly or synergistically with other compounds to induce the observed duckling toxicity.

Nagaraj *et al.* (1996) used electrocardiography to examine the acute cardiotoxic effects of moniliformin on 3-week-old broiler chickens. Each of the seven pairs of anesthetized birds (pentobarbital sodium, 40 mg/kg body weight, intramuscular) was injected intravenously with moniliformin (1 mg/kg body weight) or an equal volume of normal saline (1 ml/kg body weight), and changes in electrocardiogram were monitored for 50 minutes. Three of the seven birds injected with moniliformin died within 50 minutes post-injection. Moniliformin caused a bradycardia, which became highly significant ($P < 0.05$) within 15 minutes post-injection. The P-R, Q-T, and S-T intervals of moniliformin-injected birds were significantly lengthened throughout the 50-minute observation ($P < 0.05$). The results indicate that the moniliformin-induced mortality is due primarily to cardiac failure.

Bermudez *et al.* (1997) fed turkeys a control ration, or rations containing 200 mg FB1/kg, 100 mg M/kg, or a combination of both 200 mg FB1/kg and 100 mg M/kg feed from 1 to 21 days of age. These rations contained 0, 3.8, 1.0, and 4.8% culture material, respectively. In comparison to controls, turkeys fed FB1 had increased relative liver weights. Both aspartate aminotransferase and lactate dehydrogenase were increased in poults fed FB1. Turkeys fed M had decreased feed intake and body weight gains and increased relative heart weights in comparison to controls. Poults fed FB1 had moderate diffuse hepatocellular hyperplasia and poults fed moniliformin had a loss of cardiomyocyte cross striations. Turkeys fed the ration containing both M and FB1 had all the above changes; however, no additive or synergistic effects were evident for any single parameter measured. No treatment-related morbidity or mortality was observed in the study.

Harvey *et al.* (1997) evaluated the effects of feeding diets containing 100 mg moniliformin (M)/kg of feed from culture material and 16 mg deoxynivalenol (DON)/kg of feed from naturally contaminated wheat in growing broiler chicks from 1 day to 21 days of age. Body weight (BW), body-weight gain, and feed consumption were decreased by feeding M and M plus DON diets. Relative heart weight was increased by the M diet, whereas relative weights of proventriculus, gizzard, and heart were increased by the M plus DON diet. The M diet increased alanine transferase and aspartate transaminase activities and creatinine concentration and decreased mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (MCHC). The M and DON diet decreased glucose, hemoglobin, and MCHC. Histopathological lesions from the M diet were limited to the kidney and consisted of extensive renal tubular epithelial degeneration plus luminalmineralization. A moderation of the severity of lesions was seen in the tissues of the M plus DON-fed chicks, consisting of generally mild tubular epithelial degeneration. None of the parameters measured were affected by the DON diet. Results indicate additive or less-than-additive toxicity for most parameters when chicks were fed diets containing 100 mg M plus 16 mg DON/kg of feed. Although the concentration of M in this study was high compared with that reported for feedstuffs,

additional information on the occurrence and toxicity of M will need to be collected in order to assess the importance of M to the poultry industry.

KUBENA *et al.* (1997) evaluated the individual and combined effects of feeding diets containing 100 mg moniliformin (M) and 3.5 mg aflatoxins (AF)/kg of diet in male broiler chicks from day of hatch to 3 wk of age. When compared with controls, BW gains were reduced 29% by M, 13% by AF, and 33% by the M and AF combination. The efficiency of feed utilization was adversely affected by M independent of AF. Feeding M resulted in decreased relative weights of the bursa of Fabricius and increased relative weights of the heart, increased serum concentrations of creatinine and calcium, increased activities of alkaline phosphatase and alanine aminotransferase, and changes in hematological values. Feeding AF resulted in increased relative weights of the kidney and heart, decreased serum concentrations of total protein, albumin, cholesterol, and calcium, and decreased mean corpuscular volume. Feeding the combination of M and AF resulted in increased relative weights of the heart, decreased serum concentrations of total protein, albumin, and inorganic phosphorus, increased concentrations of creatinine and activity of alanine aminotransferase, and changes in hematological values. Results indicate additive or less than additive toxicity, but not toxic synergy, for most parameters when chicks are fed diets containing the combination of 100 mg M and 3.5 mg AF/kg of diet. The likelihood of encountering these high concentrations of these mycotoxins in finished feed is small; however, additional data on the naturally occurring concentrations of M are necessary before the importance of this mycotoxin to the poultry industry can be assessed. (Key words: moniliformin, aflatoxin, mycotoxin, toxicity, broiler)

Reams et al. (1997) induced a sudden death syndrome in chicks and poults fed diets containing Fusarium fujikuroi, formulated to contain 0-330 mg/kg moniliformin (M) with or without the maximum recommended therapeutic concentration of monensin. Lesions of monensin toxicosis were not observed. Clinical signs were referable to cardiac dysfunction (sudden death, dyspnea, cyanosis, depression). Poults and chicks dying early in the study had no gross lesions or had lesions of right ventricular dilation. Treated poults and chicks dying late in the study or euthanatized at termination of the study had lesions of bilateral myocardial hypertrophy, usually concentric. Absolute heart weights and relative heart weights, expressed as a percentage of body weight, were significantly greater in treated birds than controls ($P < 0.05$), whereas body weights were significantly less ($P < 0.05$). Microscopically, lesions progressed from acute myocardial degeneration to necrosis, fibrosis, and hypertrophy. Ultrastructural findings were consistent with the gross and microscopic lesions. Serum pyruvate concentrations were a useful indicator of M-induced cardiotoxicosis. Concentrations of serum pyruvate increased with increased concentration of dietary M, but were not affected by addition of monensin to the diet. In chicks ingesting 40-300 mg/kg M, serum pyruvate concentrations were significantly greater ($P > 0.05$) than those in controls (controls, 0.28 +/- 0.08 mmol/liter; exposed 0.38 +/- 0.11-0.55 +/- 0.13 mmol/liter). Poults ingesting 80-330 mg/kg M had significantly greater serum pyruvate concentrations than controls (controls 0.33 +/- 0.09 mmol/liter; exposed 0.43 +/- 0.13-1.00 +/- 0.006 mmol/liter). The Vetronics System was used to evaluate electrocardiographic alterations in a limited number of chicks and poults surviving to the end of the feeding trial. Electrocardiographic alterations in poults and chicks fed diets containing > or = 40 mg/kg and > or = 160 mg/kg M, respectively, were consistent with ventricular hypertrophy, myocardial injury, and hypoxia. Electrocardiographic alterations were

more striking in poults than in chicks. Altered myocardial metabolism due to M toxicosis, in conjunction with the unusual susceptibility of domestic poultry to altered cardiac metabolism, is believed to be the cause of the organ-specific lesions in these birds. These findings suggest that cardiac injury with subsequent alterations in cardiac electrical conductance may be a cause of the sudden deaths observed in poultry chronically intoxicated with dietary M.

Vesonder and Wu (1998) fermented 5 isolates of Fusarium moniliforme and two isolates Fusarium proliferatum of the Section Liseola on rice for 21 d at 25 C. Each Fusarium-fermented rice, when dried and mixed into a poultry diet (10% by weight), caused a varied degree of acute mortality in baby Pekin ducklings. The acute (death in less than 48 h) mortality correlated significantly only to the amount of moniliformin in fermented rice, thus in the diet, but not to the amount of fumonisin B1 in fermented rice. This correlation of moniliformin concentration and noncorrelation of fumonisin B1 concentrations to acute toxicity were confirmed by duckling assay using diets containing these purified mycotoxins.

Kubena *et al.* (1999) fed, beginning at 24 wk of age, control diets or diets containing 50 or 100 mg/kg moniliformin (M), 100 or 200 mg/kg fumonisin B1 (FB1), or a combination of 50 mg M and 100 mg FB1/kg of diet to White Leghorn laying hens for 420 d. The hens were then fed the control diet for an additional 60 d. At the beginning of the experiment, each treatment consisted of four replicates of six hens. Egg production was reduced by approximately 50% by the end of the second 28-d laying period and remained at approximately this level for the 420 d in only the hens fed the diet containing 100 mg M/kg feed. Production returned to control levels or above within 60 d after hens were fed the control diet. Egg weights were reduced by the 100-mg M diet during the first three 28-d laying periods before returning to weights comparable with controls. The hens in this group also had significantly lower body weights than the other treatments. Mortality was minimal except in hens fed the 100 mg M/kg diet and the 100 mg FB1/kg diet, on which approximately 20% of the hens died. The hens were artificially inseminated with semen from males fed control diets, and fertility was not affected by the dietary treatments. Importantly, toxic synergy between M and FB1 was not observed for any of the parameters measured. Results indicate that laying hens may be able to tolerate relatively high concentrations of M and FB1 for long periods of time without adversely affecting health and performance. Interestingly, hens fed the 100-mg M/kg diet were able to recover when returned to control diets. The likelihood of encountering M or FB1 at these concentrations in finished feed is small.

Morris *et al.* (1999) evaluated the effects of feeding diets containing either 20 mg deoxynivalenol (DON)/kg, 100 mg moniliformin (M)/kg, or a combination of DON and M (20 mg/kg DON and 100 mg M/kg) in growing turkey poults, from 1 to 21 d of age. Feed intake and BW gains were decreased ($P < 0.05$) by dietary treatments containing M. Feed conversion was not affected by any of the dietary treatments, and no interactive effects on performance were evident between M and DON. Absolute weights of hearts and kidneys were increased ($P < 0.05$) in poults fed diets containing M. Mean cell volume was decreased by the M and DON-M treatments; however, the decrease was much smaller in poults fed the combination DON-M treatment resulting in a significant ($P < 0.05$) DON by M interaction. Mean cell hemoglobin and mean cell hemoglobin concentrations were not affected by any of the dietary treatments. No histological lesions were seen in control poults or poults fed DON alone. Lesions associated with dietary treatments were only observed in the heart and kidney. Poults

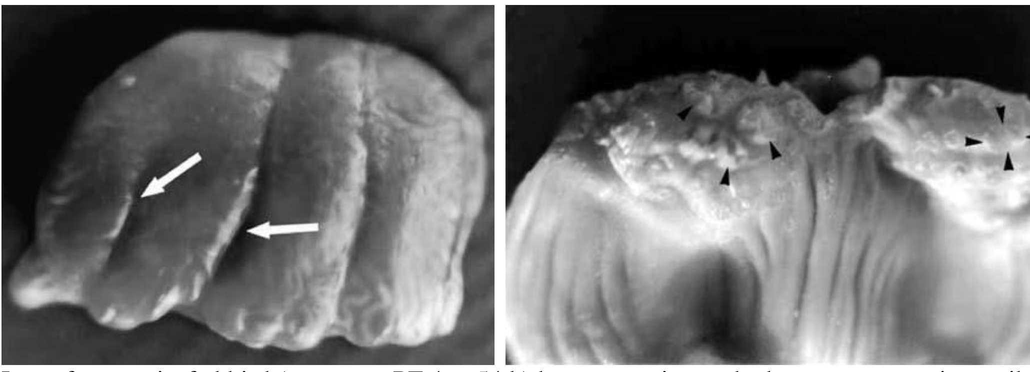
fed diets containing M alone or the DON-M combination exhibited an increased incidence of variable sized cardiomyocyte nuclei, with numerous large giant nuclei, and a generalized loss of cardiomyocyte cross striations. Isolated renal tubules in sections of kidney were noted to have mild diffuse mineralization in poults fed M and the combination DON-M treatments. None of the response variables measured were affected by DON alone. No toxic synergy was observed when these toxins were fed simultaneously to turkey poults for 21 d.

Li et *al.* (2000) evaluated effects of feeding diets containing fumonisin B1 (FB1) and moniliformin (M), singly or in combination, on performance and immune response in poults. Day-old poults were randomly assigned to one of four dietary treatments with four replicates of four poults each. Dietary treatments were 1) control; 2) 200 mg FB1, 0 mg M/kg diet; 3) 0 mg FB1, 100 mg M/kg diet; and 4) 200 mg FB1, 100 mg M/kg diet. In Experiment 1, poults were injected with 0.25 mL Newcastle disease virus (NDV) vaccine on Weeks 2 and 3 of the experiment, and anti-NDV antibody titers were measured 7 d after each injection. Compared with controls, poults fed FB1 had significantly lower ($P < 0.05$) secondary antibody response. Poults fed M and the combination of FB1 and M had significantly lower ($P < 0.05$) primary and secondary antibody response. Lower relative thymus weights were observed in poults fed diets containing FB1 or M. De-creased relative bursa and spleen weights were observed in poults fed M. In Experiment 2, poults were placed on dietary treatments for 3 wk. On Day 21, 2×106 peripheral lymphocytes were incubated with mitogens. Poults fed diets containing FB1 had a significantly lower ($P < 0.05$) proliferative response to mitogens in comparison to controls. In Experiment 3, poults were placed on the diets for 3 wk and were injected with 4.4×107 E. coli/kg body weight on Day 21. Significantly higher ($P < 0.05$) numbers of E. coli colonies were observed in the blood and tissue homogenates of poults fed M. In all three experiments, feed intake and body weight gains were significantly lower ($P < 0.05$) in turkeys fed diets containing M. Data from the present study suggest that FB1 and M are immunosuppressive in poults and that M not only suppresses immune response but also performance. However, neither synergistic nor additive effects between FB1 and M were observed for any of the parameters measured.

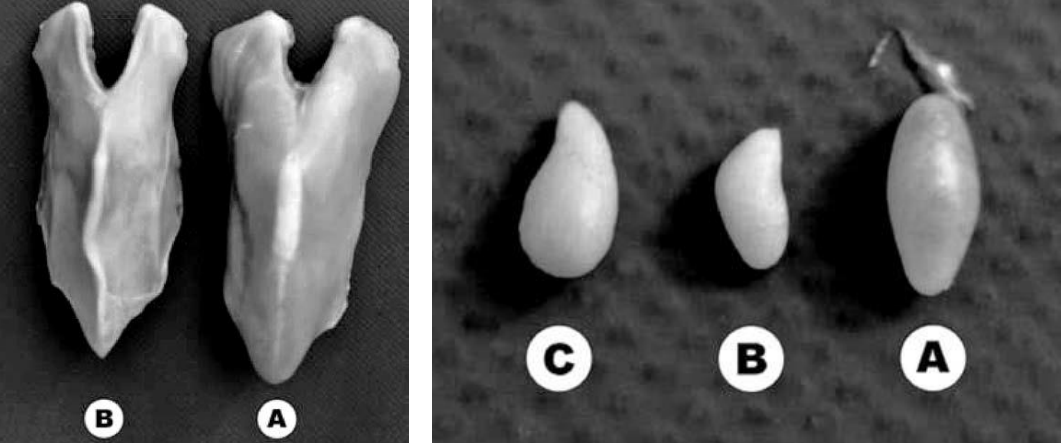
Dombrink-Kurtzman (2003) exposed turkey peripheral blood lymphocytes in vitro for 72 hours to fumonisin B1 (FB1), fumonisin B2 (FB2), hydrolyzed fumonisin B1 (HFB1), moniliformin and tricarballylic acid (TCA) (0.01-25 microg/ml). A decrease in cell proliferation, as determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] bioassay, occurred in the order: FB2 > FB1 > HFB1, with IC50 = 0.6 microM, 1 microM and 10 microM, respectively. Internucleosomal DNA fragmentation and morphological features characteristic of apoptosis were observed following exposure to fumonisin B1 and beauvericin; cytoplasmic condensation and membrane blebbing were seen by light microscopy. Tricarballylic acid and moniliformin did not interfere with cell proliferation. Results suggested that fumonisin B1 and beauvericin may affect immune functions by suppressing proliferation and inducing apoptosis of lymphocytes.

Javed *et al.* (2005) gave feed amended with autoclaved culture material (CM) of Fusarium proliferatum containing fumonisin B1 (FB1) (61-546 ppm), fumonisin B2 (FB2) (14-98 ppm) and moniliformin (66-367 ppm) to 228 male chicks in three separate feeding trials. In a fourth feeding trial, purified FB1 (125 and 274 ppm) and moniliformin (27 and 154 ppm) were given separately and in combination (137 and 77 ppm, respectively). Chicks that died during the trial periods, survivors and controls

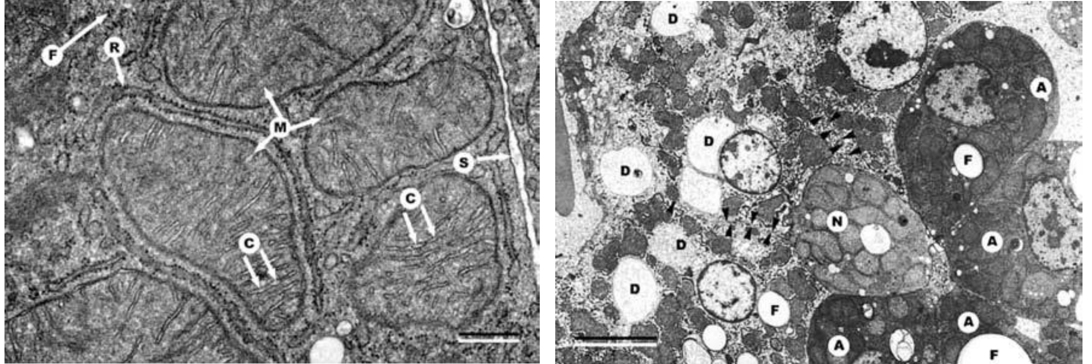
were subjected to postmortem examination. Specimens (liver, kidney, pancreas, lung, brain, intestine, testis, bursa of Fabricius, heart and skeletal muscle) were examined grossly and preserved for subsequent histopathologic and ultrastructural examination. Prominent gross lesions in affected birds fed diets amended with CM or purified FB1 and moniliformin included ascites, hydropericardium, hepatopathy, nephropathy, cardiomyopathy, pneumonitis, gizzard ulceration, and enlarged bursa of Fabricius filled with caseous material. The various concentrations of FB1 and moniliformin in the amended rations produced well-defined dose-response lesions in all groups in all four trials. Histopathologic changes included hemorrhage, leucocytic infiltration, fatty change or infiltration, individual cell necrosis and fibrosis in liver, kidneys, lungs, heart, intestines, gizzard, bursa of Fabricius and pancreas. Edema and hemorrhage were prominent in brains of treated birds. Ultrastructural changes included cytoplasmic and nuclear enlargement of cells in affected liver, lungs, kidneys, heart and pancreas. There were thickened membranes of the smooth endoplasmic reticulum, dilation of the rough endoplasmic reticulum with loss of ribosomes and vacuolated or deformed mitochondria.



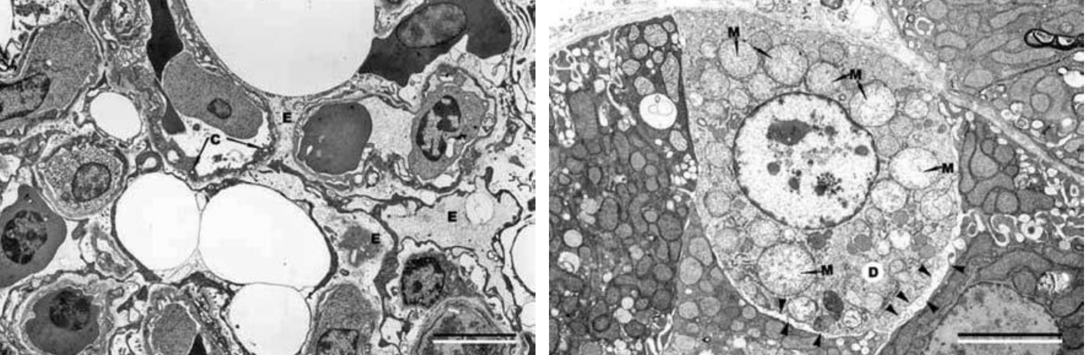
Lung from toxin-fed bird (treatment PF 4 at 54 h) has congestion and edema; note prominent rib impressions ("). Gizzard from toxin-fed bird (treatment T4 at 60 h) **Javed *et al.* (2005)**



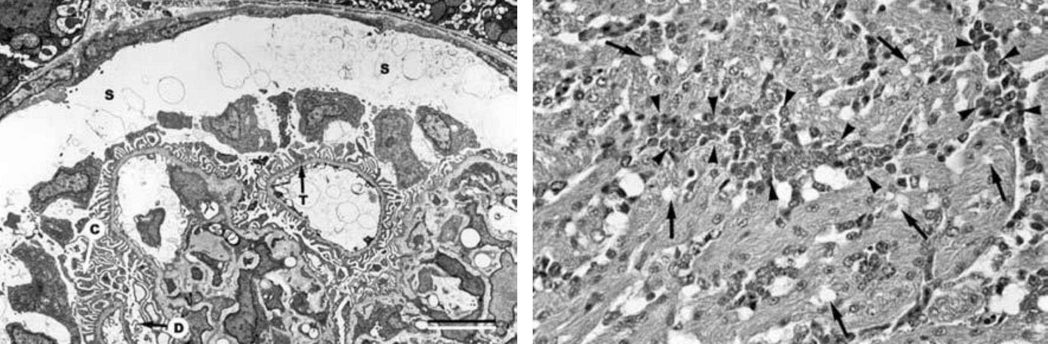
Breast of control bird (A) has straight keel bone and well-developed muscles. Breast of toxin-fed bird (B) (treatment T2 at 60 h) has markedly wavy keel bone and underdeveloped muscles., Normal testis from control bird (A) is compared to small testis (B) and misshapen, pedunculated testis (C) from toxin-fed birds (treatment T3 at 60 h). **Javed *et al.* (2005)**



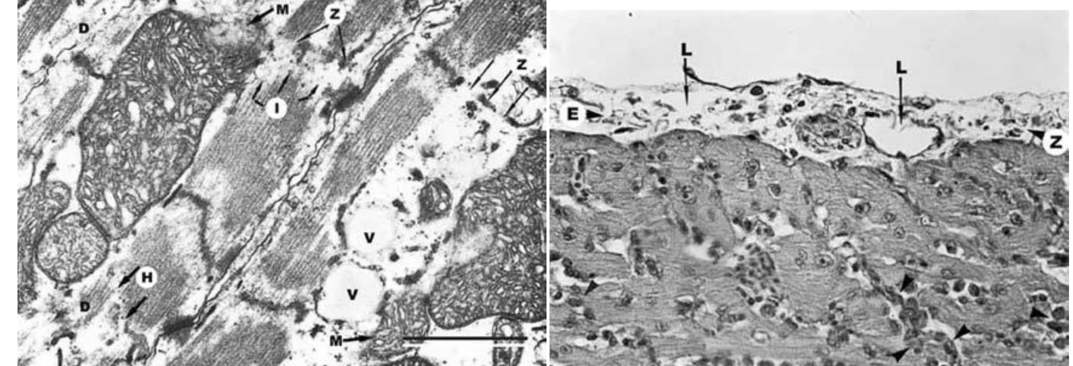
Electron micrograph of hepatocyte from toxin-fed bird (treatment T4 at 77 h) bordered by variably widened intercellular space (S) and containing mitochondria (M) with cristae (C) of slightly variable width, RER (R) with fewer attached ribosomes and increased number of free cytosolic ribosomes (F). Bar = 0.5 lm. **Javed *et al.* (2005)**



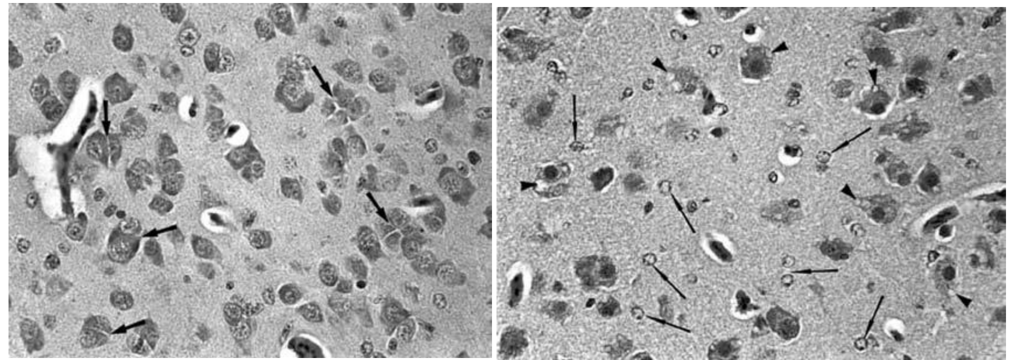
Electron micrograph of lung from toxin-fed bird (treatment T2 at 138 h) showing cytovacuolation of endothelial cells (C) and marked intercellular edema (E). Bar = 5 lm. Electron micrograph of tubular epithelial cells from toxin-fed bird (treatment T2 at 173 h) showing degenerative cell (D) with swollen rounded mitochondria (M), containing electron lucent matrix, fewer cristae, and irregular, widened intercellular spaces (m). Bar = 4 lm. **Javed *et al.* (2005)**



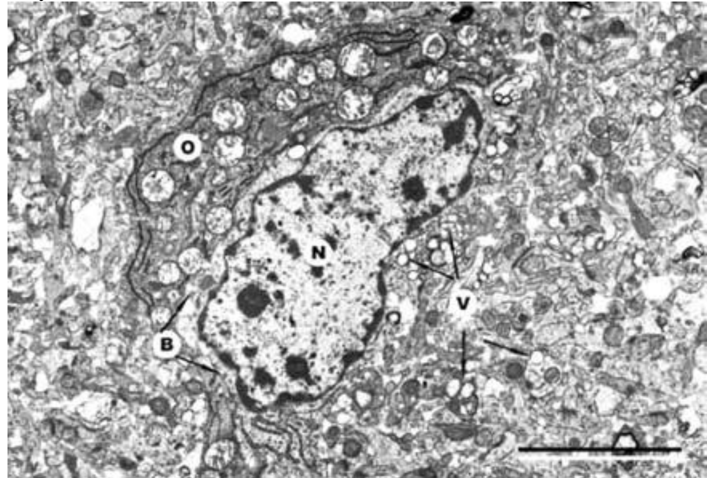
Electron micrograph of glomerular tuft from toxinfed bird (treatment T2 at 173 h) showing widened Bowman's space (S), and detached (D), thickened (T), elongated (C) and misshapen podocyte foot processes. Bar = 5 lm Heart from toxin-fed bird (treatment T2 at 3 weeks) has myofiber vacuolation ("), disorientation, fragmentation and infiltrate of heterophils, macrophages and lymphocytes (m). H&E stain, 400·.**Javed *et al.* (2005)**.



Electron micrograph of heart from toxin-fed bird (treatment T2 at 173 h) showing detachment and dissolution of myofibrils (D) with vacuolation of sarcoplasm (V), destruction of Z (Z), I (I) and H (H) bands, and mitochondrial swelling and membrane dissolution (M). Bar = 1 lm. Heart from toxin-fed bird (treatment T4 at 77 h) shows epicardium thickened by edema fluid (E) and containing dilated lymphatic channels (L), macrophages and lymphocytes and destruction of Z (Z) bands; the myocardium contains foci of heterophils and macrophages (m). H&E stain, 400·.**Javed *et al.* (2005)**



Brain from toxin-fed bird (treatment T2 at 1 week shows satellitosis ("). H&E stain, 400·.Brain from toxin-fed bird (treatment T2 at 1 week) has neuronal degeneration, reduced cellularity, astrocyte proliferation (") and cytovacuolation (m). H&E stain, 400·.**Javed *et al.* (2005)**



Electron micrograph of brain from toxin-fed bird (treatment T2 at 3 weeks) showing satellitosis with oligodendrocyte (O) adjacent to neuronal cell; note cytovacuolation (V), mitochrondrial degeneration, cytoplasm (B) and nucleus (N). Bar = 4 lm. **Javed *et al.* (2005)**

Labuda *et al.* (2005) analyzed a total of 50 samples of poultry feed mixtures of Slovak origin for fumonisin B(1) and B(2) (FB(1), FB(2)) and moniliformin (MON) using SAX-clean up procedure being detected by high pressure liquid chromatography with mass spectrometry (HPLC-MS) and diode array detection (HPLC-DAD), respectively. The samples were also simultaneously investigated

for Fusarium species occurrence, and for the capability of Fusarium isolates recovered to produce FB(1) and MON in vitro. FB1 was detected in 49 samples (98 %) in concentrations ranging from 43 to 798 microg x kg(-1), and FB(2) in 42 samples (84 %) in concentrations ranging from 26 to 362 microg x kg(-1). MON was detected in 26 samples (52 %) in concentrations that ranged from 42 to 1,214 microg x kg(-1). Only two Fusarium populations were encountered, namely F. proliferatum and F. subglutinans, of which the former was the most dominant and frequent. All 86 F. proliferatum isolates tested for FB1-production ability proved to be producers of the toxin although none of them produced MON. On the contrary, MON production was observed in a half out of 16 F. subglutinans isolates tested, yet no FB1 production was detected in this case. Despite the limited number of samples investigated during this study, it is obvious that poultry feed mixtures may represent a risk from a toxicological point of view and should be regarded as a potential source of the Fusarium mycotoxins in central Europe

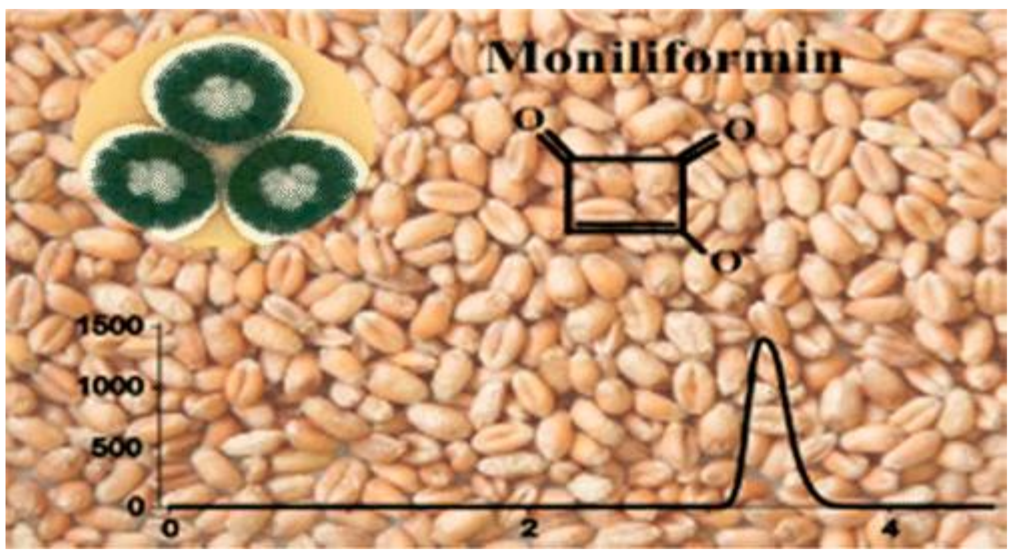
WANG *et al*. (2007) isolated Fusarium moniliform from the corn in a forage factory of Guangdong. F. moniliform was cultured in the common corn flour medium. The cu ltural condition was 25 ℃ for 10 d, then 4 ℃ for 7 d, finally 25 ℃ for 10 d. Monilifor min)was extracted by acetonitrile-water(95:5). MON were demonstrated by TLC and quantitatived by HPLC. Chickens were fed diets containing 23 mg/kg MON in poison ing assay. Thymus, bursa of Fabricius and spleen indexes and serum content of nitric oxide were determined. The results showed that compared with the control group, the thymus, bursa of Fabricius and spleen indexes in detected chickens were significantly lower(P0.05), and the serum content of nitric oxide was distinctively higher(P0.01).

Sharma *et a*l. (2008) studied the individual and combined effects of fumonisin B_1 and moniliformin on clinicopathological and cell-mediated immune response in Japanese quail. A total of 390 one-day-old quail chicks (Coturnix coturnix japonica) were divided into 4 groups (3 replicates per treatment), viz. CX, FX, MX, and FM, containing 75, 105, 105, and 105 birds, respectively. Birds in the control group (CX) were fed quail mash alone, whereas birds in group FX were fed 200 ppm of fumonisin B(1) (FB(1)) from Fusarium verticillioides culture material; group MX was fed 100 ppm of moniliformin (M) from Fusarium fujikuroi culture material; and group FM was fed a combination of 200 ppm of FB(1) and 100 ppm of M. Diets were fed from d 1 to 35 to study clinical signs, growth response, serum biochemical changes, and cell-mediated immune response. Birds fed FB(1) (FX) showed ruffled feathers and poor growth. Birds in group MX appeared more stunted than those in group FX and exhibited signs of poor feathering and decreased feed and water intake. Clinical signs observed in group FM were more or less similar to those observed in groups FX and MX. Total mortality was 12.38, 7.62, and 20.95% for groups FX, MX, and FM, respectively. Mean BW in groups FX, MX, and FM were significantly lower than those in the control group (CX) at almost all intervals. Total serum proteins, albumin, cholesterol, aspartate transaminase, lactate dehydrogenase, and creatine kinase values were higher in all treatment groups compared with the control group. Cell-mediated immune response was more or less comparable in groups CX and MX, whereas the presence of FB(1) in the diet of groups FX and FM was found to be associated with a gradual increase in skin thickness, and the mononuclear inflammatory cell response was poor as compared with groups CX and MX throughout the study. Except for mortality (additive effect) and serum aspartate transaminase values (less than an additive effect up to 14 DPF), no additive or synergistic effects were observed for any

of the other response variables measured in the current study, where all statistical differences were attributed to either one mycotoxin or the other.

Sharma *et al.* (2012) examined the effects of fumonisin B_1 (FB_1) and moniliformin (M) on the heart of Japanese quail (*Coturnix coturnix japonica*). Three hundred and ninety day-old Japanese quail were randomly divided into four groups: 1) FB_1 alone (FX), 2) M alone (MX), 3) FB_1 and M (FM), and 4) chick mash alone (CX). We used three pen replicates of 35 quail per pen in groups FX, MX, and FM and three pen replicates of 25 quail per pen in group CX. Gross and microscopic changes in the heart were studied in nine birds (three birds per replicate) from each group at weekly intervals up to 28 days postfeeding (DPF). Ultrastructural changes were studied in the heart of three birds (one bird per replicate) from each group at 21 DPF. Thinning of the heart was the only significant gross lesion in group FX. In contrast, mild-to-severe cardiomegaly was a significant finding in groups MX and FM throughout the study. Microscopically, thinning of cardiomyocytes was evident at 7 DPF in group FX. In addition to the hypertrophy of cardiomyocytes evident as early as 7 DPF, myocardial karyomegaly, nuclear hyperchromasia, and myofibril disarray exhibiting a wavy pattern were more pronounced at 28 DPF in group MX. Similar but more severe lesions were observed in the FM combination group that included myocardial hemorrhages, vacuolar changes, hypertrophy of cardiomyocytes, focal myocarditis, and loss of myofibrils cross-striations. Via transmission electron microscopy, the maximum effect of FB_1 toxicity was observed on mitochondria. In addition to an increase in the number of mitochondria, the mitochondria seemed invariably swollen and pleomorphic, although the outer membrane was intact, and the membrane cristae were usually distinct. Myofibrils seemed thinner, without much disruption in their architecture. Large numbers of vacuolar bodies of irregular size, both in the sarcoplasm and in between the myofibrils, were conspicuous in group FX. In contrast to group FX, the increase in number of mitochondria resulted in widespread separation of muscle fibers in group MX. In addition, the mitochondria were swollen and varied from round to oval to slightly elongated and occasionally forked, and vacuolation was rarely noticed in group MX. In the FM combination group, a significant increase in the number of mitochondria caused muscle fibers to look much thinner and assume a wavy pattern. We conclude that the effect of M on the heart is exaggerated in the presence of FB_1. Although the overall interactive effect of FB_1 and M was less than additive, the interactive effects between the two toxins for cardiac lesions were greater than additive to synergistic up to the second week, raising serious concerns on early age exposure to a combination of these two mycotoxins.

Hallas-Mølle *et al.* (2016) reported for the first time that moniliformin can be produced by the cereal fungus, *Penicillium melanoconidium* (4 out of 4 strains), but not in the related species in the *Viridicata* series. Moniliformin was detected in 10 out of 11 media: two agars and several cereal and bean types. Moniliformin was identified by a novel mixed-mode anionic exchange reversed phase chromatographic method which was coupled to both tandem mass spectrometry (MS) and high resolution MS. Mixed-mode chromatography showed superior peak shape compared to that of HILIC and less matrix interference compared to that of reversed phase chromatography, but during a large series of analyses, the column was fouled by matrix interferences. Wheat and beans were artificially infected by *P. melanoconidium* containing up to 64 and 11 mg/kg moniliformin, respectively, while penicillic acid, roquefortine C, and penitrem A levels in wheat were up to 1095, 38, and 119 mg/kg, respectively.



Hallas-Mølle *et al*. (2016)

References

1. Allen, N. K., H. R. Burmeister, G. A. Weaver, and C. J. Mirocha, 1981. Toxicity of dietary and intravenously administered moniliformin to broiler chickens. Poultry Sci. 60:1415–1417.
2. Bermudez AJ, Ledoux DR, Rottinghaus GE, Bennett GA. The individual and combined effects of the Fusarium mycotoxins moniliformin and fumonisin B1 in turkeys. Avian Dis. 1997 Apr-Jun;41(2):304-11.
3. Bermudez AJ, Ledoux DR, Turk JR, Rottinghaus GE. The chronic effects of Fusarium moniliforme culture material, containing known levels of fumonisin B1, in turkeys. Avian Dis. 1996 Jan-Mar;40(1):231-5.
4. Bermudez AJ, Ledoux DR, Rottinghaus GE. Effects of Fusarium moniliforme culture material containing known levels of fumonisin B1 in ducklings. Avian Dis. 1995 Oct-Dec;39(4):879-86.
5. Burmeister, H. R., A. Ciegler, and R. F. Vesonder, 1979. Moniliformin, a metabolite of Fusarium moniliforme NRRL 6322: Purification and toxicity. Appl. Environ. Microbiol. 37:11–13.
6. Dombrink-Kurtzman MA, Javed T, Bennett GA, Richard JL, Cote LM, Buck WB. Lymphocyte cytotoxicity and erythrocytic abnormalities induced in broiler chicks by fumonisins B1 and B2 and moniliformin from Fusarium proliferatum. Mycopathologia. 1993 Oct;124(1):47-54.
7. Dombrink-Kurtzman MA. Fumonisin and beauvericin induce apoptosis in turkey peripheral blood lymphocytes. Mycopathologia. 2003;156(4):357-64.
8. Engelhardt, J.A., Carlton, W.W. & Tuite, J.F. (1989). Toxicity oí Fusarium moniliforme var. subglutinans for chicks, ducklings, and turkey poults. Avian Diseases, 33, 357-360
9. Hallas-Mølle, Magnus Hallas-Møller, Kristian Fog Nielsen, and Jens Christian Frisvad* Production of the *Fusarium* Mycotoxin Moniliformin by *Penicillium melanoconidium. J. Agric. Food Chem.*, 2016, 64 (22), pp 4505–4510
10. Harvey, R. B., L. F. Kubena, G. E. Rottinghaus, J. R. Turk, H. H. Casper, and S. A. Buckley. 1997. Moniliformin from Fusarium fujikuroi culture material and

deoxynivalenol from naturally contaminated wheat incorporated into diets of broiler chicks. Avian Dis. 41:957–963

11. Javed, T., G. A. Bennett, J. L. Richard, M. A. DombrinkKurtzman, L. M. Coˆte, and W. B. Buck, 1993. Mortality in broiler chicks on feed amended with Fusarium proliferatum culture material or with purified B1, and moniliformin. Mycopathologia 123:171–184
12. Javed T, Bunte RM, Dombrink-Kurtzman MA, Richard JL, Bennett GA, Côté LM, Buck WB. Comparative pathologic changes in broiler chicks on feed amended with Fusarium proliferatum culture material or purified fumonisin B1 and moniliformin. Mycopathologia. 2005 Jun;159(4):553-64.
13. Kriek, N.P.J., W.F.O. Marasas, P. S. Steyn, S. J. van Rensburg, and M. Steyn, 1977. Toxicity of a moniliformin-producing strain of Fusarium moniliforme var. subglutinans isolated from maize. Food Cosmet. Toxicol. 15:579–587.
14. Kubena LF, Edrington TS, Kamps-Holtzapple C, Harvey RB, Elissalde MH, Rottinghaus GE. Influence of fumonisin B1, present in Fusarium moniliforme culture material, and T-2 toxin on turkey poults. Poult Sci. 1995a Feb;74(2):306-13.
15. Kubena LF, Edrington TS, Kamps-Holtzapple C, Harvey RB, Elissalde MH, Rottinghaus GE. Effects of feeding fumonisin B1 present in Fusarium moniliforme culture material and aflatoxin singly and in combination to turkey poults. Poult Sci. 1995b Aug;74(8):1295-303.
16. Kubena LF, Edrington TS, Harvey RB, Buckley SA, Phillips TD, Rottinghaus GE, Casper HH. Individual and combined effects of fumonisin B1 present in Fusarium moniliforme culture material and T-2 toxin or deoxynivalenol in broiler chicks. Poult Sci. 1997 Sep;76(9):1239-47.
17. Kubena LF, Harvey RB, Buckley SA, Bailey RH, Rottinghaus GE. Effects of long-term feeding of diets containing moniliformin, supplied by Fusarium fujikuroi culture material, and fumonisin, supplied by Fusarium moniliforme culture material, to laying hens. Poult Sci. 1999 Nov;78(11):1499-505.
18. KUBENA, L. F., R. B. HARVEY, S. A. BUCKLEY, T. S. EDRINGTON, and G. E. ROTTINGHAUS Individual and Combined Effects of Moniliformin Present in Fusarium fujikuroi Culture Material and Aflatoxin in Broiler Chicks Poultry Science 76:265–270, 1997
19. Labuda, Roman , Alexandra Parich , Elisavet Vekiru. INCIDENCE OF FUMONISINS, MONILIFORMIN AND FUSARIUM SPECIES IN POULTRY FEED MIXTURES FROM SLOVAKIA. Ann Agric Environ Med 2005, 12, 81–86
20. Ledoux, D. R., A. J. Bermudez, and G. E. Rottinghaus, 1993. Effects of Fusarium fujikuroi culture material containing known levels of moniliformin on turkey poults. Poultry Sci. 72(Suppl. 1):68. (Abstr.)
21. Ledoux, D. R., A. J. Bermudez, G. E. Rottinghaus, and J. Broomhead, 1995. Effects of feeding Fusarium fujikuroi culture material, containing known levels of moniliformin, in young broiler chicks. Poultry Sci. 74:297–305.
22. Leslie, J F , W F Marasas, G S Shephard, E W Sydenham, S Stockenström, and P G Thiel. Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and E mating populations of Gibberella fujikuroi (Fusarium moniliforme). Appl Environ Microbiol. 1996 Apr; 62(4): 1182–1187.
23. Li,Y. C., D. R. Ledoux, A. J. Bermudez, K. L. Fritsche, and G. E. Rottinghaus. The Individual and Combined Effects of Fumonisin B1 and Moniliformin on Performance and Selected Immune Parameters in Turkey Poults. Poultry Science 79:871–878, 2000
24. MORRIS, C.M., Y.C. LI, D.R. LEDOUX, A.J. BERMUDEZ, and G.E. ROTTINGHAUS (1999): The individual and combined effects of feeding moniliformin, supplied by *Fusarium* fujikuroi culture material, and deoxynivalenol in young turkey poults. Poultry Science. 78, 1110-1115)
25. Sharma, D., R. K. Asrani, D. R. Ledoux, N. Jindal, G. E. Rottinghaus, and V. K. Gupta. 2008.Individual and combined effects of fumonisin B_1 and moniliformin on

clinicopathological and cell-mediated immune response in Japanese quail. *Poult. Sci.* 87:1039–1051.

26. Nagaraj RY[1], Wu W, Will JA, Vesonder RF. Acute cardiotoxicity of moniliformin in broiler chickens as measured by electrocardiography. Avian Dis. 1996 Jan-Mar;40(1):223-7.
27. Qureshi MA, Garlich JD, Hagler WM Jr, Weinstock D. Fusarium proliferatum culture material alters several production and immune performance parameters in White Leghorn chickens. Immunopharmacol Immunotoxicol. 1995 Nov;17(4):791-804
28. Reams, R. Y., H. L. Thacker, D. D. Harrington, M. N. Novilla, G. E. Rottinghaus, G. A. Bennett, and J. Horn.A sudden death syndrome induced in poults and chicks fed diets containing *Fusarium fujikuroi* with known concentrations of moniliformin. *Avian Dis.* 41:20–35. 1997.
29. Sharma, D., R. K. Asrani, D. R. Ledoux, N. Jindal, G. E. Rottinghaus, and V. K. Gupta. 2008.Individual and combined effects of fumonisin B_1 and moniliformin on clinicopathological and cell-mediated immune response in Japanese quail. *Poult. Sci.* 87:1039–1051
30. Sharma, R. K. Asrani, D. R. Ledoux, G. E. Rottinghaus, and V. K. Gupta Toxic Interaction Between Fumonisin B_1 and Moniliformin for Cardiac Lesions in Japanese Deepa Quail. Avian Diseases / Sep 2012 / pg(s) 545-554
31. Thiel, P. G., C. J. Meyer, and W.F.O. Marasas, 1982. Natural occurrence of moniliformin together with deoxynivalenol and zearalenone in Transkeian corn. J. Agric. Food Chem. 30:308–312
32. Vesonder RF, Wu W. Correlation of moniliformin, but not fumonisin B1 levels, in culture materials of Fusarium isolates to acute death in ducklings. Poult Sci. 1998 Jan;77(1):67-72
33. WANG Kai, YANG Li-mei, HE Yong-ming, PU Wen-jun, LU Min-jie , Culture and extraction of moniliformin and immunopathological damages to chickens. Animal Husbandry & Veterinary Medicine 2007-09
34. Weibking, T., D. R. Ledoux, A. J. Bermudez, J. R. Turk, and G. E. Rottinghaus. Effects on turkey poults of feeding *Fusarium moniliforme* M-1325 culture material grown under different environmental conditions. *Avian Dis.* 39:32–38. 1995
35. Wu W, Liu T, Vesonder RF. Comparative cytotoxicity of fumonisin B1 and moniliformin in chicken primary cell cultures. Mycopathologia. 1995 Nov;132(2):111-6.

4.7.4. T-2 toxicosis

The mycotoxin T-2 is considered the most acutely toxic member of the family of the trichothecenes and exposure can occur through different routes (**Sokolovic *et al.*, 2008).**

- The avian species are considered as resistant to their toxic effects, partly because of low absorption and rapid elimination, thereby reducing the risk of persistence of residues in tissues destined for human consumption. All the data reviewed suggest that the toxicokinetics of fusariotoxins in avian species differs from those in mammals, and that variations among the avian species themselves should be assessed **(Guerre, 2015)**.

Natural occurrence in grain and feed

- Many cereal grains like wheat, corn, barley and oats are susceptible to T-2 toxin contamination and numerous studies detected T-2 as well as HT-2 toxin in different agricultural commodities [**Schothorst and van Egmond, 2004**].
- Besides the co-occurrence of HT-2 toxin in T-2 toxin contaminated grains, HT-2 toxin is furthermore described as the main metabolite after T-2 toxin application in different *in vivo* as well as *in vitro* studies [**Li** *et al.*, 2011]
- For this reason the panel on contaminants in the food chain of the European Food Safety Authority has set the tolerable daily intake for the sum of both, T-2 and HT-2 toxin, at 100 ng/kg body weight [**EFSA, 2011**)
- Warm and moist weather conditions favour plant infection with *Fusarium* spp., while improper storage and handling of grain with high moisture content can lead to T-2 toxin contamination (19, 20).
- The most important factors that influence T-2 toxin production are weather conditions, grain defects and moisture content (13 % to 22 %). T-2 toxin is produced at a wide temperature range (0 °C to 32 °C), with maximum production at temperatures below 15 °C (**Mateo et al., 2002**).

Chemical structure

- T-2 toxin is a non-volatile, low-molecular-weight compound (MW 466.52) insoluble in water and petroleum ether, but highly soluble in acetone, ethyl-acetate, chloroform, dimethyl sulphoxide, ethyl alcohol, methyl alcohol and propylene glycol **(Betina, 1984).**
- It is highly resistant to heat and UV light (**Shepard, 1988).**
- Therefore, it is not inactivated in food production and processing or by autoclaving. T-2 toxin is inactivated by heating at 200 °C to 210 °C for 30 min to 40 min, or by soaking in sodium hypochlorite - sodium hydroxidesolution for at least four hours (**Wannemacher Wiener, 1997**).
- Some bacteria and moulds have the ability to transform and detoxify T-2 toxin (**Jesenska and Sajbidorova, 1991**).

R = Ac T-2 toxin
R = H HT-2 toxin

Chemical structure of T-2 and HT-2 toxin.

- **The LD50** of T-2 is 6.3 mg/kg BW in broiler chickens (**Chi et al., 1977b**).
- **A wide range of toxic effects can be caused by chronic exposure to T-2 in animals:**
    - o Weight loss, emesis, diarrhea, lesions in liver and digestive system (**Li *et al.,* 2011).**
    - o Especially in chickens reduced egg production, impaired egg hatch and feather alterations are other symptoms of chronic exposure to T-2 **(Wyatt** *et al.,* **1975, Diaz *et al.,* 1994).**
    - o T-2 causes damage of the blood-brain barrier and causes changes in the activity of serotonine which explains the reduced feed intake (**Wang *et al.*, 1998).**
    - o An increase in brain indoleamines, e.g. serotonin, induced by T-2 can contribute to feed refusal (**MacDonald *et al.,* 1988**).
    - o T-2 causes lesions in the oral cavity which can also be a factor responsible for a decreased feed intake (**Wyatt *et al.,* 1973**).
    - o One single dose of 5 mg/kg T-2 or feeding at concentrations of 1 to 5 mg/kg T-2 for at least one week, are necessary to cause lesions in the mouth (**Sokolovic *et al.,* 2008).**.

- Broilers exposed to a relatively small amount of T-2 toxin (2 ppm) showed negative consequences of T-2 toxin in all examined organs as degenerative changes developed in small intestine mucosa, enterocites and hepatocites necroses, as well as lymphocites depletion in bursa of Fabricius (**Nesic *et al.*, 2009**).

- T-2 toxin caused abnormal position of wing, reduction in feed intake and body weight gain. In laying birds it caused delayed maturation of follicles, reduced egg production, shell thickness and hatchability in poultry **(Kurkure and Pande, 2008).**

Mode of action

- T-2 toxin have an affinity for the 60S subunit of ribosomes, which leads to inhibition of the protein synthesis at the initiation, elongation or termination step (**Rocha *et al.,* 2005)**
- T-2 toxin is active at the initiation phase, while DON acts as inhibitor of the elongation and/or termination step (**Awad *et al.*, 2008; Sokolovic et al., 2008**).
- T-2 toxin exerts other effects on eukaryotic cells such as inhibition of the RNA and DNA synthesis as well as adverse effects on the mitochondrial function (**Minervini *et al.,* 2004; Ueno, 1982**).
- T-2 toxin s can also induce apoptosis, a programmed cell death (PCD) response both in vitro and in vivo **(Minervini *et al.,* 2004; Yang et al., 2000)**.
- T-2 is a weak PCD inducer (**Shifrin and Anderson, 1999).**
- The induction of apoptosis may require both translational arrest and mitogen-activated protein kinase (MAPK) activity. MAPK's are components of a signaling cascade that regulate cell survival in response to stress (**Iordanov *et al.,* 1997**).
- T-2 induces apoptosis by activation of c-Jun N-terminal kinases (JNK), p38 and MAPK's, but the precise mechanism has not yet been elucidated (**Sokolovic et al., 2008**).
- The process is also called 'ribotoxic stress response' (**Iordanov *et al.,* 1997**).

- Trichothecenes are able to induce the production of free radicals, causing membrane and DNA damage (**Rizzo *et al.,* 1994; Atroshi *et al.,* 1997; Leal *et al.,* 1999; Vila *et al.,* 2002; Minervini *et al.,* 2005).**
- T-2 can generate higher reactive oxygen species (ROS) levels which lead to DNA damage, activation of p53 and final apoptosis in human cervical cancer cells (**Chaudhari *et al.,* 2009**).
- DNA fragmentation of leukocytes in broilers was observed after exposure to T-2 at a concentration of 13.5 mg/kg feed for 17 days (**Rezar et al., 2007**).
- The same effects were seen in broilers even at a lower concentration of 10 mg/kg T-2 after exposure for 17 days **(Frankic *et al.,* 2006**

Histological alterations have been described after ingestion of different concentrations of trichothecenes.

- More precisely, cells on the tips of the villi are destroyed and crypt epithelium is injured (**Hoerr, 1998).**
- T-2 exposure at a concentration of 0.982 mg/kg for 32 days resulted in shorter villi in the duodenum and shorter and thinner villi in the jejunum of turkey poults (**Sklan *et al.,* 2003).**

T-2 Toxin toxicokinetics

Studies with ^{3}H-T-2 toxin (radiolabelled at the C_3 position) have been conducted in different avian species.

- **In broilers**, ^{3}H-T-2 toxin (64.2 mCi/mmoL) solubilized in aqueous ethanol was administered in the crop of chickens previously fed a diet containing non-radioactive T-2 toxin (**Chi *et al.,* 1978**). After administration of ^{3}H-T-2 toxin,
    - In plasma a rebound of radioactivity was observed 24 h after administration.
    - In tissues, maximum concentration was generally observed 4 h after dosing.
    - Radioactivity in liver was two to three fold higher than in kidney, and was generally three fold higher in muscle tissue than in fat.
    - The bile contained the highest amount of T-2 toxin:
    - The ratio of radioactivity in bile *vs.* plasma was 260 and 837, respectively, 4 h and 12 h after administration.
    - Two days after administration, the concentration in plasma was still around 58% of the maximum observed at 4 h, and 28% of the maximum observed in bile at 12 h. **(Giroir *et al.,* 1991**)
- Radioactivity in hens that received a single dose reached maximum 24 h after administration.

- In multiple-dosed hens, maximum radioactivity was reached on day 3 in the white and in the shell membrane,
- The amounts in the yolk continued to increase throughout the course of the study.
- The maximum transmission of T-2 toxin and its metabolites to the eggs represented an equivalent of 0.9 µg of T-2 toxin for an exposure equivalent to 1.6 mg T-2 toxin/kg feed.(Transmission of radioactivity into eggs from laying hens administered tritium labeled T-2 toxin. **(Chi et al., 1978)**

- After administration of a single dose of 1.6 mg/kg BW to broiler chickens,
  - around 80% of the dose administered was recovered as polar metabolites in the excreta within 48 h.
  - HT-2 toxin was the main metabolite identified, followed by T-2 tetraol, T-2 triol (= deacetyl HT-2) and neosolaniol **(Yoshizawa et al., 1980).**
  - In the liver, 3′OH HT-2 was the main metabolite found at a concentration of 1370 ng/g 18 h after intraperitoneal administration of 3.5 mg/kg of T-2 toxin solubilized in water-ethanol in broilers.
  - Other metabolites, HT-2, T-2 triol, 4-deacetyl-neosolaniol (= 15 acetoxy T-2 tetraol), 15-deacetyl-neosolaniol (= 4 acetoxy T-2 tetraol), T-2 tetraol and the parent unmetabolized T-2 toxin were found at respective concentrations of 233, 210, 22, 20, 18 and 4 ng/g. In this study, 3′OH HT-2 was also the main metabolite found in the excreta **(Yoshizawa et al., 1980).**
- Direct deacetylation of T-2 toxin and deepoxidation of HT-2 and T-2 triol was also seen to occur in the excreta due to the action of the intestinal microflora (**Young** et al., 2007)

Fusarium species producing T-2 toxin

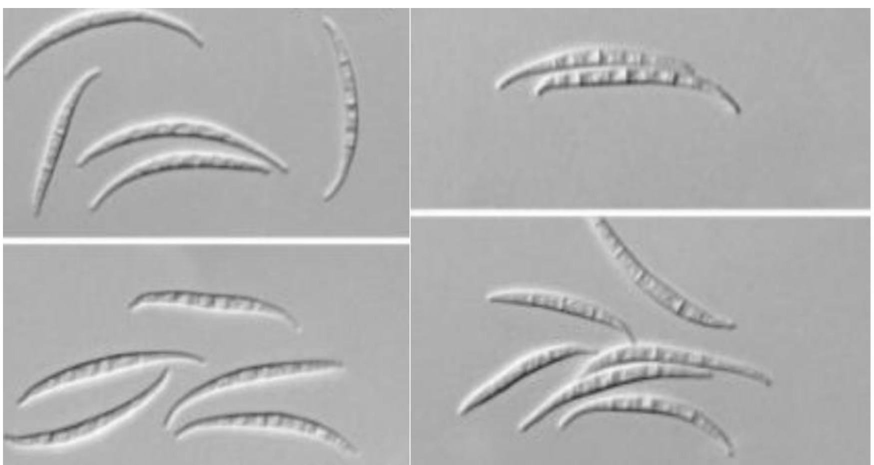
1. *Fusarium armeniacum*
2. *Fusarium equiseti*
3. *Fusarium langsethiae*
4. *Fusarium solani*
5. *Fusarium sporotrichioides:*
6. *Fusarium tricinctum*

Description of Fusarium species producing T-2 toxin

1. *Fusarium armeniacum* (G.A. Forbes, Windels & L.W. Burgess) L.W. Burgess & Summerell, Mycotaxon 75: 347 (2000)

≡Fusarium acuminatum subsp. Armeniacum G.A. Forbes, Windels & L.W. Burgess, Mycologia 85: 120 (1993)

Colonies on PDA produce white aerial mycelium, red to apricot pigment in agar, and bright orange sporodochia in the center of the culture. Some isolates produce a pionnotal form of slow-growing colonies with little aerial mycelium and abundant orange sporodochia. Macroconidia in orange sporodochia and chlamydospores formed abundantly, but microconidia are absent.

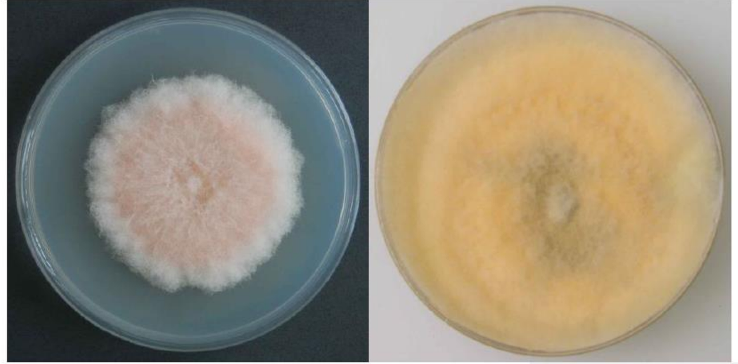


Leslie and Summerell

2. **Fusarium equiseti (Corda) Sacc., Sylloge Fungorum 4: 707 (1886)**

≡Selenosporium equiseti Corda, Icones fungorum hucusque cognitorum 2: 7, t. 9:32 (1838)
=Fusarium gibbosum Appel & Wollenw., Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft 8: 190 (1910)
=Fusarium caudatum Wollenw., Journal of Agricultural Research 2: 262 (1914) =Fusarium bullatum Sherb., Memoirs Cornell Univ. Agri. Exper. Stat. 6: 198-201 (1915)

Macroconidia: abundant in sporodochia , long , slender, dorsoventral curvature, 5-7 septa, apical cell elongate and tapering, basal cell foot-shaped. Sporodochia: orange. Microconidia: absent. Chlamydospores abundant in 2 -6 weeks, single, in pairs , in chains, or in clumps, in aerial or submerged, terminal or intercalary

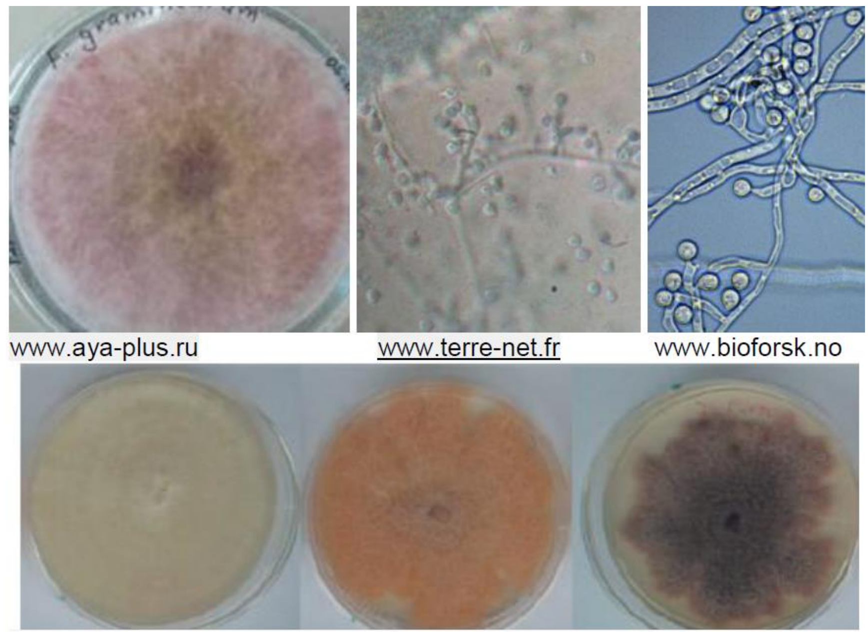


Fusarium equiseti, colony on potato sucrose agar, fungi.myspecies.info

Fusarium equiseti, macroconidia, conidiogenous cells stained in lactofuchsin.
fungi.myspecies.info

3. **Fusarium langsethiae** Torp & Nirenberg, Int. J.Food Microbiol. 95 (3): 248 (2004)

Fusarium langsethiae was initially referred to as 'powdery F. poae' due to its abundant production of small napiform to globose conidia, giving the colony a powdery-like appearance. It has spore morphology similar to F. poae. Fusarium langsethiae differs from F. poae by its slower growth, production of fewer aerial mycelia and lack of peach-like odour on synthetic media.The fungal colonies colour on synthetic solid mediarange from whitish, yellowish white, pinkish white, pale red and/or pastel red. Some of the strains can produce a pigment called aurofusarin, which is produced by nearly all strains of F. poae and F. sporotrichioides and influences colony colour development.

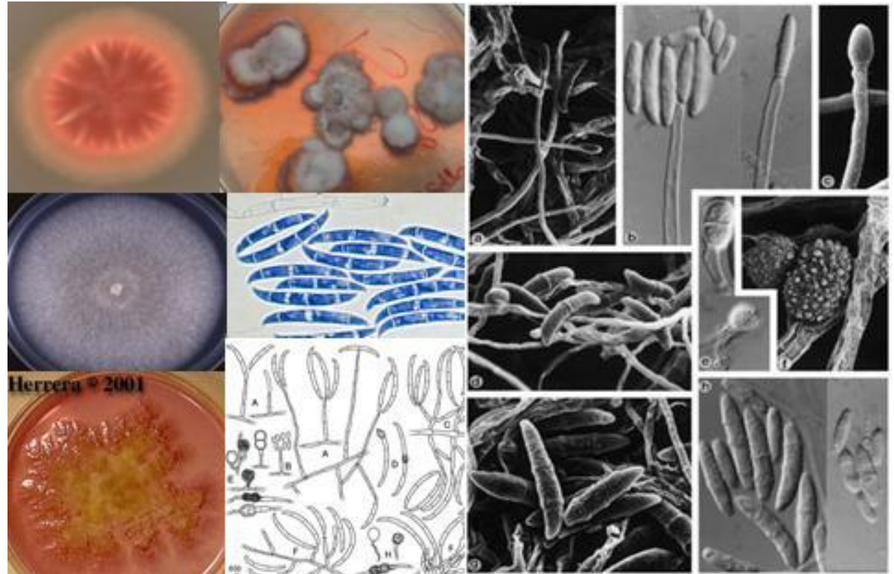


Imathiu et al., 2013

4. Fusarium solani (Mart.) Sacc., Michelia 2 (7): 296 (1881)

≡Fusisporium solani Mart., Die Kartoffel-Epidemie der letzten Jahre oder die Stockfäule und Räude der Kartoffeln: 20 (1842)
≡Fusarium solani (Mart.) Appel & Wollenw., Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft 8: 64-78 (1910)
≡Neocosmospora solani (Martius) L. Lombard & Crous, Studies in Mycology 80: 228 (2015) =Fusarium martii Appel & Wollenw., Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft 8: 83 (1910)
=Nectria cancri Rutgers, Ann. Jard. Bot. Buitenzorg, II: 59 (1913
=Fusarium striatum Sherb., Memoirs of the Cornell University Agricultural Experimental Station 6: 255 (1915)
=Fusarium solani var. minus Wollenw., Fusaria Autographice Delineata 1: 403 (1916) =Fusarium solani f. 2 W.C. Snyder, Zentralblatt für Bakteriologie und Parasitenkunde Abteilung 2 91: 174 (1934)
=Cephalosporium keratoplasticum T. Morik., Mycopath. Mycol. appl.: 66 (1939)
=Fusarium solani f. keratitis Y.N. Ming & T.F. Yu, Acta Microbiologica Sinica 12: 184 (1966) =Cylindrocarpon vaginae C. Booth, Y.M. Clayton & Usherw., Proc. Indian Acad. Sciences (Plant Sciences) 94 (2-3): 436 (1985)

Macroconidia: abundant, wide, straight or slightly curved , 3-7 septa, apical cell blunt and round, basal cell foot-shaped or cylindrical with notched end. Sporodochia: abundant, cream, blue or green. Microconidia: oval to fusiform, 0-2 sept. Chlamydospores: abundant, in 2-4 weeks, single, in pairs, in clumps or chains, terminal or intercalary

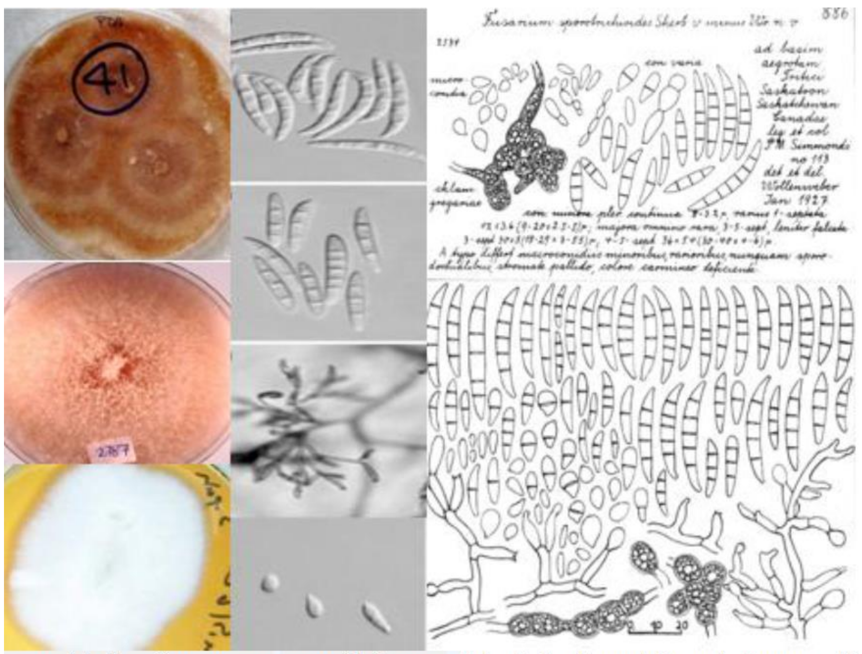


www.mycology.adelaide.edu.au, www.pf.chiba-u.ac.jp, Mycoya, Mycobank, Br J Ophthalmol. 2002, Mycobank

5. Fusarium sporotrichioides **Sherb., Memoirs of the Cornell University Agricultural Experimental Station 6: 183 (1915)**

≡Fusarium sporotrichiella var. sporotrichioides (Sherb.) Bilai, [Poisonous fungi on cereal seed]: 87 (1953)

Colonies produce profuse white to pale red mycelium. Macroconidia abundant in orange sporodochia, falcate to lunate,3-5 septate, apical cell curved and tapering, basal cell poorly developed. Microconidiaproduced from mono- or polyphialides, pyriform 0-1 septate or fusiform up to 5-septate. Chlamydospores abundant.

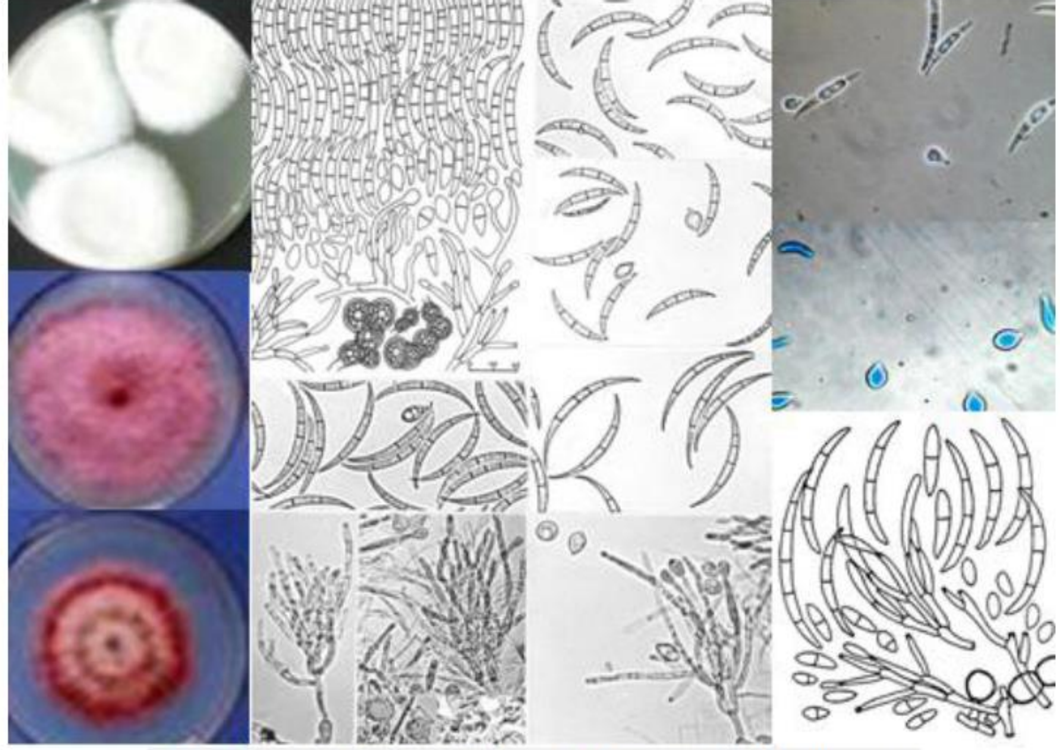


www.drjacksonkungu.com www.flickr.com John F. Leslie and Brett A. Summerell
www.marmaramedicaljournal.org G. Hagedorn, M. Burhenne & H. I. Nirenberg

6. Fusarium tricinctum (Corda) Sacc., Sylloge Fungorum 4: 700 (1886)

≡Selenosporium tricinctum Corda, Icones fungorum hucusque cogn 2: 7, t. 9:33 (1838)
≡Fusarium sporotrichioides var. tricinctum (Corda) Raillo, Fungi of the genus Fusarium: 197 (1950)
≡Fusarium sporotrichiella var. tricinctum (Corda) Bilai, [Poisonous fungi on cereal seed]: 87 (1953)
≡Fusarium sporotrichiella var. tricinctum (Corda) Bilai, Mykrobiologichnyi Zhurnal Kiev 49 (6): 7 (1987)
=Fusarium citriforme Jamal., Valt. Maatalousk. Julk.: 11 (1943)

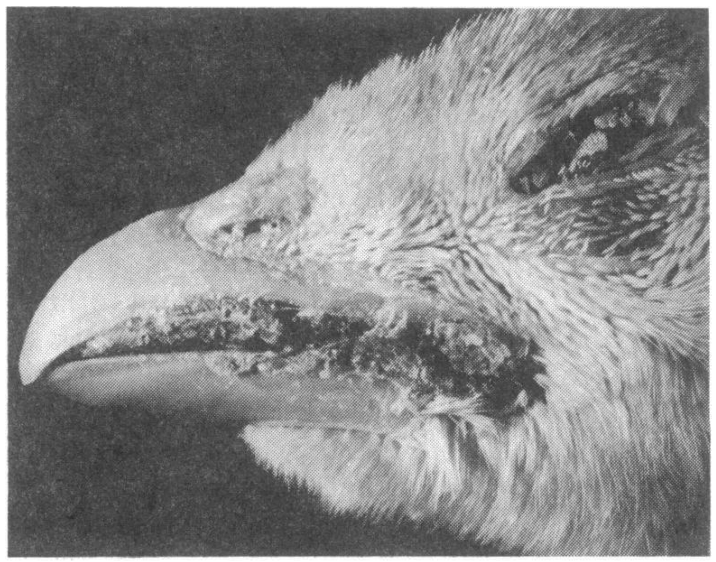
Colonies form dense white mycelium, become pink, red or purple. Sporodochia pale orange, abundant. Macroconidia abundant, slender to falcate, 3-5 –septate, apical cell curved and tapering, basa; cell foot-shaped. Microconidia abundant, napiform, oval, pyriform and citriform, 0-1-septate, may be clustered in false heads. Chlamydospores found singly or in chains



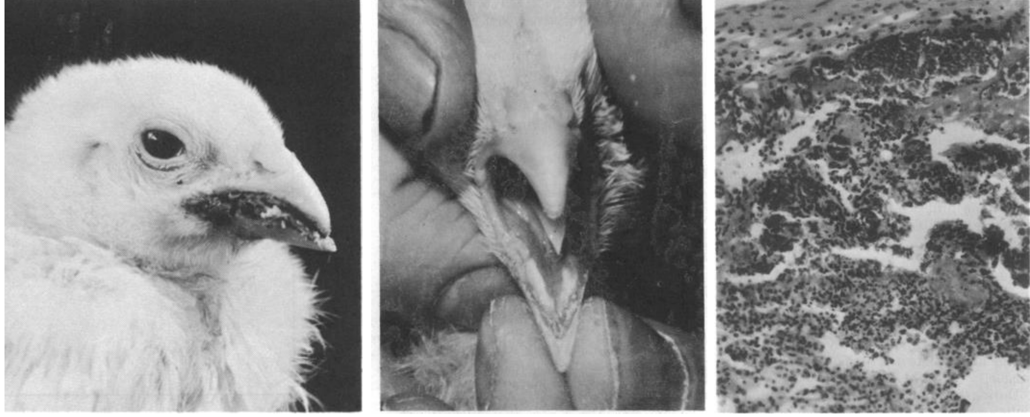
www.invasive.orgwww.andrewmccullagh.com
draaf.lorraine.agriculture.gouv.fr, en.engormix.com

Reports:

Christensen *et al.* (1972) reported that consumption of an otherwise balanced ration containing 1% of corn invaded by Fusarium tricinctum isolate 2061-C resulted in the death of 13% of turkey poults within 35 days, in decreased feed efficiency and weight gain, and moderate development of bilateral necrotic lesions at angles of the mouth, especially in those that succumbed. Consumption of a ration with 2% of corn invaded by F. tricinctum resulted in death of 60 to 83% of the birds, in greatly reduced growth and feed efficiency in the survivors, and in development of severe mouth lesions. Consumption of rations containing 5, 10, and 20% of corn invaded by the fungus resulted in death of all birds in 5 to 15 days.

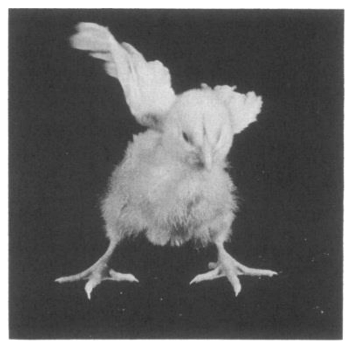
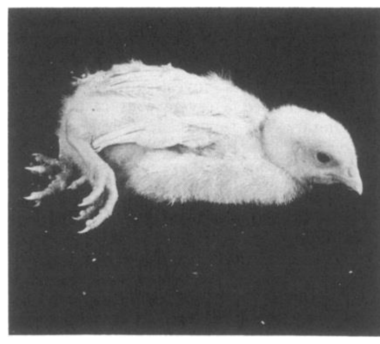
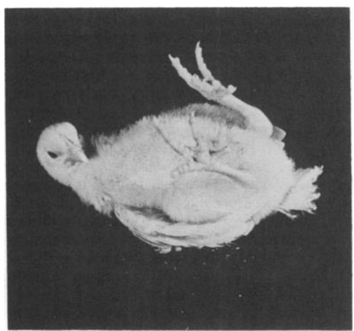


Wyatt ***et al.*** **(1972)** gave graded concentrations of dietary **fusariotoxin T-2** (0, 1, 2, 4, 8, and 16 ;tg/g, respectively) to groups of 40 chickens. Raised yellowish-white lesions on the mouth parts were produced by all concentrations, and the size of the lesions was dose-related. The growth rate was reduced significantly ($P < 0.05$) by concentrations of 4, 8, and 16 Mg/g. The mouth fluid of the affected birds contained greatly increased numbers of bacteria, including Staphylococcus epidermidis and Escherichia coli, which proved avirulent when inoculated into scarified tissue of control birds. Microscopy examinations of the lesions revealed a fibrinous surface layer, intermediate layers containing invaginations filled with rods and cocci, and a heavy infiltration of the underlying tissues with granular leukocytes. These data suggest that the role of fusariotoxin T-2 in field cases of moldy corn toxicosis should be reinvestigated since oral lesions were not mentioned in the original descriptions of the disease. However, the lesions bear some features of those characteristic of the third or septic angina stage of alimentary toxic aleukia, a nutritional toxicosis of humans produced by eating grains infested with ***F. tricinctum.***



Oral inflammatory response to dietary fusariotoxin T-2 in the young chicken.
Oral lesions produced by 4 fig of dietary fusariotoxin T-2 per g of diet.

Wyatt *et al.* (1973a) fed graded concentrations of dietary T-2 toxin (0, 1, 2, 4, 8, and 16 µg/g) to groups of 40 chickens. T-2 toxin was found to cause an abnormal positioning of the wings, hysteroid seizures, and impaired righting reflex in young chickens. The abnormal wing positioning occurred spontaneously or as the result of dropping from a height of 1 meter. The seizures could be elicited by rough handling or loud noises. The seizures and the abnormal wing posture would not occur again when the stimulus was repeated unless a rest period of 3 to 6 h was allowed. The loss of righting reflex could be demonstrated at any time. The total incidence of neural symptoms was dependent on the length of exposure to T-2 toxin and to its concentration. Neural toxicity occurred at dosages of 4, 8, and 16 µg per g of diet, which are the same doses that retard growth. This neural toxicity of T-2 toxin in chickens is similar to the neural disturbances associated with alimentary toxic aleukia, a nutritional toxicosis of humans produced by eating moldy grain. T-2 toxin has been implicated also in moldy corn toxicosis which has neural manifestations in horses and swine**.**

Wyatt *et al.* (1973b) gave graded concentrations (0, 1, 2, 4, 8, and 16 µg./g.) of dietary T-2 toxin to groups of 40 broiler chickens. A total of 240 chicks were used in these experiments. The growth rate was reduced significantly ($P < 0.05$) by concentrations of 4, 8, and 16 µg./g. but not by lower concentrations while the feed conversion ratios were unaffected by any concentration. The relative weight of the spleen was decreased and the relative weight of the pancreas was increased by the growth inhibitory concentrations. The bursa of Fabricius was reduced in relative weight by concentrations of 8 and 16 µg./g. while the relative weight of the crop was increased. The relative weight of the liver was unaffected as was its lipid content and its percent of dry matter; however, there was a dose-related increase in liver hematomas. The hemoglobin, serum proteins, serum cholesterol, serum total lipids, plasma glucose, and plasma uric acid were unaffected as were the capillary fragility, lateral shear strength of breast muscle, and liver acid phosphatase which is a marker enzyme of lysosomes. These data suggest that severe oral lesions which impair their ability to eat are the primary effect of T-2 toxin in chickens. Secondarily with greater concentrations, T-2 toxin exerts its systemic effects on the chickens.

Wyatt ***et al.* (1975)** reported that, when T-2 toxin was added at a level of 20 µg per g of feed, it caused oral lesions but no abnormal neural disturbances in young broiler chickens. T-2 toxin, when added at a level of 20 µg per g of feed, caused oral lesions but no abnormal neural symptoms in laying hens. T-2 toxin had no effect on either

hemoglobin, hematocrit values, erythrocyte count, plasma glucose, prothrombin times, or the sizes of the liver, spleen, pancreas, and heart. Lipid content of the liver was not altered. Feed consumption, however, was reduced, as were the total plasma protein and lipid concentrations and the total leukocyte count. Most important economically was the lowered egg production and a thinner egg shell. The timing and severity of the symptoms suggest that T-2 toxin causes primary oral lesions that reduce feed consumption with a consequent reduction in serum proteins and lipids, which culminate in decreased egg production. The leucopenia and thinner egg shell may be independent systemic effects of T-2 toxin in laying hens.

Puls and Greenway (1976) mentioned that **fusariotoxin T-2** was tentatively identified in barley samples that caused field outbreaks of mycotoxicosis in British Columbia**. Geese** died when fed the contaminated barley experimentally but mice were little affected after long term feeding. The methods used in the laboratory for trichothecene extraction and identification of T-2 toxin are described.

Speers *et al.* (1977) fed balanced rations containing 2.5 and 5.0% of corn invaded by Fusarium tricinctum, (with 8 and 16 p.p.m. of T-2 toxin) to White Leghorn laying hens. The ration containing 5% of the fungus-invaded corn resulted in reduced feed intake and reduction in weight gain and in egg production. Rations containing 2.5 and 5.0% of corn invaded by F. roseum 'Gibbosum' (with 25 and 50 p.p.m., respectively, of monoacetoxyscirpenol) resulted in an abrupt decrease in feed intake to 10-20% of normal, subsequent loss in weight, and cessation of egg production. Purified T-2 toxin consumed at the rate of 16 p.p.m. in the ration resulted in loss of body weight and decreased egg production; lesser amounts of T-2 toxin resulted in lessened but still detectable injurious effects. Mouth lesions developed in the birds fed these rations, their severity being proportional to the amount of toxin present.

Chi *et al.* (1978) investigated the excretion and distribution of radioactivity in 6-week-old broiler chicks intubated with a single dose of 3-^{3}H-labeled T-2 toxin during the 48-h period after dosing. Chicks excreted 6.7, 20.7, 42.1, 60.5,and 81.6% of the recovered radioactivity at 4, 8, 12, 24, and 48 h, respectively. The gastrointestinal (GI) tract contained 88.8, 74.0, 63.3, 38.0, 26.9, and 10.4% of the recovered radioactivity at 0.5, 4, 8, 12, 24 and 48 hr, respectively. The abdominal fat and heart contained the least amount of radioactivity among those tissues analyzed. The radioactivity from ^{3}H-labeled T-2 toxin reached a maximum concentration 4 hr after dosing in most tissues except for the muscle, skin, and bile; in the latter tissues, the maximum radioactivity was attained at 12 hr. The specific radioactivities (disintegrations per minute per milligram of tissue) of the blood, muscle, skin, and heart were similar throughout the 48 hr period. The bile, including the gall bladder, contained the highest specific radioactivity among organs and tissues (except GI tract) during the 48 hr period. The edible portions of the carcass contained 0.06 and 0.04 ppm of T-2 or its metabolites at 24 and 48 hr, respectively after dosing with 0.5 mg of T-2/kg body weight. The patterns of distribution and excretion suggest that T-2 toxin and/or its metabolites are excreted into the intestine through the bile and that the liver is a major organ for excretion of the toxin.

Richard *et al.* (1978) studied the effect of T-2 toxin consumption on Broad-Breasted White turkey poults and White Leghorn chicks. Groups of ten 8-day-old poults were fed rations containing T-2 at 10 ppm, 2ppm, or 0 ppm (controls) for a period of 4 weeks; a 4th group (inanition control) was fed control rations equal to the amount consumed by the group fed rations containing T-2 at 10 ppm during the previous 24

hours. A similar experimental design was used to study the effect of the toxin on 1-day-old chicks. The thymus glands of the poults given the feed containing 10 ppm were markedly decreased in size compared with thymus glands from poults in the control group, 0.182 vs 0.331 (percentage of body weight). There was no significant (P less than or equal to 0.05) decrease in thymus gland size in poults given 2 ppm or in the inanition controls. Dietary treatment did not appear to affect the size of the bursa or spleen of the poults. Histopathologic examination of thymus glands from poults given 10 ppm of T-2 revealed a depletion of cortical lymphocytes. Chicks appeared less sensitive to T-2 toxin than did the poults. There was no effect by any dietary treatment on the size of the thymus gland, bursa, or spleen of chicks. Reductions were noticed in feed efficiency and weight gain. There was no effect of T-2 toxin on agglutinating antibody formation to Pasteurella multocida bacterin..

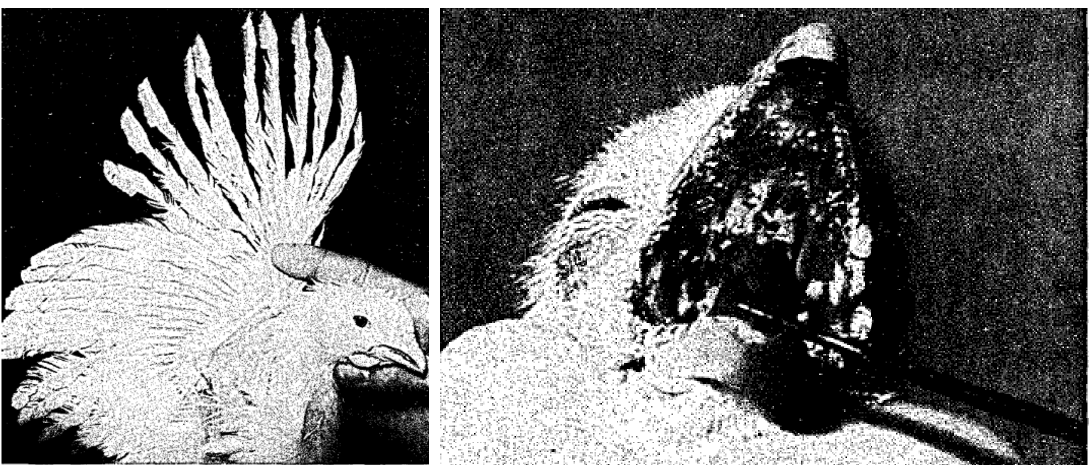
Yoshizawa *et al.* (1980) developed and applied a method for the detection of T-2 metabolites in excreta of broiler chickens administered 3H-labeled T-2 toxin. The method used acetonitrile extraction and partitioning with petroleum ether followed by chromatography on Amberlite XAD-2, Florisil, and Sep-Pak C18. The recovery of T-2 toxin added to the chicken excreta was 73% at a concentration of 0.2 microgram/g. About 80% of orally administered 3H-labeled T-2 toxin was rapidly metabolized to more polar derivatives and eliminated in the excreta within 48 h. T-2 toxin, HT-2 toxin, neosolaniol, and T-2 tetraol were detected at 0.06 to 1.13% of the total dose, 48 h after administration. Eight unknown derivatives, named TB-1 to TB-8, were quantitatively more significant than the metabolites above. TB-3 and TB-9 represented about 12 and 25% of the total dose, respectively. One of the metabolites (TB-6), 1.5% of the total dose, was identified as 4-deacetylneosolaniol (15-acetyl-3 alpha, 4 beta, 8 alpha-trihydroxy-12, 13-epoxytrichothec-9-ene).

Hoerr *et al.* (1081) studied the effect of T-2 toxin (3-hydroxy-4,15-diacetoxy-8-[3-methyl-butyrloxy]-12,13-epoxy-delta 9-trichothecene) and diacetoxyscirpenol, given by crop gavage to 7-day-old male broiler chickens. Selected birds were killed at 1, 6, 12, 18, 24, 72, and 168 hours post-treatment. The lesions induced by the two toxins were similar, but were more severe in chicks given T-2 toxin. Necrosis of lymphoid tissue and bone marrow began one hour after treatment with T-2 toxin, and was followed by rapid cell depletion. Cell repletion also was rapid, occurring by hour 24 in mildly injured tissues from birds given diacetoxyscirpenol and by hours 72 and 168 in more severely injured tissues from chickens given T-2 toxin. Hepatic lesions were multiple foci of cell necrosis resolved rapidly and the inflammatory cell reaction was minimal. Necrosis of gall bladder epithelium and secondary cholecystitis followed hepatic cell necrosis. In the alimentary tract, necrosis of the epithelium on the tips of villi in the duodenum was followed by necrosis of the epithelium of villi and crypts in the small and large intestine, and of mucosal epithelium of the proventriculus and ventriculus. Atrophy of intestinal villi and fewer mitotic figures were seen by 18 hours after treatment. The alimentary tract epithelium, however, looked normal by hour 72. Lesions in the integument, including necrosis of feather epidermis and of the follicular epidermis at the neck of the feather follicle, occurred at 12 to 24 hours after treatment.

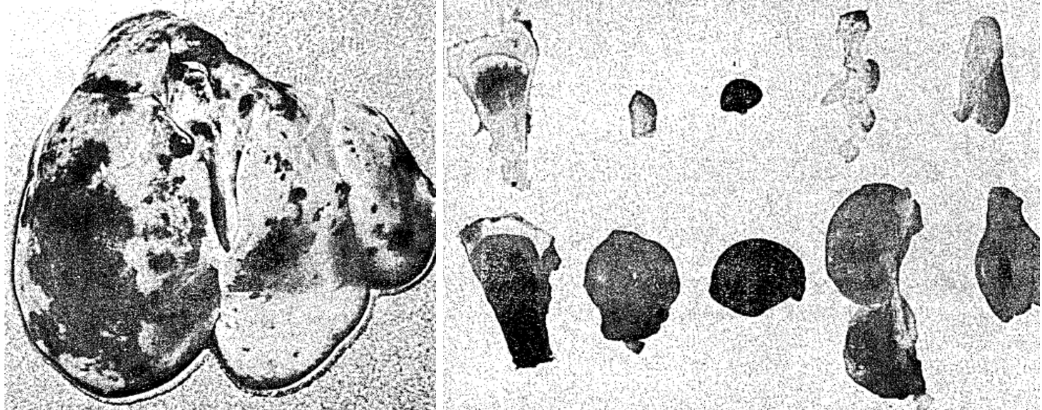
Hoerr *et al.* (1982a) evaluated the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old

chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

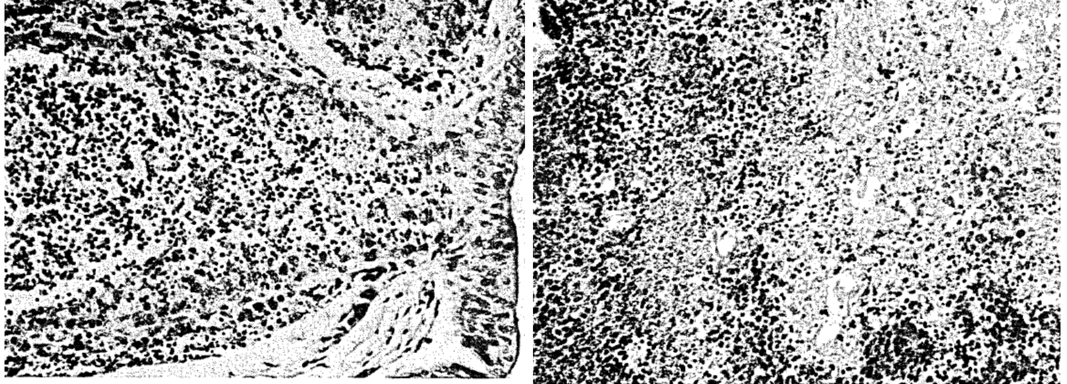
Hoerr *et al.* (1982b) carried out an experimental study on broiler chickens. ***Fusarium sporotrichiella var. sporotrichioides*** (Bilay), cultured on sterilised popcorn at 23°C and then at 8°C, 16°C and 23°C and fed as 50% of the diet, was found to be lethal to 7-day-old male broiler chickens. The 8°C culture, containing **T-2 toxin** at 50 parts per million (ppm) and **neosolaniol** at 5 ppm, was given as whole culture at dietary concentrations of 10%, 5%, 1% and 0% for 17 days and 1% for 42 days. Half the chickens that were fed the 10% diet died during the 17 days (5 ppm T-2 toxin and 0.5 ppm neosolaniol). The corresponding daily dose was 0.24 mg T-2 toxin and 0.02 mg neosolaniol/kg body weight/day. The chickens that died were dehydrated, had necrosis and depletion of lymphoid and haematopoietic tissues and necrosis of the hepatobiliary system, gastroenteric mucosa, feather epidermis and renal tubular epithelium. The survivors had anaemia, reduction of weight gain and transiently altered righting reflex. The comb and beak were pale yellow and the feather barbs were dishevelled. Survivors also had atrophied lymphoid tissues, reduced haematopoietic cellularity in the bone marrow, necrosis of oral and crop mucosa, vacuolated hepatocytes, hyperplastic bile ductules, and reduction of the thyroid follicular diameter.



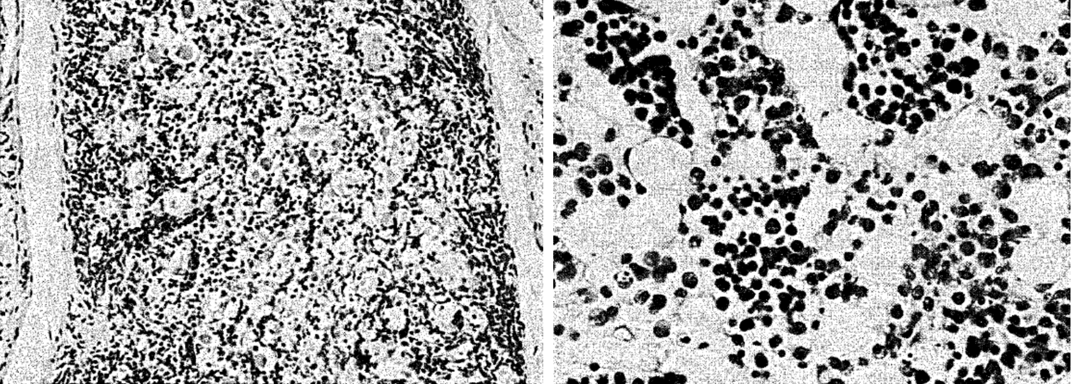
The barbs on the feathers of chickens fed Fusarium failed to spread causing a narrow blade and they were wavy and disorganized, Yellow crust on the palate and tongue of achicken fed 5% Fusarium diet for 17 days **Hoerr *et al.* (1982b)**.



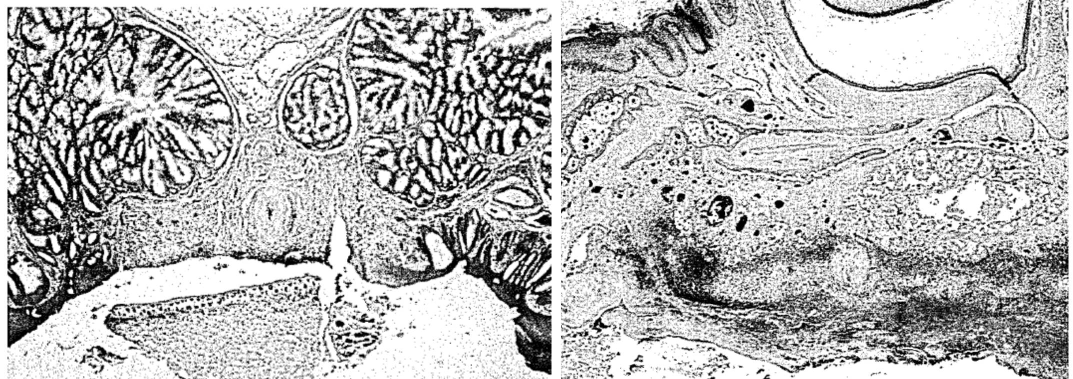
Haemorrhages in the liver of a chicken fed 25% Fusarium diet.,Pale yellow marrow and atrophied lymphoid organs (top) of a chicken fed 1% Fusarium diet for 42 days; (bottom) control tissues. **Hoerr *et al.* (1982b)**



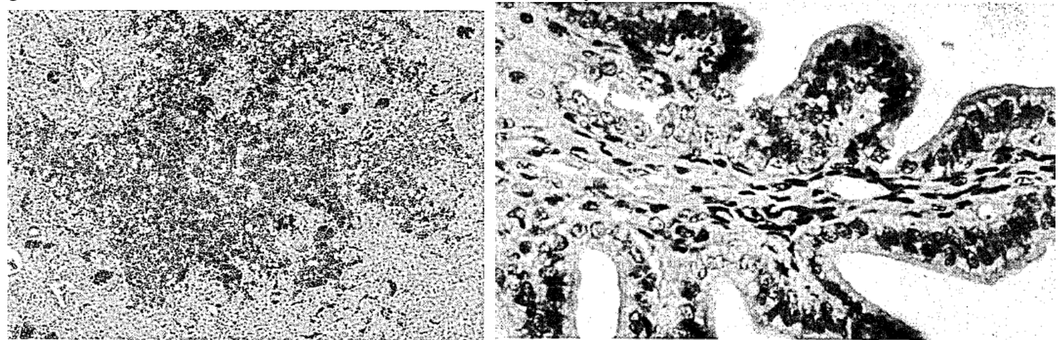
Necrosis of cortical and meduUary lymphoci Fabricius of chicken fed 50% Fusarium diet.,Necrosis of cortical and meduUary lymphocytes in ihe thymus of a chicken fed 10% Fusarium diet. **Hoerr *et al.* (1982b)**



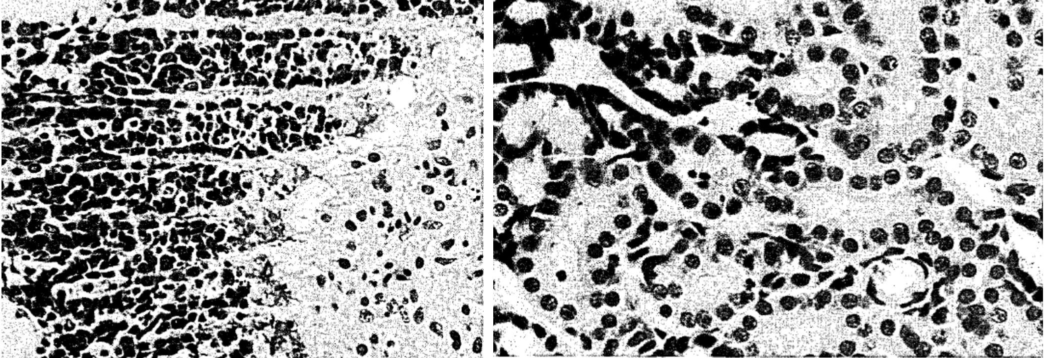
Severe depletion of lymphocytes in the thymus of a chicken fed. 50% Fusarium diet. Necrosis of haematopoietic components of the bone marrow in a chicken fed 50% Fusarium diet. **Hoerr *et al.* (1982b)**



Ulceration of the oral mucosa between the maxillary salivary duct openings in a chicken fed 1% Fusarium diet. Necrosis and ulceration of the oral mucosa with necrosis of the maxillary salivary glands in a chicken fed 107o Fusarium diet . for 15 days. **Hoerr *et al.* (1982b)**



Focal haemorrhage and necrosis in the liver of a chicken fed50% Fusarium diet for 22 hours.Basophilic cytoplasmic bodies in the gallbladder mucosalepithelium of a chicken fed 50% Fusarium diet for 18 hours. **Hoerr et al. (1982b)**

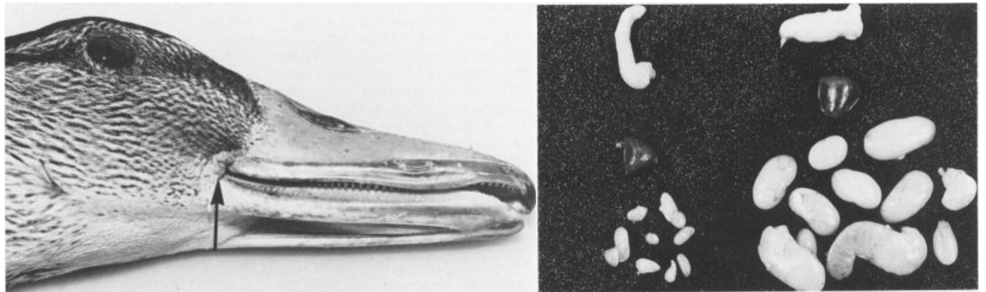


Necrosis of the barb ridges in a feather of a chicken fed 5 0% Fusarium diet. Necrosis of renal tubular epithelium in a chicken given a 5% Fusarium diet. **Hoerr *et al.* (1982b)**

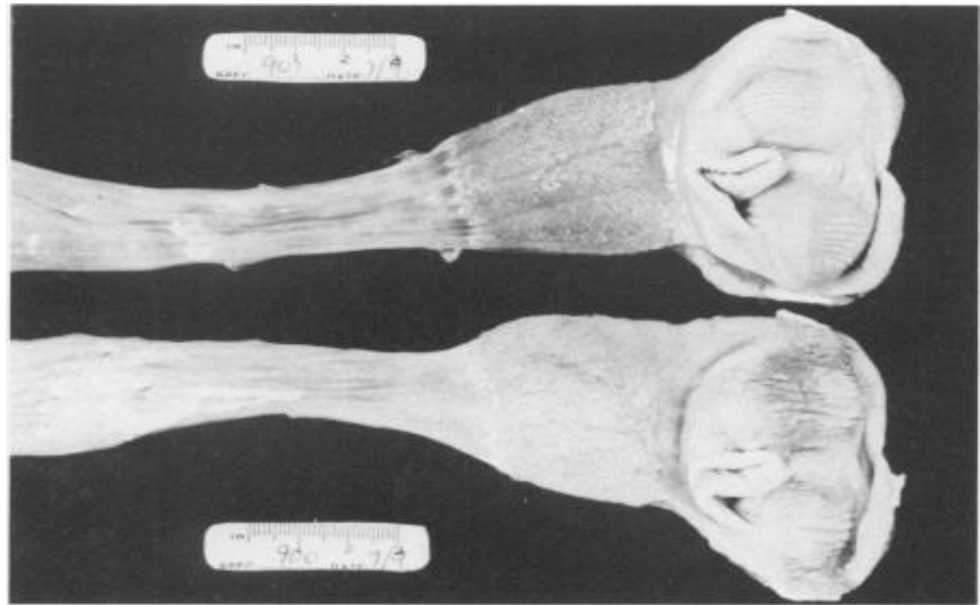
Allen *et al.* (983) fed Nicholas Large White turkey hens in egg production (10 per treatment) individually cultures of Fusarium roseum 'Gibbosum' to provide 100 ppm zearalenone, Fusarium tricinctum at a level of .1% of the diet, Fusarium roseum Alaska at a level of 2% of the diet, 100 ppm purified zearalenone, and 5 ppm purified T-2 toxin for 8 weeks. The following 4 weeks the birds were fed a control diet. Hens were inseminated every 2 weeks with .05 ml of pooled semen from males fed a control diet. After 30 days of toxin feeding, hens were innoculated with a killed Newcastle disease virus preparation. Blood samples were obtained periodically. Egg fertility and titers to Newcastle disease virus were unaffected by treatment. Egg weight was reduced by F. roseum 'Gibbosum'. F. roseum 'Gibbosum' and F. tricinctum caused decreases in feed consumption, body weight, and egg production. Egg

production was decreased by zearalenone and T-2 toxin. Hens fed F. roseum 'Gibbosum', F. tricinctum, and T-2 toxin exhibited mouth lesions that healed rapidly upon withdrawing toxic feed. Hatchability of fertile eggs was reduced by feeding F. roseum 'Gibbosum', F. tricinctum and F. roseum Alaska to 28, 78, and 49%, respectively, of control values by the end of the 8 week test period. Upon removal of toxic feed, hatchability rapidly returned to control levels. Embryo mortality occurred mainly in the first 10 days of incubation for F. roseum Alaska and the last 18 days for F. roseum 'Gibbosum' and F. tricinctum fed hens. It appears that mycotoxins other than zearalenone and T-2 toxin are responsible for reduced hatchability from feeding Fusarium cultures.

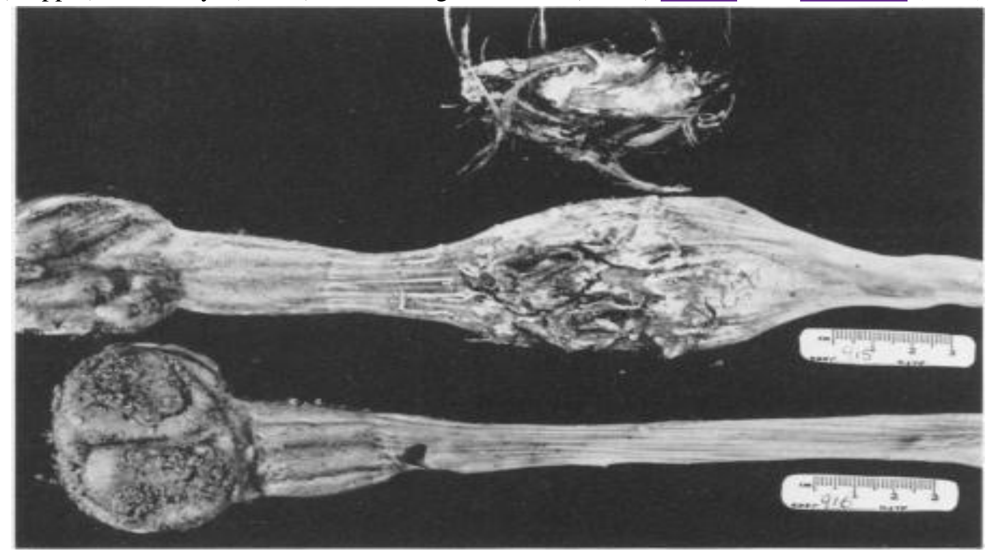
Hayes **and** Wobeser **(1983)** fed young Mallard ducks (Anas platyrhynchos) diets containing purified **T-2 toxin** at levels of 20 or 30 ppm for two or three weeks. Ingestion of T-2 toxin was associated with reduced weight gain and delayed development of adult plumage. Affected ducks developed caseonecrotic plaques throughout the upper alimentary tract, especially in oropharynx and ventriculus. Several ducks also developed severe ulcerative, proliferative esophagitis and proventriculitis. Generalized atrophy of all lymphoid tissues consistently occurred. The manifestations of T-2 mycotoxicosis in Mallard ducks were mostly attributable to irritant toxicity to the alimentary mucosa. The T-2 toxin caused neither hematopoietic suppression nor a hemorrhagic syndrome in ducks. These alimentary lesions of T-2 mycotoxicosis in ducks do not resemble diseases of native waterfowl presently being recognized in routine surveillance of waterfowl mortality in Saskatchewan.



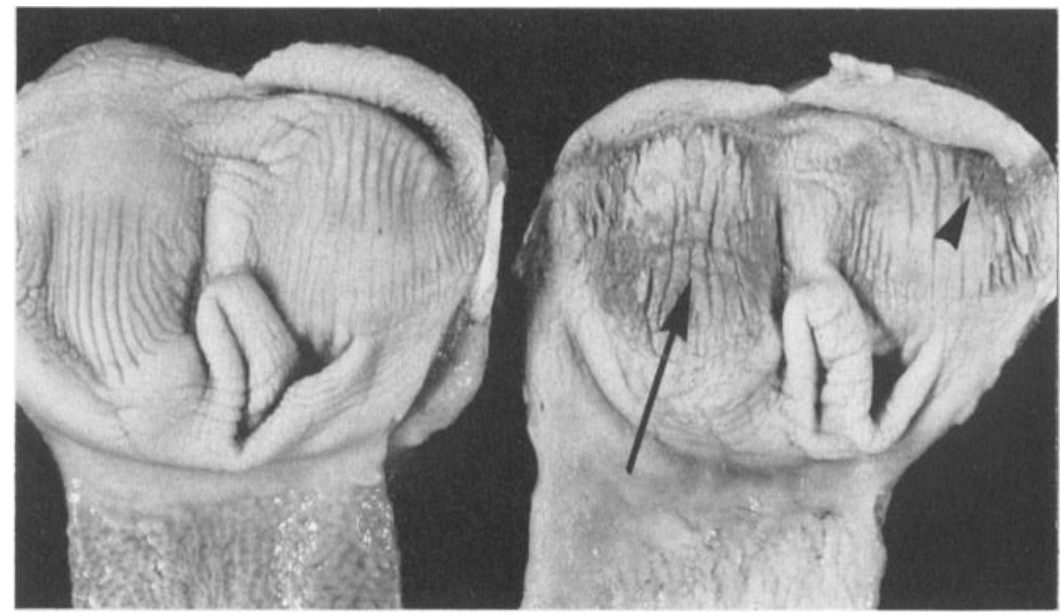
Typical mild dermatitis and hyperkeratosis at the commissure of the mouth of a duck fed T-2 toxin (30ppm) for 14 days, Marked atrophy of bursa (top), spleen (middle) and thymic lobes (below) of a duck fed T-2 toxin (20 ppm) for 21 days, Normal organs on the right, Hayes and Wobeser (1983)



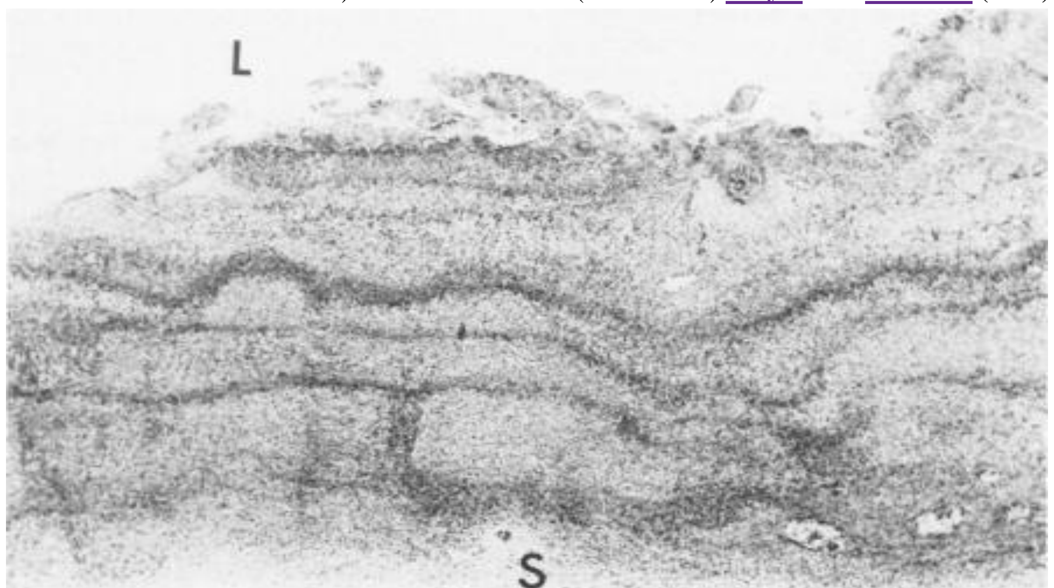
Mild fibrinous oesphagitis, catarrhal proventriculitis and ulcerative ventriculitis in a duck fed T-2 toxin (20 ppm) for 21 days (below), Normal digestive tract (above) Hayes **and** Wobeser **(1983)**



Severe esophagitis with a cast of exudate, mucus, feed and feathers in the midesophageal region of a duck fed T-2 toxin (20 ppm) for 21 days, Normal digestive tract (below) Hayes **and** Wobeser **(1983)**



Close up view of ventricular lesions induced by T-2 toxin. Note the thickened encrusted surface (arrow) and focal ulceration (arrow head) **Hayes and Wobeser (1983)**



Microscopic appearance of layers of fibrin and heterophilic exudate overlaying ulcerated esophageal submucosa of a duck fed T-2 toxin. L is the lumen, S is the submucosa. H&EX50 **Hayes and Wobeser (1983)**

Knupp *et al.* (1987) prepared hepatic microsomes from phenobarbital (PB)-treated and control rats, mice, rabbits and chickens and were incubated with T-2 toxin (100 micrograms/mg microsomal protein). Additional microsomes from PB-induced animals were incubated with T-2 toxin and the esterase inhibitor paraoxon (PA) at 2.5 nmol/mg microsomal protein. The major metabolite in microsomal preparations from both control and PB-induced rats, rabbits and mice was HT-2. In microsomes isolated from PB-treated chickens, 3'-hydroxy T-2 was the major metabolite, but 30 and 79% of the added T-2 toxin remained unmetabolized at 60 min in incubations from PB-induced and control birds, respectively. The percentage of hydroxylated metabolites formed in the microsomal preparations of the four species studied was significantly

increased following PB treatment compared with the non-treated controls. The addition of PA to the incubation system effectively inhibited the hydrolysis of the ester groups in T-2 toxin, resulting in 1.4- and 1.25-fold increases in the percentage of 3'-hydroxy T-2 in the mouse and rat microsomal samples, respectively. In the rabbit microsomal preparations, 3'-hydroxy T-2, which was not detected in the absence of PA, represented 11% of the added substrate in the PB/PA incubation samples. Addition of PA did not cause a significant change in the amount of 3'-hydroxy T-2 formed in chicken microsomal samples, since competition between hydrolysis and hydroxylation pathways for the T-2 toxin substrate was not an important factor in this species. Two new metabolites, designated RLM-2 and RLM-3 were detected in chicken, rat and mouse microsomal preparations. On the basis of gas chromatography/mass spectrometry data, the compounds were tentatively identified as isomers of 3'-hydroxy T-2.

Visconti *et al.* (1985) used gas chromatography-mass spectrometry to identify various T-2 toxin metabolites in chicken excreta and organs 18 h after intraperitoneal injection of the toxin. No trichothecenes were detected in the heart and kidneys, and only trace amounts were detected in the lungs. Most of the T-2 metabolites were found in the excreta, although considerable amounts were also found in the liver. In addition to the previously identified T-2 metabolites in chicken excreta (HT-2 toxin, 15 acetoxy T-2 tetraol, and T-2 tetraol), we found 3'-hydroxy HT-2 toxin (the major metabolite in excreta and organs), 3'-hydroxy T-2 toxin, 4-acetoxy T-2 tetraol, and trace amounts of 8-acetoxy T-2 tetraol, 3-acetoxy-3'hydroxy HT-2 toxin, and T-2 triol. Unmetabolized T-2 toxin and an unidentified isomer of T-2 tetraol monoacetate were also detected in the excreta. Most of the metabolites in the chicken are similar to those encountered in cultures of fungal species producing T-2 toxin. A comparison with T-2 toxin metabolism in the cow is also reported.

Giroir *et al.* (1991) administered a tritiated preparation of the trichothecene mycotoxin, T-2 toxin, as a single oral dose to 21-day-old male broiler (Hubbard x Hubbard) chickens and White Pekin ducks. There were few significant differences between the two species in metabolism, tissue retention, and excretion of T-2 toxin and its metabolites. On the basis of the data obtained, the differences in toxicological sensitivity to T-2 toxin known to exist between these two species cannot likely be attributed to differences in the metabolism or elimination of T-2 toxin from the body.

Ruff *et al.* (1992) fed Bobwhite and Japanese quail diets containing 1.25, 2.50, or 5.00 ppm aflatoxin; 1, 2, or 4 ppm ochratoxin A (OA); or 4, 8, or 16 ppm T-2 toxin. Aflatoxin induced mortality in bobwhites during the second and third week with 1.25 ppm (10%), 2.50 ppm (30%), and 5.00 ppm (40%), and during the same period with T-2 toxin at 8 ppm (20%) and 16 ppm (22.5%). Body weights of bobwhite quail were significantly decreased by the two higher levels of aflatoxin by 2 weeks of age, and by the two higher levels of T-2 toxin by 1 week of age. In Japanese quail, only the highest level of aflatoxin and T-2 toxin reduced body weight (by 3 weeks and by 1 week of age, respectively), and even then to a much lesser extent than in bobwhites (less than 10%). Aflatoxin did not affect feed-conversion ratio (FCR) in bobwhite

quail, but the two higher levels of T-2 toxin increased FCR. None of the toxins induced mortality or increased the FCR in Japanese quail. Aflatoxin increased liver weight in both bobwhite and Japanese quail. OA increased kidney weight in 3-week-old Japanese quail but had no effect on the kidney weight of bobwhite quail. Mouth lesions were progressively more severe in bobwhite quail fed increasing levels of T-2 toxin, but lesions were far less severe in Japanese quail.

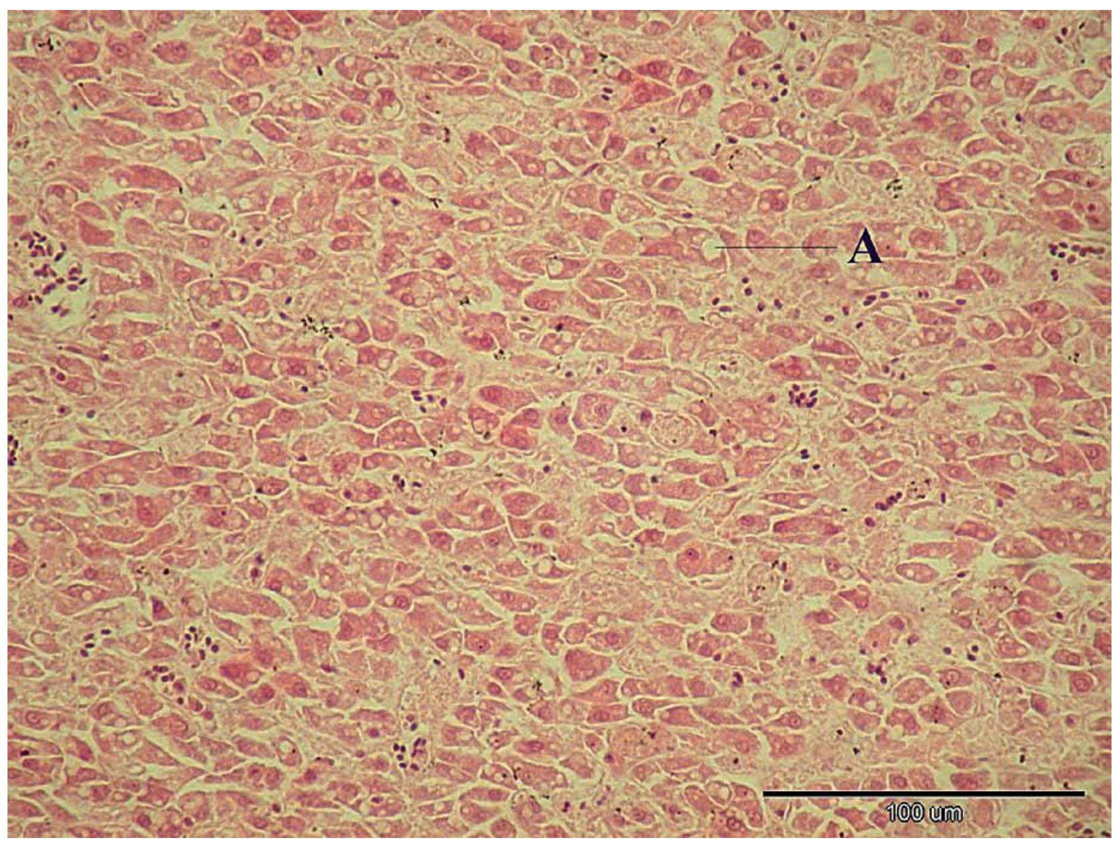
Mishra *et al.* (1996) conducted an experimental study to evaluate and record the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

Sklan *et al.* (2001) examined the effects of feeding T-2 toxin, diacetoxyscirpenol (DAS), or aflatoxin B1 at levels up to 1,000 ppb for 5 weeks on performance, health, and immune response of enterally and parenterally immunized chicks. No decreases in growth or feed efficiency were observed when T-2, DAS, or a mixture of these mycotoxins were fed for 35 days. Aflatoxin at concentrations above 800 ppb resulted in decreased growth and feed efficiency after 4 weeks. Feeding T-2 and DAS resulted in oral lesions and mild intestinal inflammation, but no other pathological or histopathological lesions. Aflatoxin caused enlargement and discoloration of liver and kidneys and mild intestinal inflammation. No effects of T-2, DAS, or aflatoxin B1 were observed on antibody production to antigens administered by enteral or parenteral routes

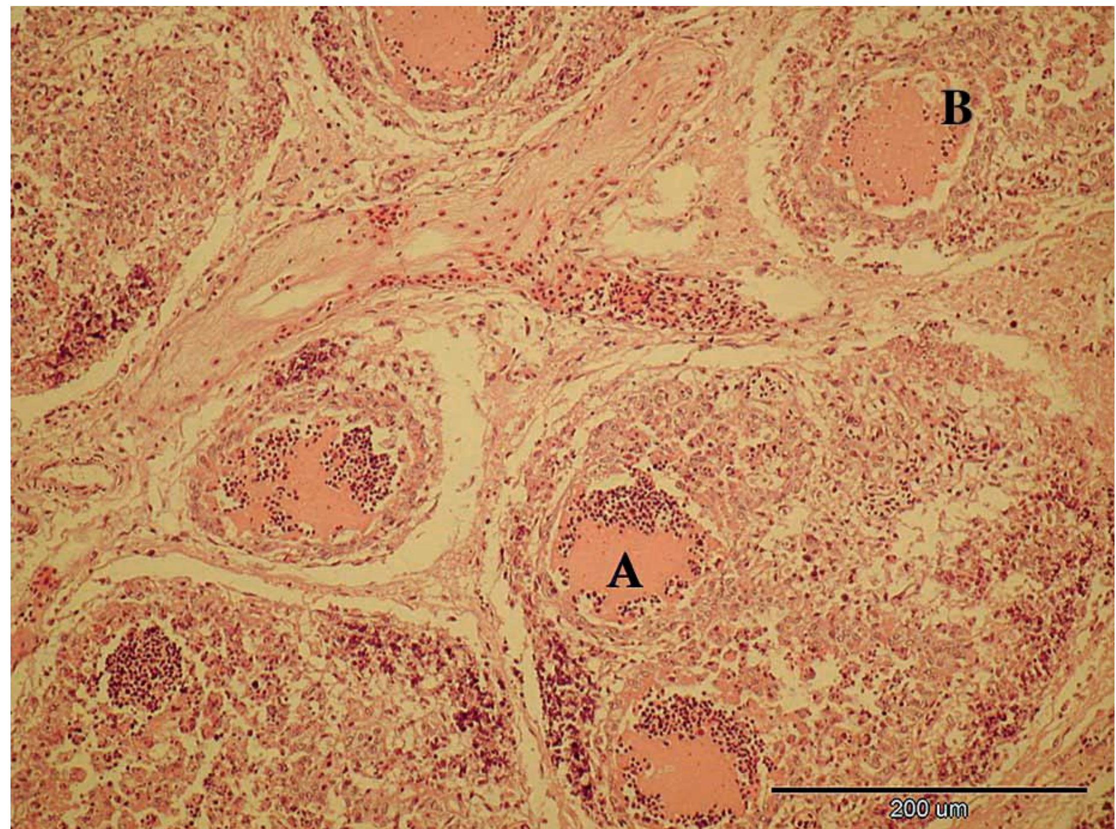
Grizzle *et al.* (2004) conducted three experiments to assess mortality rate, blood chemistry, and histologic changes associated with acute exposure to T-2 mycotoxin in adult bobwhite quail. In Experiment 1, adult quail were orally dosed with T-2 toxin to determine the lethal dose that resulted in 50% mortality of the affected population (LD_{50}), and that dose was determined to be 14.7 mg of T-2 toxin per kilogram of body weight (BW). A second experiment was performed to study the effects of 12–18 mg/kg BW T-2 toxin on blood chemistry and liver enzyme profiles. Posttreatment uric acid, aspartate aminotransferase, lactic dehydrogenase, and gamma glutamyltransferase increased as compared with pretreatment values. In contrast, posttreatment plasma total protein, cholesterol, and triglyceride levels numerically decreased as compared with pretreatment values. Changes in blood chemistry values

were consistent with liver and kidney damage after T-2 toxin exposure. In Experiment 3, histologic analyses of bone marrow, spleen, liver, small intestine, kidney, and heart were conducted on birds dosed in Experiment 2. Marked lymphocyte necrosis and depletion throughout the spleen, thymus, bursa, and gut-associated lymphoid tissue in the small intestine were observed in birds dosed with 15 and 18 mg/kg BW T-2 toxin. Necrosis of liver and lipid accumulation as a result of malfunctioning hepatocytes were also observed. Little or no morphologic change was observed in bone marrow and heart tissue.

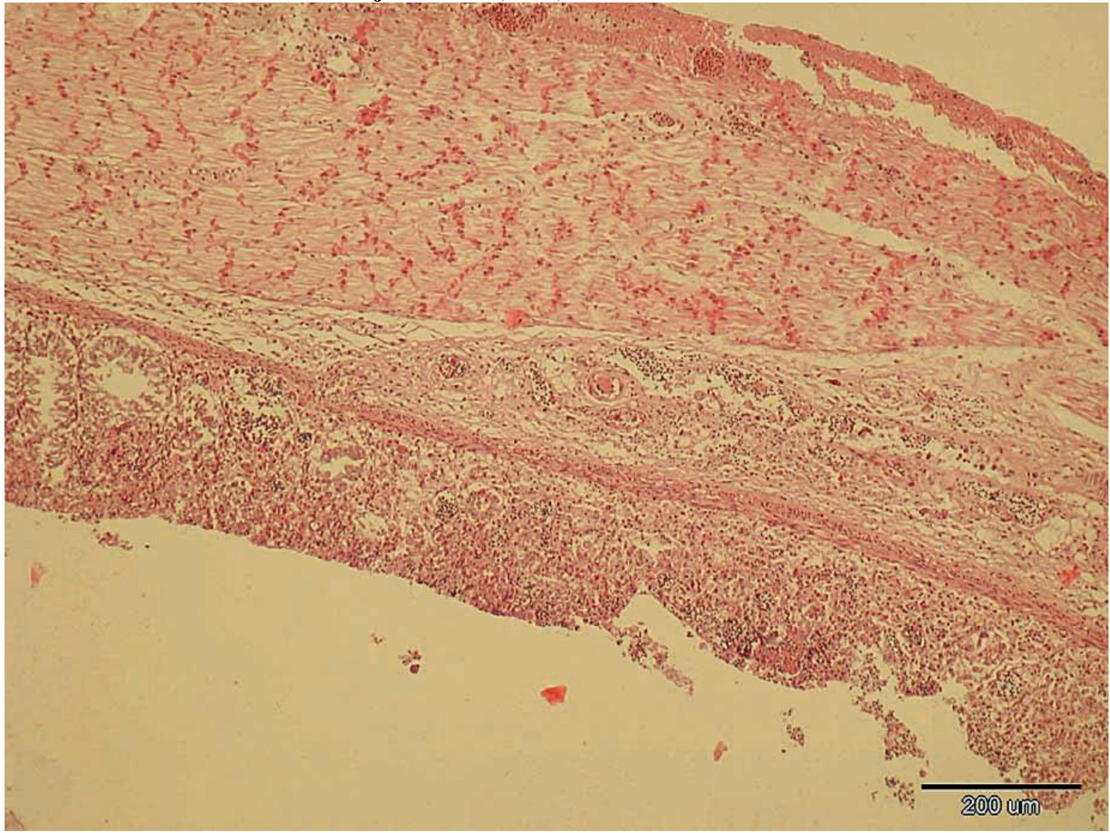
Konjevic *et al.* (2004) described the spontaneous poisoning of two Brahma chickens with T-2 toxin, diacetoxyscirpenol and deoxynivalenol. Two out of 10 chickens died under signs of depression and loss of appetite. Histopathological analysis revealed vacuolar dystrophy of the liver, necrosis and depletion of lymphocyte in the bursa of Fabricius as well as multiple necroses in the glandular stomach and gut. Even though quantities of 0.70 mg/kg T-2 in the food together with 0.50 mg/kg diacetoxyscirpenol significantly differ from the median lethal dose for chickens reported in literature (4.97 mg/kg), parasitological, virological and histopathological results indicate trichotecenes as the causative agents of this pathological condition.



The liver of a 2-month-old Brahma chicken affected by trichotecens. Advanced vacuolar dystrophy (A) of hepatocytes associated with passive hyperaemia. Haemotoxylin and eosin _ 20, bar_ 200 mm.
Konjevic *et al.* (2004)



The Bursa Fabricii of a 2-month-old Brahma chicken affected by trichotecens. Note the necrotic areas in follicles of Bursa Fabricii (A) and the markedly decreased number of lymphocyte (B). Haemotoxylin and eosin_ 40, bar_ 100 mm. **Konjevic *et al.* (2004)**



Severe necrotic typhlitis. Haemotoxylin and eosin _ 20, bar_ 200 mm. **Konjevic *et al.* (2004)**

Kamalavenkatesh *et al.* (2005) fed forty, newly hatched, unsexed broiler chicks diets containing 10 ppm cyclopiazonic acid (CPA) and 1 ppm T-2 toxin (T2) either individually or in combination for 28 days to study the immunopathological effects. Lymphoid organs revealed lymphocytolysis and lymphoid depletion in all toxin fed birds. Thymic and splenic CD+4 and CD+8 lymphocytes decreased significantly (p<0.01) in toxin fed birds when compared to the control. Thymic CD+8 lymphocytes of T2 and CPA-T2 showed significant (p<0.01) decrease from that of CPA and control groups. Splenic CD+4 and CD+8 lymphocytes showed significant (p<0.01) decrease in CPA and CPA-T2 fed groups when compared to the control. The T2 group did not differ significantly from that of control. The stimulation index (SI) of splenocytes to concavalin A revealed significant (p<0.01) decrease in all toxin fed birds. Significant (p<0.01) decrease were observed for the haemagglutination inhibition (HI) titres to Newcastle disease virus vaccine F strain (NDV) of birds fed CPA, T2 and in combination. Significant (p<0.01) interaction was found for lymphocyte subsets, SI and HI titres to NDV. The study indicated the immunosuppressive effect of these toxins either alone or in combination in broiler chicks.

Shareef (2005) evaluated and recorded the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

Venkatesh *et al.* (2005) distributed thirty-six, twenty-eight-day-old broiler chicks randomly into three groups of 12 birds each. Two groups were fed diets containing 10 ppm cyclopiazonic acid (CPA) and 1ppm T-2 toxin, respectively, to determine the mechanism of cell death in spleen and thymus at 6, 12, 24, and 36 h of post-treatment. The other group served as control. T-2 toxin treated group showed significant (P < 0.01) induction of apoptosis in thymus with peak induction at 24 h post-treatment where as, no significant differences were observed between the control and CPA groups. The CPA toxin treated group showed significant (P < 0.01) induction of apoptosis in spleen with peak induction at 24 h post-treatment. No significant differences were observed between the control and T-2 toxin group even though the latter showed a slight increase in the quantity of apoptotic cells at 36 h post-treatment in spleen. The semi-thin sections stained with toluidine blue from the spleen of CPA treated group exhibited crescent margination of chromatin against the nuclear

envelope and shrinkage of lymphoid cells without any surrounding inflammation, the characteristics of apoptosis. The apoptotic thymocytes from T-2 fed birds appeared shrunken with condensed nucleus and showed crescent margination of chromatin against the nuclear envelope without any surrounding inflammation when compared with well-defined nuclei with dispersed chromatin in normal thymocytes. Ultrastructurally, splenocytes of the CPA treated group and thymocytes of the T-2 toxin treated birds showed apoptotic bodies characterized by crescent margination of the chromatin against the nuclear envelope. The study indicates that one route of the CPA and T-2 toxin induced cell death in lymphoid organs of broiler chicken is by apoptosis.

Jaradat *et al.* (2006) studied the effect of T-2 toxin on chicken lymphocyte proliferation in the presence of mitogens and the subsequent protection with Vitamin E in both fat and water soluble forms using an MTT colorimetric assay. T-2 toxin was administered in concentrations ranging from 0 to 10ng/mL of lymphocytes in the presence of either concanavalin A (ConA) or phytohemagglutinine (PHA-M) at optimum concentration of 333ng/mL and a dilution of 1:160 for ConA and PHA-M, respectively. Lymphocyte proliferation in response to ConA and PHA-M mitogens was depressed at T-2 doses of 1ng/mL or higher ($p<0.05$). The proliferation was completely abolished at 10ng/mL when the toxin was added at 0 time, while it was decreased by 80% when the toxin was added to the lymphocytes after 24h. The addition of Vitamin E in the fat soluble form (alpha-tocopheryl acetate) did not exert any protection effect against the toxin when it was added at either 25 or 100microg. However, when the water soluble form (Trolox) was added at a concentration of (200microg) (equivalent to 100microM of alpha-tocopherol), it provided considerable protection ($p<0.05$) against T-2 toxin inhibition of lymphocyte proliferation. The difference in the effect between the two forms of Vitamin E might be related to their relative solubility in the culture media which in turn may affect their availability for protection.

Krishnamoorthy *et al.* (2006) evaluated and recorded the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

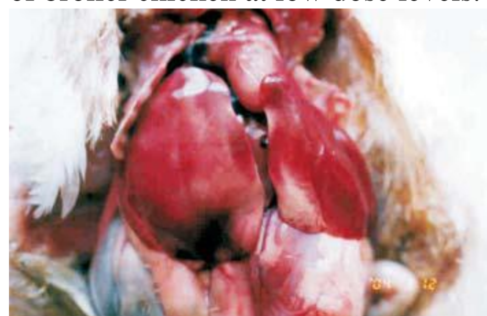
Pande *et al.* (2006) carried out an experimental study to evaluate and record the effects of T-2 toxicity alone and in association with IBV infection on

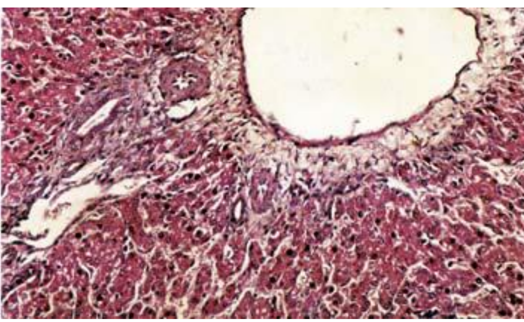
haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

Yegani *et al.* (2006) performed an experimental study to evaluate and record the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

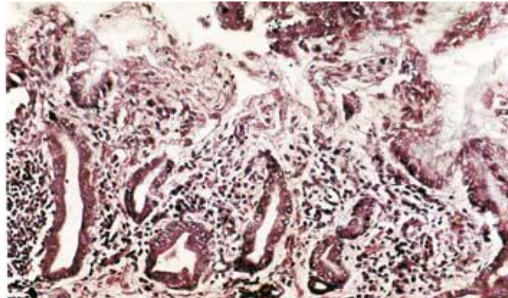
Krishnamoorthy *et al.* (2007) fed forty-eight, newly hatched, unsexed broiler chicks diets containing 45 ppm chlorpyriphos, an organophosphorus compound and 0.5 ppm T-2, a mycotoxin, singly and in combination for 28 days from day of hatch to study pathological effects. Gross, pale, enlarged liver, distended gall bladder and streaks of haemorrhages in the thigh muscles were observed in the chlorpyriphos group, while the chlorpyriphos+T-2 group showed pale and enlarged liver. Histopathological changes observed in the toxin-fed birds during 14th and 28th days of the trial were as follows: liver revealed periportal fibrosis, mononuclear cell infiltration, necrosis of hepatocytes and bile duct hyperplasia in all the toxin-fed birds. Kidney showed tubular epithelial degeneration and necrosis in chlorpyriphos and chlorpyriphos+T-2-fed birds. Hearts of all toxin treated birds showed vacuolar degeneration of myocytes. The chlorpyriphos+T-2-fed birds showed necrosis of oral mucosa with infiltration of heterophils predominantly, along with mononuclear cells. Crop mucosa showed epithelial hyperplasia and keratinisation in all treatment groups. Proventriculus showed hyperplasia of epithelial cells, glandular necrosis and infiltration of

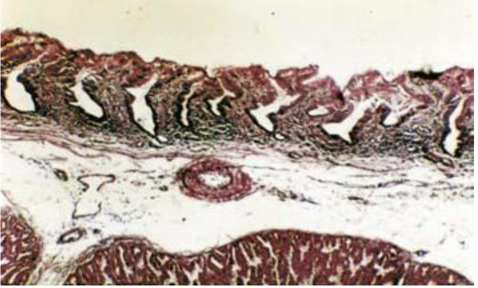
mononuclear cells in chlorpyriphos and chlorpyriphos+T-2 groups. The T-2 group showed epithelial necrosis, crypt elongation, diphtheritic membrane formation and mononuclear cell infiltration in lamina propria. Gizzard showed glandular interstitial fibrosis, infiltration of heterophils and mononuclear cells in chlorpyriphos, while T-2 groups and chlorpyriphos+T-2 groups showed interstitial glandular fibrosis and hyperplastic reaction. Intestine showed fusion of villi, necrosis, goblet cell hyperplasia and infiltration of mononuclear cells in lamina propria in all toxin-fed birds. Mononuclear cell infiltration, reduced zymogen granules and vacuolar degeneration in chlorpyriphos and chlorpyriphos+T-2 fed birds; mononuclear cell infiltration in T-2 fed birds was observed in pancreas. The chlorpyriphos group alone showed mononuclear cell infiltration in the meninges of brain. The study indicated the pathological effects of these toxins, either alone or in combination, in various organs of broiler chicken at low dose levels.



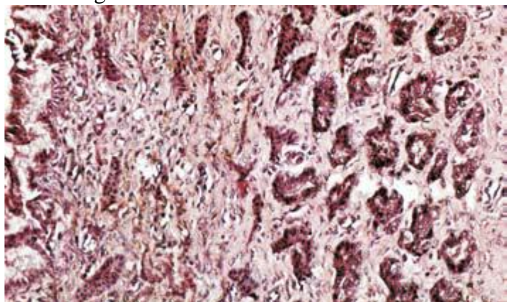


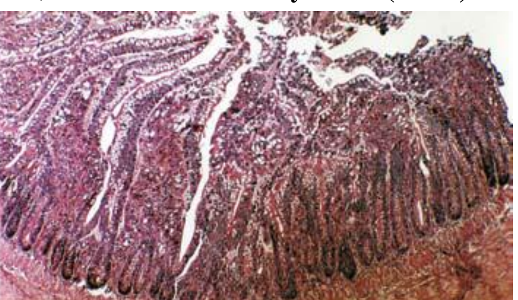
Chlorpyriphos+T-2 toxicoses. Two-week-old broiler chicken liver: pale and enlarged Chlorpyriphos+T-2 toxicoses. Two-week-old broiler chicken liver: mild periportal fibrosis and bile duct hyperplasia. H&E; ×400. **Krishnamoorthy *et al.* (2007)**





Chlorpyriphos toxicosis. Four-week-old broiler chicken proventriculus: crypt elongation and infiltration of mononuclear cells in lamina propria. H&E; ×400 T-2 toxicosis. Four-week-old broiler chicken proventriculus: shortening of villi and mononuclear cell infiltration. H&E; ×320. **Krishnamoorthy *et al.* (2007)**





Chlorpyriphos toxicosis. Two-week-old broiler chicken gizzard: glandular interstitial fibrosis. H&E; ×320. Chlorpyriphos+T-2 toxicoses. Two-week-old broiler chicken intestine: fusion of villi andgoblet cell hyperplasia. H&E; ×125. **Krishnamoorthy *et al.* (2007)**

Ogunbo *et al.* (2007) conducted two experiments to evaluate the individual and combined effects of fusaric acid (FA) and T-2 toxin (T-2) in broiler chicks and turkey

poults. In each experiment, 80 day-old birds were allotted randomly to a 2×2 factorial arrangement with treatments of 0 and 250 mg FA/kg feed and 0 and 4 mg T-2/kg feed. Diets were fed to 4 pen replicates of 5 birds each for 21 days. Feed intake and body weight gain of poults were reduced by the T-2 and the FA\T-2 combination diets. Poults fed T-2 and the FA\T-2 combination diets were also less efficient in converting feed to gain. There were no treatment effects on performance of broilers. Poults fed FA and the FA\T-2 combination diets had increased heart weights, whereas chicks fed FA and the FA\T-2 combination diets had increased kidney weights. Poults fed the combination FA\T-2 diet had higher serum Mg. Uric acid concentrations were higher in chicks fed the FA and FA\T-2 combination diets. Oral lesions were present in chicks (68%) and poults (100%) fed T-2 with or without FA. Data indicate no toxic synergy when FA and T-2 were fed simultaneously to broilers and turkeys at these dietary concentrations.

Rezar *et al.* (2007) evaluated the effects of different concentrations of T-2 toxin in feed on performance, lipid peroxidation, and genotoxicity in vivo. For a 17-d period, T-2 toxin was added to the diet of the chickens. Fifty 22-d-old male broiler chickens were divided into 5 groups that were supplemented with different concentrations of T-2 toxin: control (0.0 mg/kg of feed), T 0.5 (0.5 mg/kg of feed), T 1.5 (1.5 mg/kg of feed), T 4.5 (4.5 mg/kg of feed), and T 13.5 (13.5 mg/kg of feed). Deoxyribonucleic acid fragmentation in spleen leukocytes, malondialdehyde in plasma and liver, total plasma antioxidative status, glutathione peroxidase activity, and total serum Ig (IgA and IgG) were measured. Feed consumption and BW gain decreased when the concentration of T-2 toxin was 4.5 and 13.5 mg/kg of feed. Compared with the control group, the rate of DNA damage increased significantly in the group fed 13.5 mg of T-2 toxin/kg of feed. In contrast to DNA fragmentation, indicators of oxidative stress did not show differences between groups fed T-2 toxin and the control. More serum IgA was detected in the group T 13.5 compared with the control, whereas there were no differences in serum IgG levels. The results of the present study indicate that impaired performance, DNA fragmentation in spleen leukocytes, and elevated serum IgA levels induced by T-2 toxin are dose-dependent. Based on our results, we could not confirm the hypothesis that oxidative stress is among the mechanisms by which T-2 toxin induces DNA fragmentation.

Young *et al.* (2007) monitored the degradation of 12 trichothecene mycotoxins by chicken intestinal microbes by liquid chromatography-ultraviolet-mass spectrometry under positive ion atmospheric pressure chemical ionization. Two pathways were observed: deacylation and deepoxidation. Essentially complete conversions to the deepoxy metabolites were observed for the non-acylated trichothecenes 4-deoxynivalenol, nivalenol, and verrucarol. However, deacetylation was the predominant pathway for the monoacetyl trichothecenes 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol (15ADON), and fusarenon X. Small amounts of the deepoxy metabolites were observed from 15ADON and large amounts from 15-monoacetoxyscirpenol where steric hindrance protected the C-15 acetyl groups from enzymatic attack. Diacetylated trichothecenes diacetoxyscirpenol and neosolaniol exhibited only deacetylation. The larger isovaleryl functionality was resistant to removal and deepoxidation was the prevalent reaction in HT-2 toxin and T-2 triol, whereas T2 toxin showed only deacetylation.

Girish ***et al.*** **(2008a)** conducted an experiment to investigate the effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on performance, hematology, metabolism, and immunological parameters of turkeys. The efficacy of polymeric glucomannan mycotoxin adsorbent (GMA) in preventing these adverse effects was also evaluated. Three hundred 1-d-old male turkey poults were fed wheat-, corn-, and soybean mealbased starter (0 to 3 wk), grower (4 to 6 wk), developer (7 to 9 wk), and finisher (10 to 12 wk) diets formulated with uncontaminated grains, contaminated grains, and contaminated grains + 0.2% GMA. Feeding contaminated grains significantly decreased BW gains during the grower and developer phases, and GMA supplementation prevented these effects. There was no effect of diet, however, on feed intake or feed efficiency. The feeding of contaminated grains reduced total lymphocyte counts at wk 3 ($P < 0.05$). Dietary supplementation with GMA increased plasma total protein concentrations compared with controls and birds fed the contaminated diet. Plasma

Krishnamoorthy ***et al.*** **(2008)** observed that wing feathers of broiler chickens which were fed 1 ppm T- 2 toxin mixed feed for 28 days were seen shortened with clear primary and secondary wing feathers on the 2nd and 4 th week of age. This pattern of wing growth was attributed to the effect of T-2 toxin on protein synthesis as a result of liver damage and inhibition of amino acid synthesis, indirectly affecting the growth of wing feathers in broiler chickens. Mortality rate of 5% due to T-2 toxicosis was also observed by Balachandra et al. (2008) in broiler chickens.

Sokolovic ***et al.*** **(2007)** developed a protocol for detection of DNA damage induced by T-2 toxin in chicken blood cells. Chickens were administered orally with T-2 toxin and the samples of whole blood were collected at 24 h post treatment. The DNA damage was determined by an increase in the comet parameters in tested animals. Our results show that T-2 toxin had induced significant DNA damage in treated chicken as compared with control animals, indicating that the assay can be used for the assessment of primary DNA damage caused by mycotoxins.

SOKOLOVI ***et al.*** **(2008a)** reviewed the incidence and toxic effects of T-2 toxin in poultry. They mentioned that toxic effects of **T-2 toxin** in poultry include inhibition of protein, DNA, and RNA synthesis, cytotoxicity, immunomodulation, cell lesions in the digestive tract, organs and skin, neural disturbances and low performance in poultry production (decreased weight gain, egg production, and hatchability). Concentrations of T-2 toxin in feed are usually low, and its immunosuppressive effects and secondary infections often make diagnosis difficult. If at the onset of the disease, a change in diet leads to health and performance improvements in poultry, this may point to mycotoxin poisoning. Regular control of grain and feed samples is a valuable preventive measure, and it is accurate only if representative samples are tested.

Sokolović ***et al.*** **(2008b)** mentioned that T-2 toxin is the most toxic type A trichothecene mycotoxin. It is the secondary metabolite of the Fusarium fungi, and is common in grain and animal feed. Toxic effects have been shown both in experimental animals and in livestock. It has been implicated in several outbreaks of human mycotoxicoses. Toxic effects in poultry include inhibition of protein, DNA, and RNA synthesis, cytotoxicity, immunomodulation, cell lesions in the digestive

tract, organs and skin, neural disturbances and low performance in poultry production (decreased weight gain, egg production, and hatchability). Concentrations of T-2 toxin in feed are usually low, and its immunosuppressive effects and secondary infections often make diagnosis difficult. If at the onset of the disease, a change in diet leads to health and performance improvements in animals, this may point to mycotoxin poisoning. Regular control of grain and feed samples is a valuable preventive measure, and it is accurate only if representative samples are tested. This article reviews the incidence and toxic effects of T-2 toxin in poultry.

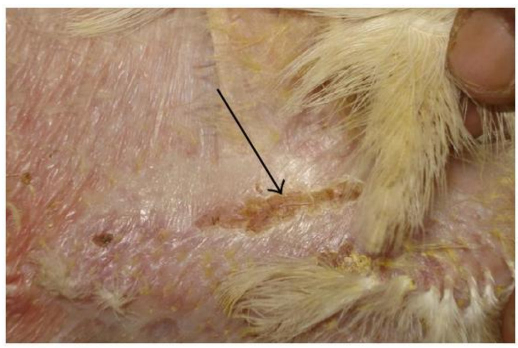
Kalantari and Moosavi (2010), in their review on T-2 toxin, mentioned that, the major attribute of T-2 toxin is that it inhibist protein synthesis which is followed by a secondary disruption of DNA and RNA synthesis. T-2 toxin affects the actively dividing cells such as those lining the gastrointestinal tract, skin, lymphoid and erythroid cells. It can decrease antibody levels, immunoglobolines and certain other humoral factors. In addition, in this review article acute and chronic effects on health, toxicokinetics, regulatory matters related to its use as a potential warfare and treatment strategies that may be undertaken will be briefly covered

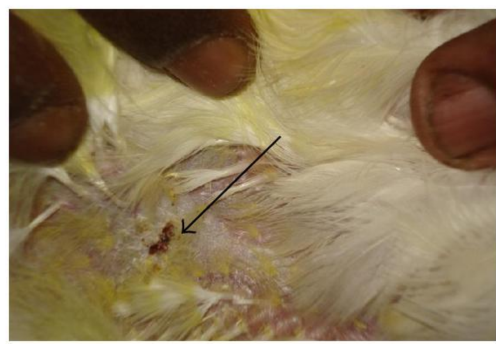
Yohannes *et al.* (2010) evaluated and recorded the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection.

Nesic *et al.* (2009) performed a 21-day-long experiment on 160 one-day-old 'Ross' broiler chicks. This research was done with the aim of investigating pathomorphological changes in broilers exposed to a relatively small amount of T-2 toxin (2 ppm) and the possibility of prevention and/or alleviating adverse effects of T-2 toxin using various feed additives. Pathohistological examination showed negative consequences of T-2 toxin in all examined organs as degenerative changes developed in small intestine mucosa, enterocites and hepatocites necroses, as well as lymphocites depletion in bursa of Fabricius. Disparately from inorganic (Minazel-plus, Mz) and organic (Mycosorb, Ms) adsorbents, which did not provoke protective effects, in liver, small intestine and bursa of Fabricius of broilers who were given feed with T-2 toxin and mixed adsorbent (Mycofix-plus, Mf), mostly preserved structure of these organs could be noted.

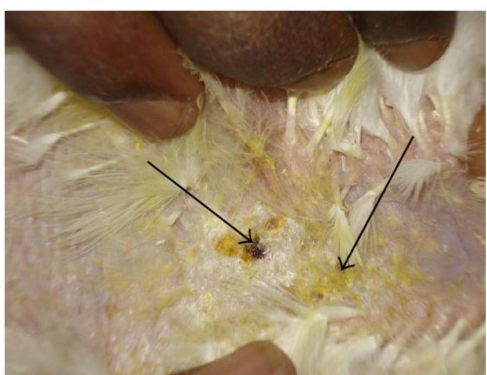
Ramasamy *et al*. (2010) studied the immunoprotective effect of seabuckthorn berries and glucomannan against T-2 toxin-induced immunodepression in 15-day-old chicks.

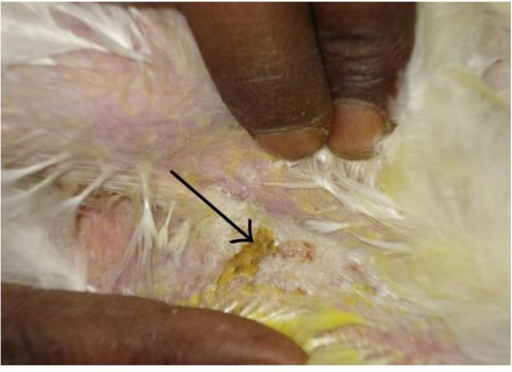
T-2 toxin was produced in the laboratory by growing Fusarium sporotrichioides MTCC 2081 on wheat. T-2 toxin was fed to birds at 1 ppm level of the diet. The powdered seabuckthorn berries were added at 400 and 800 ppm levels, and glucomannan added at 1 g/kg of feed. All the treatments were continued up to 28 days. The immunoprotective effects of seabuckthorn and glucomannan were assessed by evaluating humoral immune reaction against NCD vaccine (haemagglutination test and immunoglobulin estimation), serum immunoglobulin levels, phagocytic index, and DTH reaction against DNFB between day 25 and day 28 of experiment. There was significant ($P < .05$) decrease in non-specific immunity in T-2 toxin-treated group as evidenced by a reduction in phagocytic index, DTH reaction, HI titer, and total serum Ig compared to the healthy control group. A significant increase ($P < .05$) in HI titer and total serum Ig was seen in seabuckthorn and glucomannan fed group. A significant ($P < .05$) increase in DTH reaction and non-specific immune response was seen in seabuckthorn and glucomannan fed birds. The present investigation revealed that the seabuckthorn alone protected the immunosuppressant action of T-2 toxin, but seabuckthorn and glucomannan in combination provided an additive protection against T-2 toxicity.



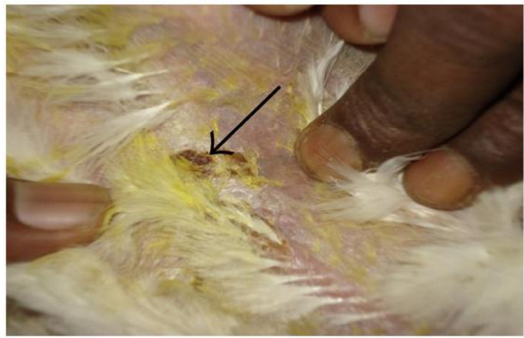
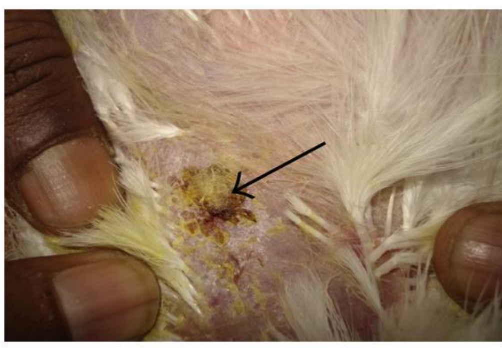


DTH reaction of skin (control): increased thickness and scab formation.,DTH reaction of skin (T-2 toxin fed bird): minimal skin thickness and scab formation. **Ramasamy *et al.* (2010)**

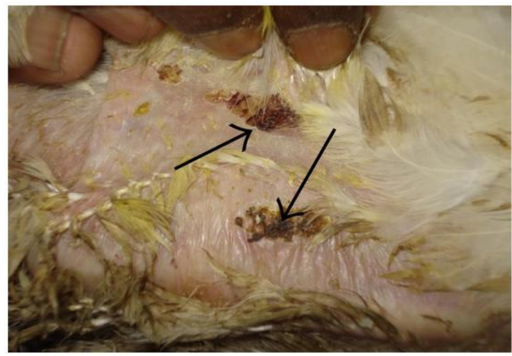




DTH reaction of skin (T-2 toxin plus GM fed bird): mild increase in skin thickness and scab formation compare to toxin-fed bird., DTH reaction of skin (T-2 toxin plus SBT 400 ppm): mild increase in skin thickness and scab formation compare to toxin fed bird. . **Ramasamy *et al.* (2010)**

DTH reaction of skin (T-2 toxin plus SBT 800 ppm): moderate increase in skin thickness, ulceration, and scab formation compare to toxin-fed bird., DTH reaction of skin (T-2 toxin plus SBT 400 ppm plus GM): severe increase in skin thickness, ulceration, induration, and scab formation compare to toxin-fed bird. **Ramasamy *et al*. (2010)**



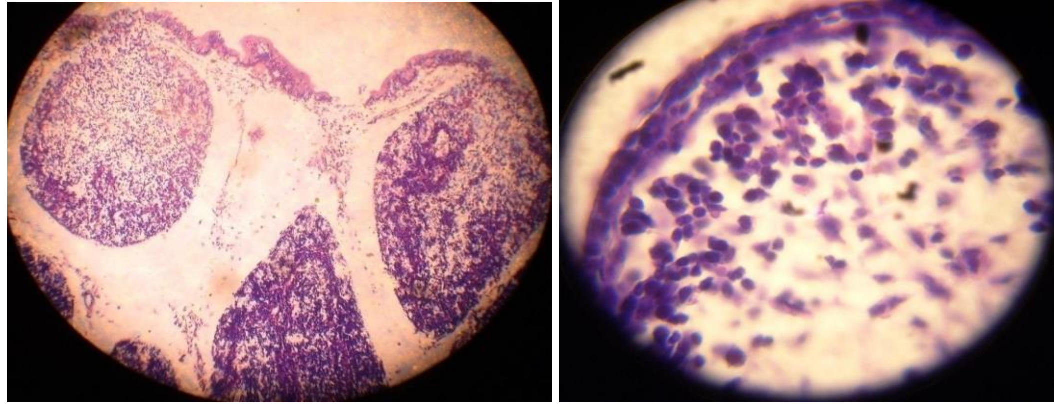
DTH reaction of skin (T-2 toxin plus SBT 800 ppm plus GM fed bird): severe increase in skin thickness, erythema, ulceration, induration, and large area of scab formation compare to toxin fed bird.

Xue et *al.* (2010) investigated the immunopathological effects of combinations of ochratoxin A (OTA) and T-2 toxin on broilers. Four hundred eighty 1-d-old broilers were randomly assigned to 4 groups, each group consisting of 4 duplicates each with 30 broilers. The 4 groups were fed the following diets for 4 wk: group 1 = basal diet (control, mycotoxin-free); group 2 = basal diet + 2,000 mg/kg of Mycofix Plus; group 3 = basal diet + 0.25 mg/kg of OTA and 0.5 mg/kg of T-2; and group 4 = basal diet + 0.25 mg/kg of OTA and 0.5 mg/kg of T-2 + 2,000 mg/kg of Mycofix Plus. The feeding of OTA-T-2 toxin diets reduced ($P < 0.05$) the level of anti-Newcastle disease virus antibody titers by 10.4%. When broilers were administered lipopolysaccharide, the results of real-time PCR showed that broilers fed OTA-T-2 toxin reduced the cytokine mRNA expression levels of interleukin-2 and interferon-γ to some extent but not significantly ($P > 0.05$). The concentrations of interleukin-2 and interferon-γ in serum were significantly decreased ($P < 0.05$) by OTA-T-2 toxin combination. Histopathological studies demonstrated that OTA-T-2 toxin combination caused abnormalities in the thymus, bursa of Fabricius, spleen, and liver. Ochratoxin A-T-2 toxicity could be counteracted by Mycofix Plus partially but not significantly ($P >$ 0.05). The concentrations of OTA and T-2 toxin used in this study are under the maximum tolerated levels recommended by Canadian Food Inspection Agency. Our study clearly put the standard and detoxification method for these toxins into question. We suggest that it may be time to reduce the maximum allowable limits of

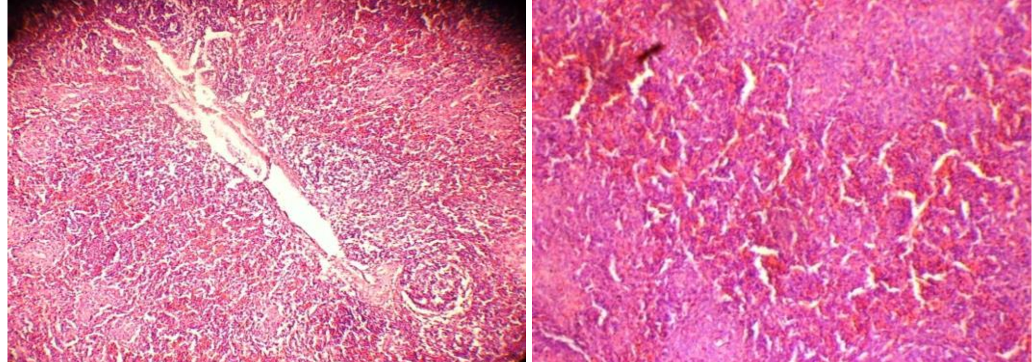
OTA and T-2 mycotoxins in feeds to improve animal health and the safety of the food chain.

Manafi *et al.* (2012) studied the synergistic effects of two contaminating mycotoxins aflatoxin (AF) and T-2 toxin (T-2) in the feed of poultry on the performance of broiler chickens were studied individually and in combination, by using one hundred and sixty eight day-old commercial broiler chicks obtained from a commercial hatchery and randomly separated into four groups in 2X2 Complete Randomized Design of three replicates and fourteen chicks per replicate, with dietary treatments of 0.0 (control), 0.5μg/g AF, 2.0μg/g T-2 and their combination (0.5 μg/g AF+2.0 μg/g T-2). The chicks were housed in deep litter independent conventional system with feed and water ad libitum throughout the experimental study. The toxin treated birds exhibited a significant (P 0.05) decrease in total serum protein, albumin and uric acid. The serum alanine amino transferase (ALT) levels were decreased and antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) were also decreased significantly (P 0.05). These findings were more severe in the combined group of AF and T-2. Results indicated that the presence of AF and T-2 in the diet may have a very severe synergistic effect on these measured factors of the commercial chicks.

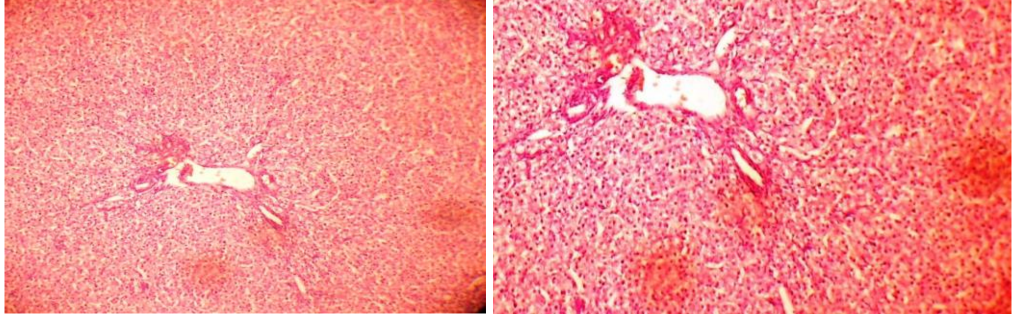
Shereen (2012) studied the histopathological changes of some internal organs in broilers fed **T-2 Toxin**. Forty, one-day-old male broiler chicks (Ross 308), were randomly distributed at one day of age to 2 experimental groups consisting of 10 birds with two replicates for 35 days. Group 1 fed control diet with no T-2 toxin (negative control), while group 2 fed T-2 toxin contaminated diet at a rate of 4 ppm. Scarifying birds done at the end of the experiment, bursa of Fabricius, spleen, liver, kidney and intestine, were sectioned for microscopical examination. Results showed that T-2 toxin, was hepatotoxic, nephrotoxic, toxic to lymphatic tissue, haemopoetic tissue, and gastrointestinal tissues. And these organs are considered to be the target organs for T-2 toxin which primarily affected during T-2 toxicosis.



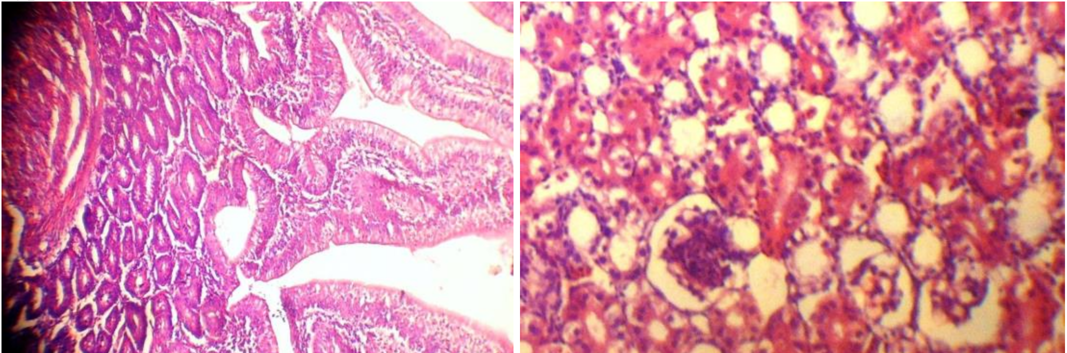
Histopathological depletion and atrophy of lymphoid tissue in the cortex and medulla , lymphocytolysis. lymphoid necrosis and interfollicular odema and mild fibrosis in bursa of Fabricius of broilers fed T-2 toxin.(35X), Magnification showing reduction in the cortical area, lymphocytolysis, and necrosis in more than 50 per cent of cells of medulla of bursa of Fabricius of broilers fed T-2 toxin. (200X) **Shereen (2012)**



Pathohistological picture of spleen show isolated lymphoid cell depletion and necrosis in broilers fed T-2 toxin.(65X)**,** Pathohistological picture of spleen show diffuse hyperaemia and haemorrhagic foci in broilers fed T-2 toxin.(200X) **Shereen (2012)**



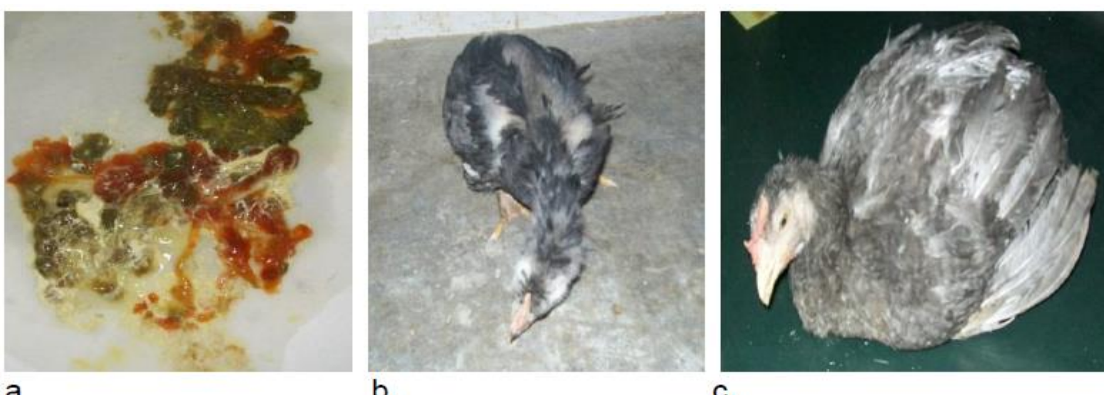
Pathohistological examination of liver from broilers fed T-2 toxin, revealed cytoplasmic vacuolation in moderately swollen hepatocytes.(145X)Magnification of showing periportal fibrosis, bile duct hyperplasia, and hepatocytes , vacculation in broilers fed T-2 toxin. (145X) **Shereen (2012)**



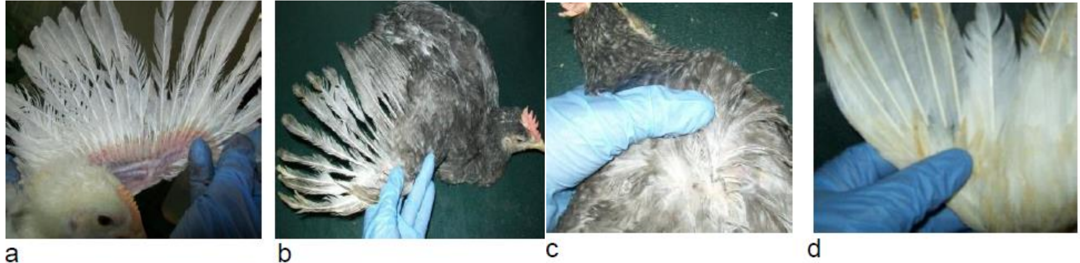
Pathohistological examination of small intestine showing shortening, fusion of Villi, and goblet cell hyperplasia in broilers fed T-2 toxin.(35X)**,** Chickens fed T-2 toxin showed tubular epithelial degeneration and necrosis of affected kidneys(165X) **Shereen (2012)**

Yohannes *et al.* (2012) assessed the effects of **T-2** mycotoxin at dose level of 2 ppm in association with infectious bronchitis virus (IBV) infection on growth performance and clinical signs of broiler chickens. A total of 128 one-week-old chicks were classified into four groups and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and untreated (control) for a period of 6 weeks. The treatment groups exhibited variable degrees of dullness, lethargy, and dehydration, intensity of which increased with duration of treatment. The T-2 toxicated birds, in addition, showed thin and haemorrhagic droppings. The birds treated with T-2+IBV exhibited severe weakness, recumbency, extending and dragging of neck on the ground and gasping (respiratory distress) at 48 h post IBV infection. Characteristic ruffled, thin shafted, uneven and fewer hairs of feathers were also noticed in almost all toxicated birds. Body weights were lower in toxin groups since 2 nd week of toxin feeding. In IBV groups, however, birds did not show much

difference from control, but it was higher than toxin groups. The mean percent body weight gains (BWG) and feed conversion ratios (FCR) in all treatment groups were significantly reduced from 3 weeks of toxin feeding or 3rd day post infection (DPI) onwards.



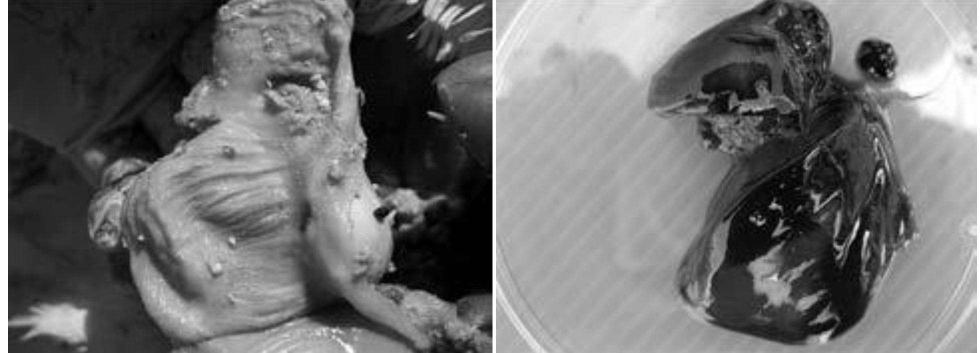
Clinical signs manifested by different groups at different interval. a. Toxin group, 4 WTF, Thin mucoid and haemorrhagic droppings. b. T-2+IBV group, chick at 4 (10) WTF (DPI) showed depressed, unable to move and respiratory distress. C. T-2+IBV group, chicks at 5 WTF showed weakness, depression, stretching feather and open mouth indicating respiratory distress. **Yohannes *et al.* (2012)**



Narrow, thin and ruffled feather. a. T-2 Group, 5 WTF, Narrow and stunt feathers growth. b and c. T-2+IBV group, 5 (17) WTF (DPI), narrow feathers and loss of back hair. d. Control group, 5 week: Normal feather growth. **Yohannes *et al.* (2012)**

K a p e t a n o v *et al.* (2013) underlined the significance of clinical and pathological diagnosis supported with laboratory analysis that gave an objective causative diagnosis. On the farm, the disease occurred suddenly and with total cessation of feed consumption. First cases were recorded in the flock at the age of 42 weeks. Grouping, intensive breathing and lying with overstretched legs and extended neck were symptoms observed in birds. Evident necrosis of beak tips and painful multi-focal necrosis in oral cavity were recorded during the clinical examination. On section, dark unclothed blood was first observed. Other postmortem findings included: filled gizzard with mucosal erosions and easy-removable cuticle, enlarged congested liver with multi-focal necrosis and subcapsulary bleeding. The mortality rate increased by 4%, and the drop of laying rate was by about 18%. The fertility rate decreased by 22%. There was the increased number of rejected hatching eggs, 12%. Culture of the complete diet resulted in approximately 150000 colonies per 1g of Fusarium. T-2 was detected by using ELISA in concentration of 480 µg/kg, which corresponded to the upper limit of maximum permitted concentrations for chickens, according to national legislations. This bylaw interpretation of “tolerable” concentrations of mycotoxins provokes controversy among experts and public.

Flock grouping in island– Necrotic process in oral cavity. **K a p e t a n o v *et al.* (2013)**



Gizzard: cuticle and mucosal erosions. Liver: subcapsularry bleeding **K a p e t a n o v *et al.* (2013)**

Khmelnitskiy and Korzunenko (2013) carried out a study to determine the detoxification activity of combined sorbent preparation consisted of anthracite, saponite and inactivated yeasts on the mixed chickens' mycotoxicosis, thirty, two-weeks-old chickens cross "Ross 308" were divided into three groups: A (control); B (T-2 toxin and deoxynivalenol); C (T-2 toxin, deoxynivalenol and the combined sorbent preparation). Chickens were weighed every week, hematological and serum biochemical investigations were provided at 28-th and 42-nd day of chicken`s age. Applying of the combined sorbent preparation in T-2 toxin and deoxynivalenol mixed chickens toxicosis at 3 % by weight of the feed, neutralizes the negative effects of mycotoxins on the bird. It manifests high yield carcass weight and lowers the feed conversion, with almost no variations in hematological and serum biochemical parameters of blood

Shang *et al.* (2013) reported that T-2 toxin significantly induced CYP1A4 and CYP1A5 expression in chicken embryonic hepatocyte cells. The enzyme activity assays of CYP1A4 and CYP1A5 heterologously expressed in HeLa cells indicate that only CYP1A5 metabolizes T-2 to 3′OH-T-2 by the 3′-hydroxylation of isovaleryl groups. *In vitro* enzyme assays of recombinant CYP1A5 expressed in DH5α further confirm that CYP1A5 can convert T-2 into TC-1 (3′OH-T-2). Therefore, CYP1A5 is critical for the metabolism of trichothecene mycotoxin in chickens

Osselaere (2013) studied the absolute oral bioavailability and the toxicokinetic parameters of deoxynivalenol, T-2 and zearalenone in broilers. Toxins were administered intravenously and orally in a two-way cross-over design. For deoxynivalenol a bolus of 0.75 mg/kg BW was administered, for T-2 toxin 0.02 mg/kg BW and for zearalenone 0.3 mg/kg BW. Blood was collected at several time points. Plasma levels of the mycotoxins and their metabolite(s) were quantified using

LC-MS/MS methods and toxicokinetic parameters were analyzed. Deoxynivalenol has a low absolute oral bioavailability (19.3%). For zearalenone and T-2 no plasma levels above the limit of quantification were observed after an oral bolus. Volumes of distribution were recorded, i.e. 4.99 L/kg, 0.14 L/kg and 22.26 L/kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. Total body clearance was 0.12 L/min.kg, 0.03 L/min.kg and 0.48 L/min.kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. After IV administration, T-2 toxin had the shortest elimination half-life (3.9 min), followed by deoxynivalenol (27.9 min) and zearalenone (31.8 min).

Osselaere (2013) studied the effects of the mycotoxin T-2 on hepatic and intestinal drug-metabolizing enzymes (cytochrome P450) and drug transporter systems (MDR1 and MRP2) in poultry. Broiler chickens received either uncontaminated feed, feed contaminated with 68 µg/kg or 752 µg/kg T-2 toxin. After three weeks, the animals were euthanized and MDR1, MRP2, CYP1A4, CYP1A5 and CYP3A37 mRNA expression were analyzed using qRT-PCR. Along the entire length of the small intestine no significant differences were observed. In the liver, genes coding for CYP1A4, CYP1A5 and CYP3A37 were significantly down-regulated in the group exposed to 752 µg/kg T-2. For CYP1A4, even a contamination level of 68 µg/kg T-2 caused a significant decrease in mRNA expression. Expression of MDR1 was not significantly decreased in the liver. In contrast, hepatic MRP2 expression was significantly down-regulated after exposure to 752 µg/kg T-2. Hepatic and intestinal microsomes were prepared to test the enzymatic activity of CYP3A. In the ileum and liver CYP3A activity was significantly increased in the group receiving 752 µg/kg T-2 compared to the control group. The results of this study show that drug metabolizing enzymes and drug transporter mechanisms can be influenced due to prolonged exposure to relevant doses of T-2.

Yohannes *et al.* (2013) evaluated and recorded the effects of T-2 toxicity alone and in association with IBV infection on haematobiochemical parameters. A total of 128 one-week-old chicks were divided into four groups of 32 birds each and were treated respectively with T-2 toxin alone, IBV alone, T-2 toxin and co-infected with IBV, and no treatment (control) for a period of 6 weeks. Haematologically, the birds treated with T-2 toxin developed anaemia as indicated by significant decrease in haemoglobin levels, total erythrocyte counts and packed cell volume values; leucopenia, lymphocytopenia heterophilia and thrombocytopenia. The IBV infected birds exhibited lymphocytophilia and heteropoenia; the degrees of severity of leucopenia, lymphocytopenia heterophilia and thrombocytopenia were more pronounced in T-2+IBV groups. The serum biochemistry revealed hypoproteinemia and hypoalbuminemia in all the treated groups consistently. Besides, hypoglobulinemia and increased levels of alanine aminotransferase in T-2+IBV, and increased levels of alkaline phosphatase in toxin group alone were recorded. The changes in biochemical parameters were more in magnitude in the combination treatment group and their severity increased with duration of treatment. It was concluded that T-2 toxin made the birds more susceptible to IBV infection

Balogh *et al.* (2015) investigated the effect of two different contamination levels of T-2 toxin (1.5 or 3.4 mg/kg feed) in a 28- days feeding trial on body weight, relative weight of liver and spleen, and some lipid peroxidation and glutathione redox parameters of 14-days old broiler chicken. The results showed that T-2 toxin decreased significantly the body weight at both contamination levels and showed a

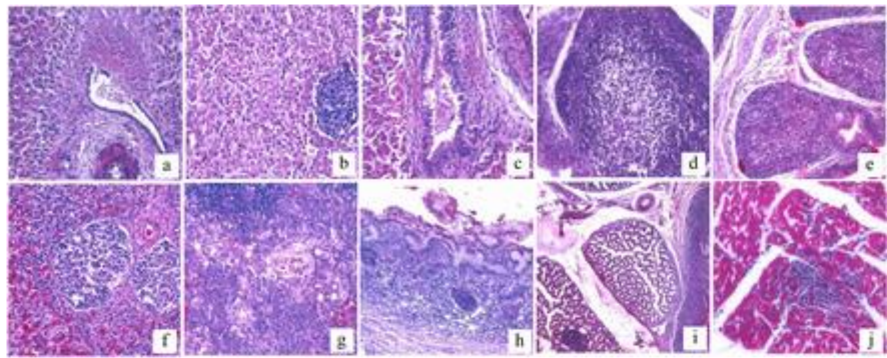
dose-dependent tendency. Relative weight of liver increased till the end of the trial, while relative weight of spleen was lower at both samplings at lower level of T-2 toxin exposure. Initial phase of lipid peroxidation (conjugated dienes and trienes) was not detected in the liver, but as product of later phase, thiobarbituric acid reactive substances increased significantly, except in the liver. Glutathione content on day 14 was higher in liver homogenate as compared to the control at the lower T-2 toxin contamination level. On day 28 it was higher in blood plasma at the higher and in liver homogenate at both levels of T-2 contamination. Glutathione peroxidase activity on day 14 was significantly higher in liver and spleen homogenates as compared to the control at the lower level of T-2 toxin contamination. On day 28, significantly higher activity was found at both T-2 toxin contamination levels in liver homogenate, and at the lower contamination level in spleen homogenate as compared to the control. The results revealed that T-2 toxin exposure initiates lipid peroxidation and activates the glutathione redox system as well, but the changes were irrespective of the dose- and partly duration of the exposure.

Guerre (2015) mentioned that , despite the fact that avian species are highly exposed to fusariotoxins, the avian species are considered as resistant to their toxic effects, partly because of low absorption and rapid elimination, thereby reducing the risk of persistence of residues in tissues destined for human consumption. This review focuses on the main fusariotoxins deoxynivalenol, T-2 and HT-2 toxins, zearalenone and fumonisin B1 and B2. The key parameters used in the toxicokinetic studies are presented along with the factors responsible for their variations. Then, each toxin is analyzed separately. Results of studies conducted with radiolabelled toxins are compared with the more recent data obtained with HPLC/MS-MS detection. The metabolic pathways of deoxynivalenol, T-2 toxin, and zearalenone are described, with attention paid to the differences among the avian species. Although no metabolite of fumonisins has been reported in avian species, some differences in toxicokinetics have been observed. All the data reviewed suggest that the toxicokinetics of fusariotoxins in avian species differs from those in mammals, and that variations among the avian species themselves should be assessed.

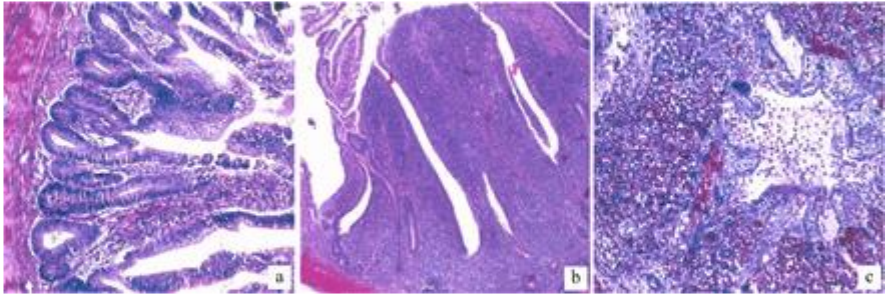
Manafi *et al.* (2015) conducted an experiment using 192 day-old Ross 308 chicks, divided into 4 groups of 4 replicate consisting 48 birds. Group I was fed a control diet, Group II was fed control diet supplemented with 0.5 ppm T-2 toxin for 5 weeks, Group III was fed control diet supplemented with 8×10^8 cfu/mL of *Mycoplasma gallisepticum*, and group IV was fed control diet supplemented by T-2 toxin and *Mycoplasma gallisepticum*. Body weight and feed conversation ratio (FCR), relative organ weights, clinical signs, biochemical characteristics, and gross and histopathological lesions were recorded in the experimental groups at the end of the second and fifth weeks of age. Body weight and relative weights of bursa of Fabricius, thymus, and spleen decreased and FCR increased significantly ($P \leq 0.05$), but the relative weights of liver and kidney showed no significant decrease ($P \leq 0.05$) in the serum total proteins, albumin, and increase in aspartate aminotransferase and alanine transaminase were observed in T-2 toxin and T-2 accompanied with Mycoplasma fed birds when compared to the control group. Liver was enlarged, friable, and yellowish discoloration with distended gall bladder was noticed. Lymphoid organs such as bursa of Fabricius, thymus, and spleen were atrophied in group II and group IV throughout the study. Microscopically, liver showed vacuolar degeneration of hepatocytes, with increased Kupffer cell activity, bile duct epithelial hyperplasia, and infiltration of inflammatory cells. Kidney showed vacuolar

degeneration of tubular epithelium along with pyknotic nuclei. Lymphoid organs showed lymphocytolysis and depletion with prominent reticuloepithelial cells. Proventriculus revealed desquamation of villous epithelial cells and lymphoid infiltration in submucosa. Heart showed mild hemorrhage with infiltration of inflammatory cells. Lung showed edema and inflammatory cells in the bronchioles. Trachea showed desquamation and erosions of mucosa. Proliferation of mucosal glands with increased mucous secretion was obvious. Air sacs showed thickening with presence of inflammatory cells and edema.

Morphology of affected and normal organs. (a) left side- group II, enlarged and congested liver, right-side normal liver on d 21; (b) group II, atrophy of thymus, bursa and enlarged liver on d 35; (c) Control, normal thymus, bursa, and liver on d 35 **Manafi *et al.* (2015)**



Morphology of different chicken organs All H.E. 200× except e and j 100× (a) liver, group II, periportal hepatocytes degeneration, and necrosis along with individualization of hepatocytes on d 14; (b) liver, group II, focal area showing degeneration and necrosis of hepatocytes with infiltration of multinucleated cells. Also note increased nucleus of Kupffer cells on d 28; (c) liver, group IV, periportal infiltration of inflammatory cells and fibrosis with mild bile duct epithelial hyperplasia on d 28; (d) bursa of Fabricius, group II, lymphocytolysis, and depletion of lymphocytes with prominent reticuloepithelial cells in medullary area on d 7; (e) bursa of Fabricius, group II, lymphocytolysis, and depletion of lymphocytes with prominent reticuloepithelial cells. Also note cystic transformation with infiltration of inflammatory cells on d 35; (f) spleen, group II, showing proliferation of lymphoblasts and formation of secondary lymphoid nodule on d 35; (g) thymus, group II, medullary area showing massive lymphocytolysis, esinophilic debris with hemorrhage on d 7; (h) proventriculus, group II, desquamation and degeneration of villus epithelium with infiltration of lymphoid cells into submucosa on d 28; (i) proventriculus, group IV, shortening of villi with increased mucus production on d 28; (j) heart, group II, degeneration of cardiac myocytes, and infiltration of mononuclear cells on d 35 **Manafi *et al.* (2015)**



Morphology of intestine, cecum, and lung of broilers. (a) Intestine, group II, desquamation, and degeneration of villous epithelium with increased in mucous secretion, H.E. 200×, on d 21; (b) cecal tonsils, group II, lymphoid hyperplasia with destruction of villi due to infiltration of lymphoid cells, H.E. 50×, on d 28; (c) lung, group IV, alveolar, and bronchial epithelium showing degeneration and necrosis with cellular debris in the bronchiole, H.E. 200×, on d 35 **Manafi *et al.* (2015)**

Kufuor-Mensah *et al.* (2016) conducted four trials to determine the effects of T-2 toxin on vaccinal immunity against MD. Day-old, white leghorn chicks of Avian Disease and Oncology Laboratory line $15I_5 \times 7_1$ were treated daily for 7 days via crop gavage with T-2 toxin at a sublethal dose of 1.25 mg/kg body weight. Treated and untreated chicks were also vaccinated with turkey herpesvirus (HVT) at hatch and were challenged with the JM strain of MD virus (MDV) at 8 days of age. Chickens were tested for HVT viremia at 1 wk post vaccination immediately before challenge, and for HVT and MDV viremia at 3 wk post challenge. Chickens were observed for the development of MD lesions and mortality within 8 wk of age. T-2 toxin significantly reduced body weight and titers of HVT viremia within 7 days after hatch. T-2 toxin shortened the incubation period for the development of MD lesions and mortality, but only in unvaccinated chickens. The percent MD protection in T-2–toxin-treated, HVT-vaccinated chickens ranged from 82% to 96% and was comparable to that in HVT-vaccinated untreated control chickens (89%–100%). The data suggest that exposure of chickens to sublethal doses of T-2 toxin for 7 consecutive days after hatch may influence the development of 1) HVT viremia; and 2) MD lesions and mortality, but only in unvaccinated chickens.

References

1. **Allen NK**, **Peguri A**, **Mirocha CJ**, **Newman JA**. Effects of fusarium cultures, T-2 toxin, and zearalenone on reproduction of turkey females. **Poult Sci.** 1983 Feb;62(2):282-9.
1. Atroshi, F., Rizzo, A., Biese, I., Veijalainen, P., Antila, E., Westermarck, T., 1997. T-2 toxin-induced DNA damage in mouse livers: the effect of pretreatment with coenzyme Q10 and alpha-tocopherol. Mol Aspects of Med 18 Suppl, S255-258.
2. Awad, A., Ghareeb, K., Böhm, J., Razzazi, E., Hellweg, P., Zentek, J., 2008. The Impact of the Fusarium Toxin Deoxynivalenol (DON) on Poultry. Int J Poult Sci 7, 827-842.
3. Balogh , Krisztián, Andrea Bócsai , Csilla Pelyhe , Erika Zándoki , Márta Erdélyi , Judit Szabó-Fodor and Miklós Mézes. J. Poult. Sci., 52: 176-182, 2015
4. Bhandarkar A (2006). Effects of T-2 toxin on growth, performance and haematobiochemical alterations in broires. Indian J. Exp. Biol., 44: 86-88
5. Chaudhari, M., Jayaraj, R., Bhaskar, A.S., Lakshmana Rao, P.V., 2009. Oxidative stress induction by T-2 toxin causes DNA damage and triggers apoptosis via caspase pathway in human cervical cancer cells. Toxicology 262, 153-161. Rezar et al., 2007).
6. Chi M.S., Robison T.S., Mirocha C.J., Swanson S.P., Shimoda W. Excretion and tissue distribution of radioactivity from tritium-labeled T-2 toxin in chicks. Toxicol. Appl. Pharmacol. 1978;45:391–402.
7. Chi M.S., Robison T.S., Mirocha C.J., Behrens J.C., Shimoda W. Transmission of radioactivity into eggs from laying hens (Gallus domesticus) administered tritium labeled T-2 toxin. Poult. Sci. 1978;57:1234–1238.

8. Christensen, C. M., R. A. Meronuck, G. H. Nelson, and J. C. Behrens. 1972. Effects on turkey poults of rations containing corn invaded by fusarium tricinctum (cda.) Sny. & Hans. Appli. Microbiol. 23:177–179.
9. Diaz, G.J., Squires, E.J., Julian, R.J., Boermans, H.J., 1994. Individual and Combined Effects of T-2 Toxin and Das in Laying Hens. Brit Poultry Sci 35, 393-405. Giroir L.E., Ivie G.W., Huff W.E. Comparative fate of the tritiated trichothecene mycotoxin, T-2 toxin, in chickens and ducks. Poult. Sci. 1991;70:1138–1143.
10. Frankic T, Pajk T, Rezar V, Levart A and Salobir J. The role of dietary nucleotides in reduction of DNA damage induced by T- 2 toxin and deoxynivalenol in chicken leukocytes. Food and Chemical Toxicology, 44: 1838-1844. 2006
11. Grizzle, Judith M., David B. Kersten, Malcolm D. McCracken, Allan E. Houston, and Arnold M. Saxton. Determination of the Acute 50% Lethal Dose T-2 Toxin in Adult Bobwhite Quail: Additional Studies on the Effect of T-2 Mycotoxin on Blood Chemistry and the Morphology of Internal Organs. Avian Diseases 48(2):392-399. 2004
12. Guerre, P. (2015). Fusariotoxins in Avian Species: Toxicokinetics, Metabolism and Persistence in Tissues. *Toxins*, *7*(6), 2289–2305.
13. Hayes MA, Wobeser GA. Subacute toxic effects of dietary T-2 toxin in young mallard ducks. Can J Comp Med. 1983 Apr;47(2):180-7.
14. Hoerr FJ, Carlton WW, Yagen B. Mycotoxicosis caused by a single dose of T-2 toxin or diacetoxyscirpenol in broiler chickens. Vet Pathol. 1981 Sep;18(5):652-64.
15. Hoerr. F. J., W. W. Carlton, B. Yagen and A. Z. Joffe, "Mycotoxicosis Caused by Either T-2 Toxin or Diacetoxyscirpenol in the Diet of Broiler Chickens," Fundamental Applied Toxicology, Vol. 2, 1982a, pp. 121-124.
16. Hoerr. F. J., W. W. Carlton, B. Yagen and A. Z. Joffe, "Mycotoxicosis Caused by Either T-2 Toxin or Diacetoxyscirpenol in the Diet of Broiler Chickens," Fundamental Applied Toxicology, Vol. 2, 1982b, pp. 121-124.
17. Iordanov MS, Pribnow D, Magun JL, Dinh TH, Pearson JA, Magun BE. 1997. Ribotoxic stress response: activation of the stress activated protein kinase JNK1 by inhibitors of the peptidyl transferase reaction and by sequence-specific RNA damage to the -sarcin/ricin loop in the 28S rRNA. Molecular and Cellular Biology 17:3373–3381
18. Jaradat ZW, Viià B, Marquardt RR. Adverse effects of T-2 toxin on chicken lymphocytes blastogenesis and its protection with Vitamin E. Toxicology. 2006 Aug 15;225(2-3):90-6.
19. Kalantari H1*, Moosavi M. REVIEW ON T-2 TOXIN. *Jundishapur Journal of Natural Pharmaceutical Products 2010; 5(1): 26-38*
20. Kamalavenkatesh P[1], Vairamuthu S, Balachandran C, Manohar BM, raj GD. Immunopathological effect of the mycotoxins cyclopiazonic acid and T-2 toxin on broiler chicken. Mycopathologia. 2005 Feb;159(2):273-9.
21. Kapetanov, Miloš, C. Kapetanov, Dubravka V. Potkonjak, Igor M. Stojanov, Milica M. Živkov – Baloš, Sandra M. Jakšić Dubravka V. Potkonjak, Igor M. Stojanov, Milica M. Živkov – Baloš, Sandra M. Jakšić. CLINICAL AND PATHOMORPHOLOGICAL DIAGNOSTICS OF MYCOTOXICOSIS IN PARENT POULTRY FLOCK CAUSED BY T-2 TRYCHOTECENE. / Jour. Nat. Sci, Matica Srpska Novi Sad, № 124, 137—143, 2013
22. Khmelnitskiy ,G. O. and V. D. Korzunenko EFFECT OF COMBINED SORBENT PREPARATION ON GROWTH, PERFORMANCE AND HAEMATOBIOCHEMICAL ALTERATIONS IN BROILER CHICKENS UNDER THE INFLUENCE OF T-2 TOXIN AND DEOXYNIVALENOL MIXED TOXICOSIS. Bangl. J. Vet. Med. (2013). 11 (1) : 7-11

23. Knupp C.A., Swanson S.P., Buck W.B. Comparative *in vitro* metabolism of T-2 toxin by hepatic microsomes prepared from phenobarbital-induced or control rats, mice, rabbits and chickens. Food Chem. Toxicol. 1987;25:859–865
24. Konjevic, D., E. Srebocan, A. Gudan, I. Lojkic, K. Severin, and M. Sokolovic. A pathological condition possibly caused by spontaneous trichotecene poisoning in Brahma poultry; first report. *Avian Pathol.*33(3):377–380. 2004.
25. Krishnamoorthy, P., S. Vairamuthu, C. Balachandra and B. Muralimanohar, "Chlorpyriphos and T-2 Toxin Induced Haemato-Biochemical Alterations in Broiler Chicken," International Journal of Poultry Science, Vol. 5, No. 2, 2006, pp. 173-177. doi:10.3923/ijps.2006.173.177]
26. Krishnamoorthy, P., S. Vairamuthu, C. Balachandran, B. Muralimanohar: Pathology of chlorpyriphos and T-2 toxin on broiler chicken. Vet. arhiv 77, 47-57, 2007.
27. Kufuor-Mensah, E., W. M. Reed, S. Sleigh, J. Pestka, A. M. Fadly, and J. R. Dunn Effects of T-2 Toxin on Turkey Herpesvirus–Induced Vaccinal Immunity Against Marek's DiseaseAvian Diseases 60(1):56-62. 2016
28. Leal, M., Shimada, A., Ruiz, F., Gonzalez de Mejia, E., 1999. Effect of lycopene on lipid peroxidation and glutathione-dependent enzymes induced by T-2 toxin in vivo. Toxicol Lett 109, 1-10.
29. Li, Y., Wang, Z., Beier, R.C., Shen, J., De Smet, D., De Saeger, S., Zhang, S., 2011. T-2 toxin, a trichothecene mycotoxin: review of toxicity, metabolism, and analytical methods. J Agric Food Chem 59, 3441-3453
30. MacDonald, E.J., Cavan, K.R., Smith, T.K., 1988. Effect of acute oral doses of T-2 toxin on tissue concentrations of biogenic amines in the rat. J Anim Sci 66, 434-441. Madheswaran R, Balachandran C, Murali-Manohar B (2005). Pathological effects of feeding aflatoxin and T-2 toxin in Japanese Quail. Indian J. Vet. Pathol., 29(1): 23-26.
31. Madheswaran, R., C. Balachandran and B. M. Manohar, "Pathological Effects of Feeding Aflatoxin and T-2 Toxin in Japanese Quail," Indian Journal of Veterinary Pathology, Vol. 29, No. 1, 2005, pp. 23-26.
32. Manafi, M., B. Umakantha, K. Mohan and H.D. Narayana Swamy . Synergistic Effects of Two Commonly Contaminating Mycotoxins (Aflatoxin and T-2 Toxin) on Biochemical Parameters and Immune Status of Broiler Chickens. World Applied Sciences Journal 17 (3): 364-367, 2012
33. Manafi,M, , N. Pirany, M. Noor Ali, M. Hedayati, S. Khalaji, and M. Yari Experimental pathology of T-2 toxicosis and mycoplasma infection on performance and hepatic functions of broiler chickensPoultry Science first published online April 23, 2015 doi:10.3382/ps/pev115
34. Minervini, F., Fornelli, F., Flynn, K.M., 2004. Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. Toxicol in Vitro 18, 21-28.
35. Mishra, U. K., P. K. Dwarakanath, S. P. Agrawal and M. I. Hossain, "Effect of T-2 Toxin on Haemostatic Profile in Growing Chickens," Indian Veterinary Journal, Vol. 73, No. 11, 1996, pp. 1133-1137.
36. Nesic , Vladimir, Darko Marinkovic, Ksenija Nesic, Radmila Resanovic. (2009) Examination of the efficacy of various feed additives on the pathomorphological changes in broilers treated with T-2 toxin. *Zbornik Matice srpske za prirodne nauke*, 49-54.
37. Pande, V.V., N. V. Kurkure and A. Bhandarkar, "Effects of T-2 Toxin on Growth, Performance and Haematobiochemical Alterations in Briores," Indian Journal of Experimental Biology, Vol. 44, 2006, pp. 86-88.
38. Puls, R. and J A Greenway. Fusariotoxicosis from barley in British Columbia. II. Analysis and toxicity of syspected barley. *Canadian Journal of Comparative Medicine*. 1976;40(1):16-19.

39. Ogunbo SO, Broomhead JN, Ledoux DR, Bermudez AJ, Rottinghaus GE (2007). The individual and combined effects of Fusaric acid and T-2 toxin in broiler and turkeys. Int. J. Poult. Sci., 6(7): 484-488
40. Osselaere, Ann Influence of deoxynivalenol and T-2 toxin on the intestinal barrier and liver function in broiler chickens, Dissertation Ghent University, Faculty of Veterinary Medicinem 2013
41. Ramasamy,T., C. Varshneya, and V. C. Katoch, "Immunoprotective Effect of Seabuckthorn (Hippophae rhamnoides) and Glucomannan on T-2 Toxin-Induced Immunodepression in Poultry," Veterinary Medicine International, vol. 2010, Article ID 149373, 6 pages, 2010.
42. Rezar, V., Frankic, T., Narat, M., Levart, A., Salobir, J., 2007. Dose-dependent effects of T-2 toxin on performance, lipid peroxidation, and genotoxicity in broiler chickens. Poultry Sci 86, 1155-1160.
43. Richard JL, Cysewski SJ, Pier AC, Booth GD. Comparison of effects of dietary T-2 toxin on growth, immunogenic organs, antibody formation, and pathologic changes in turkeys and chickens. Am J Vet Res. 1978 Oct;39(10):1674-9.
44. Rizzo, A.F., Atroshi, F., Ahotupa, M., Sankari, S., Elovaara, E., 1994. Protective effect of antioxidants against free radical-mediated lipid peroxidation induced by DON or T-2 toxin. Zentralbl Veterinarmed A 41, 81-90.
45. Rocha, O., Ansari, K., Doohan, F.M., 2005. Effects of trichothecene mycotoxins on eukaryotic cells: A review. Food Addit Contam 22, 369-378.
46. Ruff MD, Huff WE, Wilkins GC. Characterization of the toxicity of the mycotoxins aflatoxin, ochratoxin, and T-2 toxin in game birds. III. Bobwhite and Japanese quail. Avian Dis. 1992 Jan-Mar;36(1):34-9.
47. Shang S, Jiang J, Deng Y. Chicken Cytochrome P450 1A5 Is the Key Enzyme for Metabolizing T-2 Toxin to 3′OH-T-2. *International Journal of Molecular Sciences*. 2013;14(6):10809-10818. doi:10.3390/ijms140610809.*
48. Shareef A. M., "Some Immunosuppressive Effect of T-2 Toxin in Broiler Chicks," Iraqi Journal of Veterinary Sciences, Vol. 19, No. 2, 2005, pp. 99-108.
49. Shifrin, V.I., Anderson, P., 1999. Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. J Biol Chem 274, 13985-13992.
50. Shereen K. K. Histopathological changes of some internal organs in broilers fed T-2 Toxin. AL-Qadisiya Journal of Vet.Med.Sci. Vol./11 No./2 2012
51. Sklan, D., Klipper, E., Friedman, A., Shelly, M., Makovsky, B., 2001. The effect of chronic feeding of diacetoxyscirpenol, T-2 toxin, and aflatoxin on performance, health, and antibody production in chicks. J Appl Poultry Res 10, 79-85.
52. Sklan, D., Shelly, M., Makovsky, B., Geyra, A., Klipper, E., Friedman, A., 2003. The effect of chronic feeding of diacetoxyscirpenol and T-2 toxin on performance, health, small intestinal physiology and antibody production in turkey poults. Brit Poultry Sci 44, 46-52.
53. Sklan, D, Klipper, E., Friedman, A., Shelly, M., Makovsky, B. THE EFFECT OF CHRONIC FEEDING OF DIACETOXYSCIRPENOL, T-2 TOXIN, AND AFLATOXIN ON PERFORMANCE, HEALTH, AND ANTIBODY PRODUCTION IN CHICKS. 2001 J. Appl. Poultry Res. 10:79–85
54. Sokolovic, M., Garaj-Vrhovac, V., Simpraga, B., 2008a. T-2 toxin: Incidence and toxicity in poultry. Arh Hig Rada Toksikol 59, 43-52.2008
55. Sokolovic M, Garaj-Vrhovac V, Ramic S, Simpraga B. Chicken nucleated blood cells as a cellular model for genotoxicity testing using the comet assay. Food Chem Toxicol. 2007 Nov;45(11):2165-70.
56. Speers GM, Mirocha CJ, Christensen CM, Behrens JC. Effects on laying hens of feeding corn invaded by two species of Fusarium and pure T-2 mycotoxin. Poult Sci. 1977 Jan;56(1):98-102.

57. Ueno,Y., M. Nakajima, K. Sakai, K. Ishii, N. Sato, and N. Shimada, “Comparative toxicity of trichothecene mycotoxins: inhibition of protein synthesis in animal cells,” Journal of Biochemistry, vol. 74, pp. 285–292, 1973.
58. Uneo, Y. 1983. Trichothecenes- Chemicals, Biological and Toxicological Aspects. Y. Uneo, ed. Kodansha, Tokyo, Japan.
59. Venkatesh PK, Vairamuthu S, Balachandran C, Manohar BM, Raj GD. Induction of apoptosis by fungal culture materials containing cyclopiazonic acid and T-2 toxin in primary lymphoid organs of broiler chickens. Mycopathologia. 2005 Apr;159(3):393-400.
60. Vila, B., Jaradat, Z.W., Marquardt, R.R., Frohlich, A.A., 2002. Effect of T-2 toxin on in vivo lipid peroxidation and vitamin E status in mice. Food Chem Toxicol 40, 479-486.
61. Visconti A., Mirocha C.J. Identification of various T-2 toxin metabolites in chicken excreta and tissues.Appl. Environ. Microbiol. 1985;49:1246–1250
62. Wang, J., Fitzpatrick, D.W., Wilson, J.R., 1998. Effect of T-2 toxin on blood-brain barrier permeability monoamine oxidase activity and protein synthesis in rats. Food Chem Toxicol 36, 955-961.
63. Wyatt, R. D., B. A. Weeks, P. B. Hamilton, and H. R. Burmeister. 1972. Severe oral lesions in chickens caused by ingestion of dietary fusariotoxin T-2. Appl. Microbiol. 24(2):251–257Wyatt, R.D., Hamilton, P.B., Burmeist.Hr, 1973. Effects of T-2 Toxin in Broiler Chickens. Poultry Sci 52, 1853-1859.
64. Wyatt, R.D., Hamilton, P.B., Burmeister, H.R., 1975. Altered feathering of chicks caused by T-2 toxin. Poultry Sci 54, 1042-1045.
65. Yang, G.H., Jarvis, B.B., Chung, Y.J., Pestka, J.J., 2000. Apoptosis induction by the satratoxins and other trichothecene mycotoxins: relationship to ERK, p38 MAPK, and SAPK/JNK activation. Toxicol Appl Pharm 164, 149-160.
66. Yegani, M., T. K. Smith, S. Leeson and H. J. Boermanst, “Effect of Feeding Grains Naturally Contaminated with Fusarium Mycotoxins on Performance and Metabolism of Broiler Breeders,” Poultry Science, Vol. 85, 2006, pp. 1541-1549.
67. Yohannes, T. A., “Clinicopathological, Pathological and Immunological Studies on Experimental T-2 Mycotoxicosis and IBV Infection in Broiler Chicken,” M.V.Sc. Thesis, Indian Veterinary Research Institute, India, 2010.
68. Yohannes T, Sharma AK, Singh SD, Goswami TK. Immunopathological effects of experimental T-2 mycotoxocosis in broiler chicken co-infected with infectious bronchitis virus (IBV). Vet Immunol Immunopathol. 2012 May 15;146(3-4):245-53.
69. Yohannes, T., A. Sharma, S. Singh and V. Sumi, "Experimental Haematobiochemical Alterations in Broiler Chickens Fed with T-2 Toxin and Co-Infected with IBV," *Open Journal of Veterinary Medicine*, Vol. 3 No. 5, 2013, pp. 252-258.
70. Young J.C., Zhou T., Yu H., Zhu H., Gong J. Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food Chem. Toxicol. 2007;45:136–143.
71. Yoshizawa T., Swanson S.P., Mirocha C.J. T-2 metabolites in the excreta of broiler chickens administered 3H-labeled T-2 toxin. Appl. Environ. Microbiol. 1980;39:1172–1177.
72. Xue , C. Y., G. H. Wang, F. Chen, X. B. Zhang, Y. Z. Bi, and Y. C. Cao Immunopathological effects of ochratoxin A and T-2 toxin combination on broilersPoultry Science (2010) 89 (6): 1162-1166

4.7.5. Zearalenone (ZEN)

Zearalenone is a nonsteroidal oestrogenic mycotoxin biosynthesized through a polyketide pathway by a variety of Fusarium fungi, including F. graminearum (Gibberella zeae), F. culmorum, F. cerealis, F. equiseti, F. crookwellense and F. semitectum, which are common soil fungi, in temperate and warm countries, and are regular contaminants of cereal crops worldwide (**Bennett and Klich, 2003).**

- ZEN was given the trivial name zearalenone as a combination of G. **zeae**, resorcylic acid lactone, **-ene** (for the presence of the C-10 to C-2 double bond), and **-one**, for the C-60 ketone (**Urry** et al., **1966**).
- ZEN) is also known as RAL and F-2 mycotoxin
- ZEN exists widely in many cereal crops such as corn, barley, wheat, oats, sorghum, and sesame seeds, as well as in hay and corn silage. These are all ingredients in many food products for human or animal nutrition.
- ZEN has an estrogenic effect that causes alterations in the reproductive tract of laboratory animals (mice, rats, and guinea pigs) and farm animals
- ZEN has a high binding affinity for estrogen receptors. It is biologically potent, but it is hardly toxic
- The mechanism of the estrogenic effects of ZEN appears to be mediated via binding of this mycotoxin or its metabolites to the cytoplasmic estrogen receptor
    - increasing cell proliferation
    - resulting in uterine hyperplasia as well as cervical and vaginal metaplasia
- The immune system is a potential target for estrogenic endocrine disruptors

Chemical and physical properties

- Zearalenone is a white crystalline solid. It exhibits blue-green fluorescence when excited by long wavelength ultraviolet (UV) light (360 nm) and a more intense green fluorescence when excited with short wavelength UV light (260 nm).
- In methanol, UV absorption maxima occur at 236 (e = 29,700), 274 (e = 13,909) and 316 nm (e = 6,020).
- Maximum fluorescence in ethanol occurs with irradiation at 314 nm and with emission at 450 nm. Solubility in water is about 0.002 g/100 mL.
- ZEN is slightly soluble in hexane
- ZEN is progressively more soluble in benzene, acetonitrile, methylene chloride, methanol, ethanol, and acetone.
- ZEN is also soluble in aqueous alkali.
- ZEN heat-stable
- ZEN chemical formula andmolar mass:

Chemical structure of zearalenone and its analogues (Shier et al., 2001)

Compound	R_1	R_2	1', 2'	R_3	R_4	Relative oestrogenicity
α-zearalenol	OH	OH	trans	H, α-OH	H	92.0
α-zearalanol	OH	OH	dihydro	H, α-OH	H	18.0
2,4-o-dimethyl-8-hydroxyzearalenone	OCH_3	OCH_3	trans	O	OH	3.8
β-zearalanol	OH	OH	dihydro	H, β-OH	H	3.5
6-amino-zearalenone	OH	OH	trans	H, NH_2	H	3.2
zearalanone	OH	OH	dihydro	O	H	2.5
6'-acetyl-β-zearalanol	OH	OH	dihydro	H, β-OAc	H	2.0
zearalenone	OH	OH	trans	O	H	1.0
2,4-dideoxy-zearalene	OH	H	dihydro	H_2	H	0.8
cis-zearalenone	OH	OH	cis	O	H	0.7
zearalane	OH	OH	dihydro	H_2	H	0.5
4-o-methyl-zearalenone	OH	OCH_3	trans	O	H	0.5
β -zearalenol	OH	OH	trans	H, β-OH	H	0.4
6'-acetyl-β-zearalenol	OH	OH	trans	H, β-OAc	H	0.3
2-o-methyl-zearalenone	OCH_3	OH	trans	O	H	0.2
2,4-o-dimethyl-zearalenone	OCH_3	OCH_3	trans	O	H	0.2
4-acetyl-zearalenone	OH	OAc	trans	O	H	0.2
4-sulfate zearalenone[1]	OH	OSO_3^-	trans	O	H	
zearalenone-4-glucoside[2]	OH	Glc	trans	O	H	
zearalenone-4-malonyl-glucoside[2]	OH	MalGlc	trans	O	H	
zearalenone-4-dihexoside[2]	OH	DiGlc	trans	O	H	
zearalenone-4-hexosyl-pentoside[2]	OH	GlcXyl	trans	O	H	
α-zearalenol-4-glucoside[2]	OH	Glc	trans	H, α-OH	H	
α-zearalenol-4-malonyl-glucoside[2]	OH	MalGlc	trans	H, α-OH	H	
α-zearalenol-4-dihexoside[2]	OH	DiGlc	trans	H, α-OH	H	
α-zearalenol-4-hexosyl-pentoside[2]	OH	GlcXyl	trans	H, α-OH	H	
β-zearalenol-4-glucoside[2]	OH	Glc	trans	H, β-OH	H	
β-zearalenol-4-malonyl-glucoside[2]	OH	MalGlc	trans	H, β-OH	H	
β-zearalenol-4-dihexoside[2]	OH	DiGlc	trans	H, β-OH	H	
β-zearalenol-4-trihexoside[2]	OH	TriGlc	trans	H, β-OH	H	
β-zearalenol-4-hexosyl-pentoside[2]	OH	GlcXyl	trans	H, β-OH	H	

[1] Reported by Plasencia and Mirocha (1991).
[2] Reported by Berthiller *et al.* (2007).

Toxicokinetics

The word toxin is almost certainly a misnomer because zearalenone, while biologically potent, is hardly toxic; rather, it sufficiently resembles 17β-estradiol, the principal hormone produced by the human ovary, to allow it to bind to estrogen receptors in mammalian target cells. Zearalenone is better classified as a nonsteroidal

estrogen or mycoestrogen. Sometimes it is called a phytoestrogen (**Kuiper-Goodman et al., 1987**).

- **In laying hens**, studies of the toxicokinetics and of the persistence of zearalenone were conducted using ^{14}C- and ^{3}H-radiolabeled toxin. uniformly labeled ^{14}C- zearalenone was solubilized in propylene glycol and administered in the crop (1.54 μCi equivalent to 10 mg/kg), after which feed and water were provided *ad libitum* [**Dailey *et al.*, 1980].**
    - T_{max} in plasma was observed 4 h post dosing, at concentration of 820 ng/g, then radioactivity decreased continuously to reach 12 ng/g 72 h after administration.
    - levels in red blood cells increased over time to reach 2690 ng/g 48 h post dosing.
    - The highest level of radioactivity was measured in the bile 24 h after administration, and the bile: plasma ratio was 264.
    - After the gastrointestinal tract, the liver and kidneys showed the highest levels of contamination measured 4 h and 2 h after administration, at an equivalent zearalenone level of 3970 and 3410 ng/g, respectively.
    - The mean concentration in muscle was 100 ng/g, whereas the mean concentration in fat was 300 ng/g, and the levels of radioactivity in both tissues remained relatively constant from 4 h to 72 h after administration.
    - Approximately 1% of the dose was found in the egg and in the clutch 24 h after administration, and this level persisted until day 3, when more than 99% of radioactivity was recovered.
    - The use of different extraction solvents combined with the use of glucuronidase revealed that around one-third of the dose excreted was unchanged zearalenone, while another third was excreted as more polar metabolites that could be the conjugated form with glucuronic acid

- **In boilers**, ^{3}H-zearalenone (labelled at 5′ and 3′ position) was used in boilers previously fed a diet containing 100 mg/kg of unlabeled zearalenone for one week. The toxin was solubilized in ethanol and administered into the crops at a dose equivalent to 5 mg/kg BW. Water and feed were provided *ad libitum* throughout the experiment **(Mirocha *et al.,* 1987).**
    - C_{max} in plasma occurred 0.5 h after administration, with a rebound 8 h later, after which the concentration decreased slowly.
    - The highest radioactivity was observed in the bile 8 h after administration, when the bile: plasma ratio was 1462.
    - After the gastro-intestinal tract and the gizzard, the liver and kidneys had the highest concentrations of labelled compounds.

- The highest radioactivity in the muscle and fat was observed 24 h after administration, with values close to those observed in plasma at the same time.
- The average recovery of the radioactivity two days after administration was 83%. Analysis of the metabolites formed revealed major differences between birds, with the levels of α- and β-zearalenol close to that of unmetabolized zearalenone.

- **In broilers**: the toxin was administered in the crop at a dose of 0.3 mg/kg BW with no feed provided for a period of 6 h before and 4 h after administration of the toxin. Zearalenone, α- and β-zearalenol, zearalenone, and α- and β-zearalenol were analyzed in plasma using LC/MS with LOD < 0.1 ng/mL.
  - o Only traces of α-zearalenol were found. The $T_{1/2elim}$observed when the toxin was administered by the intravenous route was 31.8 min, and α-zearalenone was detected at a non-quantifiable level (**Osselaere *et al.,* 2013)**

- **In male turkey poults**, the administration of a diet containing 800 mg zearalenone/kg for a period of two weeks resulted in
  - The detection of zearalenone in different tissues, with the liver and the kidney being the most contaminated at 276 and 122 ng/g, respectively.
  - The amounts of α-zearalenol in the liver and kidney were higher than those of zearalenone, 2715 and 477 ng/g, respectively, whereas only traces of β-zearalenol were observed (**Olsen *et al.*, 1986**)

- **At lower levels** of zearalenone exposure, the presence of α-zearalenol as the main metabolite of zearalenone was confirmed in the excreta and in tissues. In broilers fed with a diet containing less than 0.5 mg zearalenone/kg, α-zearalenol was seen to be the main metabolite, and only low levels of β-zearalenol were measured in excreta (**Dänicke *et al.* (2001)**

- **In laying hens** fed with a diet containing 1.57 mg zearalenone and 17.63 mg DON/kg, the concentrations of zearalenone and α-zearalenol in the liver were respectively 2.1 and 3.7 ng/g (**Dänicke *et al.*, 2002)**
  - o α-zearalenol was also the main metabolite found in the bile of Pekin ducks fed with a diet containing low level of zearalenone (less than 0.1 mg/kg) and DON (up to 7 mg/kg), whereas less β-zearalenol was excreted and unchanged zearalenone remained the most abundant compound
  - o Analysis of liver and bile fluid in laying hens fed zearalenone strengthened this hypothesis, the sulfate of α-zearalenol being more abundant than the glucuronide conjugate, even if the

glucuronide of zearalenone was most abundant than the sulfate conjugate

- o No transmission of zearalenone and its metabolites to eggs was found when laying hens were fed a diet containing 1.57 mg zearalenone and 17.63 mg DON/kg The respective detection limits for zearalenone, α- and β-zearalenol, zearalenone, and α- and β-zearalenol by HPLC/FLD were 1, 0.5, 3, 20, 20 and 40 ng/g.

The rate of reduction of zearalenone into α- and β-zearalenol was compared in geese, ducks, guinea-fowl, chickens, laying hens, and quail **(Kolf-Clauw *et al.,* 2008).**

- ZEN reduction was lowest in geese
- ZEN reduction was highest in quail.
- α-zearalenol was the main metabolite formed in all the avian species,
- α:β ratio ranged from 1.8 in quail to 5.3 in chicken

Tolerance of chickens to zearalenone.

In chickens administered 15 g of zearalenone per kg of body weight at a single oral dose survived the treatment and did not show any noticeable gross or histopathological signs (**Chi *et al,* 1980).**

- On day 10 after zearalenone dosing, there was no difference between control and zearalenone-treated chickens in body weight and weights of the oviduct, comb, and liver.
- Birds treated with zearalenone had significantly ($P < 0.05$) lower serum calcium but significantly ($P < 0.01$) higher serum phosphorus concentrations than control chickens.
- Hematological criteria (hematocrit, erythrocyte, leukocyte, and differential leukocyte counts) and serum cholesterol were similar between two groups.
- Chickens appeared to tolerate a large single oral dose of zearalenone;
- The 50% lethal dose of the toxin for growing chickens is greater than 15 g/kg of body weight.

Mechanism of action

Zearalenone, has been demonstrated to possess estrogenic properties which cause uterine proliferation in rats and hyperestrogenism in swine and turkeys

- ZEN acts as agonist for estrogens.

- ZEN is metabolized by the liver, gastrointestinal mucosa, erythrocyte or intestinal bacteria to be α-zeararenoru and β-zeararenoru.
- These substances are further metabolized to be α-zeararenoru and β-zeararenoru. This metabolic reaction greatly varies depending on the animal spice.
- The binding activity for the estrogen receptor of uterus cytosols functions best in α-zeararenoru, followed by α-zeararenoru, β-zeararenoru, Zearalenone and β-zeararenoru in this order.

Zearalenone producing Fusarium spcies

1. *Fusarium crookwellense*
2. *Fusarium culmorum*
3. *Fusarium dactylidis*
4. *Fusarium equiseti*
5. *Fusarium graminearum*
6. *Fusarium pseudograminearum*
7. *Fusarium semitectum*
8. *Fusarium solani,*

Description of zearalenone producing Fusarium spcies

1. Fusarium crookwellense **Burgess, Nelson & Toussoun, Transa. Brit. Mycol. Soci.79,498 (1982)**

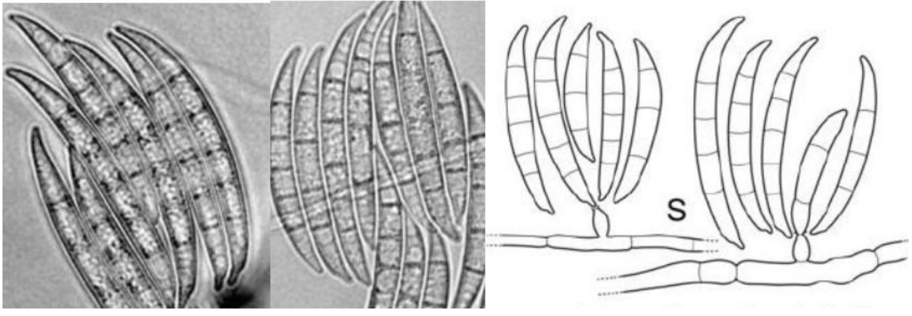
=Fusisporium cereale Cooke, Grevillea 6 (40): 139 (1878)
=Fusisporium cerealis Cooke, Grevillea 6 (40): 139 (1878) =Gibberella roseum f. cerealis (Cooke) W.C. Snyder & H.N. Hansen, American Journal of Botany 32: 664 (1945)

Macroconidia: abundant, pronounced dorsal curvature and straight ventrally, 5 septa, apical cell curved and tapering and pointed , basal cell foot-shaped. Sporodochia: pale orange –dark brown, abundant. Microconidia: absent. Chlamydospores : abundant after 4-6 weeks, smooth, in chains and clusters

John F. Leslie and Brett A. Summerell

2. Fusarium dactylidis. Takayuki Aoki, Martha M. Vaughan , Susan P. McCormick Mark Busman,Todd J. Ward, Amy Kelly Kerry O'Donnell, Peter R. Johnston and David M. Geise. at Mycologia, 2014

Colonies produce abundant loose to densely floccose aerial mycelium ; reverse white at margin, reddish pigmentation centrally, reddish white, pale red to violet brown. Sporodochia formed on agar surface. Sporodochial conidia formed directly from phialides on substrate hypha. Sporodochial conidiophores form conidia on monophialides. Macroconidia of a single type, typically falcate and curved, dorsiventral, 1–7-septate, usually widest at or slightly above the midregion of their length, tapering and curving equally toward both ends, with an acute apical cell and a distinct basal foot cell. Upper and lower halves of conidia nearly symmetrical. Chlamydospores and sclerotia absent, but round intercalary or terminal cell swellings sometimes present in hyphae or older conidia.



3. Fusarium semitectum Berk. & Ravenel, Grevillea 3 (27): 98 (1875)

≡Pseudofusarium semitectum (Berk. & Ravenel) Matsush., Icones Microfu Matsus lectorum: 119 (1975)
=Fusisporium incarnatum Roberge ex Desm., Annales des Sciences Naturelles Botanique 11: 274 (1849)
=Fusarium semitectum var. semitectum (1875)
=Fusisporium pallidoroseum Cooke, Grevillea 6 (40): 139 (1878)
=Fusarium semitectum var. majus Wollenw., Fusaria Autographice Delineata 3: 907-910 (193]

Macroconidia: abundant, slender, curved dorsal surface, 3-5 septa, apical cell curved and tapering , basal cell foot-shaped. Sporodochia: orange. Microconidia: pyriform, 1-septa, mesoconidia spindle-shaped, 3-5 septa. Chlamydospores: globose

file.scirp.org, ecoport.org, Galería de imágenes, EcoPort Picture, Databank

Reports:

Chi *et al.* (1980) studied acute toxic effects of purified zearalenone in growing female White Leghorn chickens. In the first experiment, zearalenone in gelatin capsules was administered to 10 chickens (zearalenone-treated chickens [ZC]) in a single oral dose of 15.0 g/kg. Another 10 control chickens (CC) received empty gelatin capsules. All chickens survived the 10-day experiment and did not show any noticeable gross or histopathological lesions. There were no differences between CC and ZC in weight gain, oviduct, comb and liver weights, hematological parameters, and serum cholesterol. ZC had significantly less (P less than 0.05) serum calcium but significantly greater (P less than 0.01) serum phosphorus than CC. In the second experiment, zearalenone was administered orally or intramuscularly (pectoral muscle) at levels of 0, 50, 200, 400, and 800 mg/kg for 7 consecutive days. The oviduct weight increased with increasing toxin levels in both orally (OZC) and intramuscularly (IZC) administered groups: there were more pronounced effects in the IZC. The liver weight increased and comb weight decreased in IZC. The relative estrogenic biopotency of zearalenone in IZC, using estradiol dipropionate as a standard, was 1.37%. The results of this experiment demonstrate that chickens are highly tolerant to zearalenone and that the estrogenic effects of the toxin are greater when it is administered in multiple doses than in a single dose and in IZC than in OZC.

Allen *et al.* (1981) incorporated variable quantities of zearalenone (0, 10, 25, 50, 100, 200, 400, or 800 mg/kg diet) into a practical laying hen diet and fed to 30-week-old White Leghorn females in egg production. During the 3 week pretest and 8 week experimental periods hens were inseminated weekly with .05 ml of pooled semen from males fed normal diets. Zearalenone was without effect on egg production, egg size, feed consumption, change in body weight, fertility, hatchability of fertile eggs, growth of progeny to 3 weeks of age, comb, weight, oviduct weight, heart weight, liver weight, spleen weight, egg shell thickness, Haugh units, blood hematology, serum calcium, serum inorganic phosphorus, and serum alkaline phosphatase. Zearalenone above 50 mg/kg of diet caused reduced serum cholesterol. In a reciprocal study, adult male New Hampshire chickens were fed diets containing 0, 100, or 800 mg/kg zearalenone for an 8 week period. Semen was collected and inseminated into White Leghorn females fed normal diets. Zearalenone was without effect on fertility or hatch of fertile eggs resulting from matings of these males. Zearalenone resulted in reduced serum inorganic phosphorus, serum cholesterol, and serum alkaline phosphatase in males. Histological examination of a number of tissues in both males and females revealed no changes due to zearalenone feeding. It is concluded that zearalenone up to 800 mg/kg of diet is without effect on reproductive performance of mature chickens.

Allen *et al.* (1983) fed Nicholas Large White turkey hens in egg production (10 per treatment) individually cultures of Fusarium roseum 'Gibbosum' to provide 100 ppm zearalenone, Fusarium tricinctum at a level of .1% of the diet, Fusarium roseum Alaska at a level of 2% of the diet, 100 ppm purified zearalenone, and 5 ppm purified T-2 toxin for 8 weeks. The following 4 weeks the birds were fed a control diet. Hens

were inseminated every 2 weeks with .05 ml of pooled semen from males fed a control diet. After 30 days of toxin feeding, hens were innoculated with a killed Newcastle disease virus preparation. Blood samples were obtained periodically. Egg fertility and titers to Newcastle disease virus were unaffected by treatment. Egg weight was reduced by F. roseum 'Gibbosum'. F. roseum 'Gibbosum' and F. tricinctum caused decreases in feed consumption, body weight, and egg production. Egg production was decreased by zearalenone and T-2 toxin. Hens fed F. roseum 'Gibbosum', F. tricinctum, and T-2 toxin exhibited mouth lesions that healed rapidly upon withdrawing toxic feed. Hatchability of fertile eggs was reduced by feeding F. roseum 'Gibbosum', F. tricinctum and F. roseum Alaska to 28, 78, and 49%, respectively, of control values by the end of the 8 week test period. Upon removal of toxic feed, hatchability rapidly returned to control levels. Embryo mortality occurred mainly in the first 10 days of incubation for F. roseum Alaska and the last 18 days for F. roseum 'Gibbosum' and F. tricinctum fed hens. It appears that mycotoxins other than zearalenone and T-2 toxin are responsible for reduced hatchability from feeding Fusarium cultures.

Olsen *et al.* (1986) fed six male turkey poults (3 weeks of age) a starter ration artificially contaminated with 800 mg zearalenone/kg for a 2-week period to examine zearalenone metabolism and residues in various tissues, excreta, and blood plasma. Zearalenone had no effect on either feed consumption or body weight gain. All the birds fed zearalenone frequently showed strutting behavior, displayed an increased size and coloration of caruncles and dewlaps, and had swollen vent tissue. None of these signs were seen among six control birds fed uncontaminated starter feed. Hormone analysis, however, revealed that the testosterone concentrations in blood plasma were the same in both controls and treated birds. Analysis after 14 days of feeding showed that most of the dietary zearalenone had been metabolized into alpha-zearalenol. Levels of zearalenone and alpha-zearalenol were: blood plasma 66 +/- 27 and 194 +/- 80 ng/ml, excreta 182 +/- 33 and 644 +/- 86 micrograms/g, lung 56 +/- 45 and 202 +/- 161 ng/g, heart 57 +/- 40 and 238 +/- 121 ng/g, kidney 122 +/- 25 and 477 +/- 53 ng/g, and liver 276 +/- 54 and 2715 +/- 590 ng/g, respectively. Only traces of beta-zearalenol could be detected in plasma, excreta, and the various tissues. The percentage alpha-zearalenol of total zearalenone plus alpha-zearalenol rose significantly in both blood plasma and excreta during the experimental period. Almost all zearalenone and alpha-zearalenol was found conjugated in blood plasma, and the conjugates consisted of both glucuronides and sulfate conjugates. Approximately 65% of all zearalenone and alpha-zearalenol in excreta was found to be conjugated.

Branton *et al.* (1989) carried out an experiment to determine the effect of corn vs. grain sorghum on performance of laying hens. Egg production decreased significantly in the grain sorghum-fed hens in each of two trials starting 24 weeks after the trials began. Necropsy of chickens fed both diets revealed buccal ulceration at the ventral aspect of the oral cavity and squamous metaplasia of the esophageal glands and submaxillary salivary glands. Lesions were much more severe in the sorghum-fed birds than in the corn-fed birds. Analysis of the grain sorghum and corn revealed the presence of mycotoxins. Zearalenone and deoxynivalenol were present in the grain sorghum, and a lower amount of deoxynivalenol and a trace of aflatoxin B1 were found in the corn. Although mycotoxin levels were low, interaction between these mycotoxins and others may have decreased egg production.

Maryamma *et al.* (1992) intubated [^{3}H]Zearalenone into the crops of 7-week-old broiler chickens, and its distribution was monitored at 0, 0.5, 4, 8, 12, 24, and 48 hr. Metabolic products were measured by gas chromatography-mass spectroscopy and radioimmunoassay. The average recovery of administered radioactivity was 83%. Of the edible tissue, the greatest accumulation of radioactivity occurred in the liver 0.5 hr (0.94%) after administration after which it fell off quickly so that by 48 hr only a trace (0.07%) of radioactivity was found. Muscle, abdominal fat, skin, and heart contained only trace quantities of radioactivity. The bile and gall bladder (4.2%) and excreta (87%) contained the major portion of radioactivity. The excreta was the major avenue of elimination. Total zearalenone and α- and β-zearalenols found in the excreta at 0.5 hr after administration were 0.5 to 3.86 ppm, 8.8 to 2.5 ppm at 4 hr, 12.5 to 121 ppm at 8 hr, 10.1 to 82.7 ppm at 12 hr, 1.6 to 122 ppm at 24 hr, and 39 to 43.5 ppm at 48 hr. The concentration in the muscle ranged from 23 to 25 ppb at 0.5 hr to 4 ppb at 48 hr; the maximum residue found was 111 ppb. The muscle contained only zearalenone and no zearalenol. Zearalenone and α- and β-zearalenols were found in the liver with concentrations ranging between 57 and 1103 ppb when measured by RIA and 17.3 and 2543 ppb when measured by GC-MS. The identity of all metabolites was confirmed by mass spectroscopy. The data suggest little danger from residue in the edible portions. Moreover, analysis of the feces can be used as an indicator of intoxication. The presence of zearalenone and metabolites in tissues was also analyzed with HPLC/FLD or UV detection following high levels of exposure. After administration of 10 mg zearalenone/kg BW by intubation of the crop in broilers, 416, 207, and 170 ng/g of residues were measured in the kidneys, liver, and muscles, respectively

Keshavarz (1993) conducted an experiment to determine the effects of feeding corn contaminated naturally with deoxynivalenol (DON, vomitoxin) on performance of laying hens and growing chicks. Ten dietary regimens used in the laying hen experiment contained incremental levels of 0-2.1 ppm DON and 0-0.42 ppm zeralenone. Six dietary regimens used in the growing chick experiment contained 0 or 2.1 ppm DON and 0-0.42 ppm zeralenone. The criteria used for evaluating the effect of dietary treatments were body weight, body weight gain, egg production, feed consumption, feed conversion, egg weight, egg grades, shell quality, albumen quality, fertility and hatchability, organ weight, and presence of lesions in the mouth. No adverse effects were observed in laying hens or growing chicks for any of these parameters even at the highest levels of DON contamination, which provided 2.1 ppm DON and 0.42 ppm zeralenone in the finished feeds. The data indicate that growing chicks and laying hens are relatively insensitive to corn contaminated naturally with 2-3 ppm DON and 0.4-0.6 ppm zeralenone, and having specifications similar to the corn samples used in this study. The results do not support the notion that corn contaminated with more than 0.5 ppm DON should be rejected for use in poultry feeds.

Dänicke *et al.* (2001) carried out two experiments out with male broilers to examine excretion kinetics of zearalenone (ZON) and its metabolites and their occurrence in blood plasma and bile fluid after a single oral dose of ZON (approximately 6 micrograms/kg BW) from naturally contaminated wheat (406 micrograms ZON per kg). In addition, this ZON bolus was administered either in the absence or presence of a detoxifying agent (Mycofix-Plus, Biomin GmbH, Herzogenburg, Austria). Specimens were sampled after administration of the zearalenone bolus at different times of up to 48 h. Excretion of zearalenone and alpha-zearalenol as the only

detectable metabolite of ZON peaked at approximately 6.5 h after administration of the bolus. Cumulative excretion of both substances amounted to approximately 58% of ZON intake after 48 h, when a plateau was achieved. The incomplete recovery could have been due to a partial total degradation of ZON in the digestive tract, undetected sulfate conjugates of ZON or its metabolites, to other unknown and undetected metabolites or to incomplete analytical recovery from the matrix, and needs to be examined further. Peak concentrations of zearalenone and alpha-zearalenol in bile were detected in the time period of approximately 2 to 6 h after bolus, whereas ZON and metabolite concentrations in blood plasma were around or lower than the detection limits. Mycofix-Plus supplementation seemed to have only minor or no effects on the parameters examined.

Dänicke *et al.* (2002) carried out 16-wk experiment with laying hens to examine the effects of feeding of mycotoxin-contaminated maize (CM) on performance, nutrient digestibility, weight of organs, serum chemical parameters, and antibody titers to Newcastle disease virus (NDV) in serum. Also tested were fimbrien antigen K88 in egg yolk and zearalenone (ZON) residues in eggs and tissues. The Fusarium-toxin-contaminated maize contained 17,630 microg deoxynivalenol and 1,580 microg ZON/kg. Moreover, Mycofix Plus (MP), a so-called detoxifying agent, was added to both the uncontaminated control (UCM) and to the CM diet (70% dietary maize inclusion). Each of the four resulting diets (UCM, UCM-MP, CM, CM-MP) was tested on 25 laying hybrids (Lohmann Brown). Feeding of the CM diets significantly depressed feed intake compared to the control groups by approximately 5%. This was mainly due to the effects observed at the beginning of the experiment. Daily egg mass production/hen was 56.6, 58.4, 53.9, and 55.2 g in groups UCM, UCM-MP, CM and CM-MP, respectively. Nutrient digestibility and metabolizability of gross energy were slightly depressed by feeding the CM diets and improved by MP addition. Feeding of the CM diets resulted in a significant decrease in serum titers to NDV and to an increase in yolk titers to antigen K88. No residues of ZON or of its metabolites were found in yolk, albumen, abdominal fat, breast meat, follicles greater than 1 cm in diameter, ovaries including follicles smaller than 1 cm in diameter, magnum, and serum. ZON and alpha-zearalenol (alpha-ZOL) were detected in livers of hens fed the CM diets at mean concentrations of 2.1 and 3.7 microg/kg, respectively. It was concluded that feeding maize which was highly contaminated with Fusarium mycotoxins adversely influenced performance of hens and modulated immune response. At the given level of zearalenone and at the indicated detection limits, no residues of ZON and its metabolites were found in eggs. The effects of the tested detoxifying agent were quite mycotoxin-independent.

Swamy *et al.* (2002) fed three hundred sixty, 1-d-old male broiler chicks, diets containing grains naturally contaminated with Fusarium mycotoxins for 56 d. The four diets included control (0.14 mg/kg deoxynivalenol, 18 mg/ kg fusaric acid, < 0.1 mg/kg zearalenone), low level of contaminated grains (4.7 mg/kg deoxynivalenol, 20.6 mg/kg fusaric acid, 0.2 mg/kg zearalenone), and high level of contaminated grains without (8.2 mg/kg deoxynivalenol, 20.3 mg/kg fusaric acid, 0.56 mg/kg zearalenone) and with (9.7 mg/kg deoxynivalenol, 21.6 mg/kg fusaric acid, 0.8 mg/kg zearalenone) 0.2% esterified-glucomannan polymer derived from Saccharomyces cerevisiae1026 (E-GM). Body weight gain and feed consumption responded in a significant quadratic fashion to the inclusion of contaminated grains during the finisher period. Efficiency of feed utilization, however, was not affected by diets. The feeding of contaminated grains in the finisher period also caused significant linear

increases in blood erythrocyte count and serum uric acid concentration and a significant linear decline in the serum lipase activity. Dietary inclusion of contaminated grains resulted in a significant quadratic effect on serum albumin and y-glutamyltransferase activity. Blood hemoglobin and biliary IgA concentrations, however, responded in significant linear and quadratic fashions. Supplementation of E-GM counteracted most of the blood parameter alterations caused by the Fusarium mycotoxin-contaminated grains and reduced breast muscle redness. It was concluded that broiler chickens may be susceptible to Fusarium mycotoxicoses when naturally contaminated grains are fed containing a combination of mycotoxins.

Dänicke *et al.* (2003) carried out a growth experiment with male broilers from d 1 to d 35 of age in order to evaluate the effects of the addition of a detoxifying agent (Mycofix Plus, Biomin GmbH, Herzogenburg, Austria) at different dietary proportions of wheat (0, 16.5, 33, 49.5 and 66%) contaminated with Fusarium mycotoxins (21.2 mg of deoxynivalenol and 406 microg of zearalenone, ZON, per kg of wheat) on growth performance, nutrient and zearalenone balance and clinical-chemical parameters. 2. An increase in dietary mycotoxin concentration resulted in a linearly related decrease in feed intake, a slight decrease in weight gain and an improvement in feed to gain ratio. 3. Apparent protein digestibility and net protein utilisation were higher in diets containing exclusively Fusarium toxin-contaminated wheat than control diets. 4. The proportions of beta-zearalenol, alpha-zearalenol and ZON of total ZON metabolites in excreta of broilers fed on the diets containing the Fusarium toxin-contaminated wheat were approximately 3, 21 and 76%. 5. Serum antibody titres to Newcastle disease virus decreased in a linear fashion with increasing mycotoxin concentration in the diets, whereas other clinical-chemical serum parameters (liver cell and muscle cell necrosis indicating enzymes, haemoglobin, haematocrit, magnesium, inorganic phosphate) were not influenced by increasing Fusarium toxin concentrations. 6. Supplementation of the diets with Mycofix Plus decreased performance in a manner independent of mycotoxin concentration. Moreover, some clinical-chemical serum parameters were significantly altered due to Mycofix Plus but also independently of the dietary mycotoxin concentration.

Dänicke *et al.* (2004) fed diets with increasing proportions of Fusarium-toxin-contaminated wheat to **Pekin ducks** for 49 d in order to titrate the lowest effect level. Dietary **deoxynivalenol** (DON) and **zearalenone** (ZON) concentrations were successively increased up to 6 to 7 mg/kg and 0.05 to 0.06 mg/kg, respectively. Feed intake, live weight gain and feed to gain ratio were not influenced by dietary treatment. Gross macroscopic inspection of the upper digestive tract did not reveal any signs of irritation, inflammation or other pathological changes. The weight of the bursa of Fabricius, relative to live weight, decreased in a dose-related fashion. Activities of glutamate dehydrogenase and gamma-glutamyl-transferase in serum were either unaffected or inconsistently affected by dietary treatments. Concentrations of DON and of its de-epoxydised metabolite in plasma and bile were lower than the detection limits of 6 and 16 ng/ml, respectively, of the applied high performance liquid chromatography (HPLC) method. ZON or its metabolites were not detectable in plasma and livers (detection limits of the HPLC method were 1, 0.5 and 5 ng/g for ZON, alpha-zearalenol (alpha-ZOL) and beta-zearalenol (beta-ZOL), respectively). Concentrations of ZON, alpha-ZOL and beta-ZOL in bile increased linearly with dietary ZON concentration. The mean proportions of ZON, alpha-ZOL and beta-ZOL of the sum of all three metabolites were 80, 16 and 4%, respectively. Taken together, it can be concluded that dietary DON and ZON concentrations up to 6 and 0.06

mg/kg, respectively, did not adversely affect performance and health of growing Pekin ducks.

Swamy ***et al.*** **(2004)** conducted an experiment to investigate the effects of feeding grains naturally contaminated with Fusarium mycotoxins on growth and immunological parameters of broiler chickens. Three hundred sixty, 1- d-old male broiler chicks were fed 1 of 4 diets containing grains naturally contaminated with Fusarium mycotoxins for 56 d. The diets included (1) control; (2) low level of contaminated grains (5.9 mg/kg d**eoxynivalenol** (DON), 19.1 mg/kg **fusaric acid** (FA), 0.4 mg/kg **zearalenone**, and 0.3 mg/kg 15-acetyldeoxynivalenol; (3) high level of contaminated grains (9.5 mg/kg DON, 21.4 mg/kg FA, 0.7 mg/kg zearalenone, and 0.5 mg/kg 15-acetyldeoxynivalenol); and (4) high level of contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GM polymer). Body weight gains and feed consumption of chickens fed contaminated grains decreased linearly with the inclusion of contaminated grains during the grower phase (d 21 to 42). Efficiency of feed utilization, however, was not affected by diet. Production parameters were not significantly affected by the supplementation of GM polymer to the contaminated grains. Peripheral blood monocytes decreased linearly in birds fed contaminated grains. The feeding of contaminated diets linearly reduced the B-cell count at the end of the experiment, whereas the T-cell count on d 28 responded quadratically to the contaminated diets. The feeding of contaminated diets did not significantly alter serum or bile immunoglobulin concentrations, contact hypersensitivity to dinitrochlorobenzene, or antibody response to SRBC. Supplementation with GM polymer in the contaminated diet nonspecifically increased white blood cell count and lymphocyte count, while preventing mycotoxin-induced decreases in B-cell counts. It was concluded that broiler chickens are susceptible during extended feeding of grains naturally contaminated with Fusarium mycotoxins.

Sypecka ***et al.*** **(2004)** assessed the potential for the *Fusarium* mycotoxins 4-deoxynivalenol (DON) and zearalenone (ZON) to enter the human food chain through contaminated eggs was assessed using a controlled feed study. Four groups of laying hens (eight in each group) were fed a diet that included differing amounts of naturally contaminated wheat containing DON (≈20 mg kg^{-1}) and ZON (0.5 mg kg^{-1}). Eggs were collected and pooled from each group on a daily basis. Pooled samples were analyzed by liquid chromatography with mass spectrometry detection (LC-MS/MS). The method allowed DON, other type B trichothecenes, ZON, and its metabolites to be determined in a single multi-residue analysis. The selectivity of the MS/MS procedure allowed cleanup to be minimized (for DON, cleanup by immunoaffinity column was used) or eliminated (for ZON). The limits of detection of 0.01 μg kg^{-1} for DON and 0.1 μg kg^{-1} for ZON in eggs were lower than previously published methods. None of the samples analyzed had detectable levels of ZON or its metabolites. Although maximum levels of DON contamination (10 mg kg^{-1} feed) were relatively high, no adverse effects were observed on egg production. On the basis of the determined DON levels in the hen's diet and the determined levels of DON in the corresponding eggs, transmission rates of 15 000:1, 18 000:1, and 29 000:1 for treatment levels 5, 7.5, and 10 mg DON kg^{-1} feed, respectively, were found. These results show that, although eggs could be a human exposure route for DON, the levels are insignificant compared to the other sources, although the presence of metabolites of DON was not studied.

Chowdhury ***et al.*** **(2005)** examined the effects of feeding grains naturally contaminated with Fusarium mycotoxins on hematology and immunological indices and functions of laying hens and the possible protective effect of feeding a polymeric glucomannan mycotoxin adsorbent (GMA). One hundred forty-four laying hens were fed for 12 wk with diets formulated with (1) uncontaminated grains, (2) contaminated grains, or (3) contaminated grains + 0.2% GMA. Fusarium mycotoxins such as **deoxynivalenol** (DON, 12 mg/kg), 15-acetyl-DON (0.5 mg/kg), and **zearalenone** (0.6 mg/kg) were identified in the contaminated diets arising from contaminated grains grown in Ontario, Canada. The concentrations of DON arising from naturally contaminated grains in this study were similar to purified mycotoxin fed to experimental mice. The chronic feeding of Fusarium mycotoxins induced small decreases in hematocrit values, total numbers of white blood cells, lymphocytes including both CD4+ and CD8+ T lymphocytes and B lymphocytes, and biliary IgA concentration. Supplementation of diets containing feedborne mycotoxins with GMA prevented the reduction in total number of B lymphocytes in the peripheral blood and the reduction in biliary IgA concentration. In addition, the delayed-type hypersensitivity response to dinitrochlorobenzene was increased by feed-borne mycotoxins, whereas IgG and IgM antibody titers to sheep red blood cells were not affected by diet. We concluded that chronic consumption of grains naturally contaminated with Fusarium mycotoxins at levels likely to be encountered in practice were not systemically immunosuppressive or hematotoxic; however, mucosal immunocompetence needs to be explored further.

Labuda ***et al. (*****2005)** analyzed a total of 50 samples of poultry feed mixtures of Slovakian origin for eight toxicologically significant Fusarium mycotoxins, namely **zearalenone** (ZON), A-trichothecenes: **diacetoxyscirpeno**l (DAS), T-2 toxin (T-2) and HT-2 toxin (HT-2) and B-trichothecenes: deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON) and nivalenol (NIV). The A-trichothecenes and the B-trichothecenes were detected by means of high pressure liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS) and gas chromatography electron capture detection (GC-ECD), respectively. Reversed phase-high performance liquid chromatography with a fluorescence detector (RP-HPLC-FLD) was used for ZON detection. The most frequent mycotoxin detected was T-2, which was found in 45 samples (90%) in relatively low concentrations ranging from 1 to 130 microg kg(-1) (average 13 microg kg(-1)), followed by ZON that was found in 44 samples (88%) in concentrations ranging from 3 to 86 microg kg(-1) (average 21 microg kg(-1)). HT-2 and DON were detected in 38 (76%) and 28 (56%) samples, respectively, in concentrations of 2 to 173 (average 18 microg kg(-1)) for HT-2 and 64 to 1230 microg kg(-1) sample (average 303 microg kg(-1)) for DON. The acetyl-derivatives of DON were in just four samples, while NIV was not detected in any of the samples investigated. In as many as 22 samples (44%), a combination of four simultaneously co-occurring mycotoxins, i.e. T-2, HT-2, ZON and DON, was revealed. Despite the limited number of samples investigated during this study poultry feed mixtures may represent a risk from a toxicological point of view and should be regarded as a potential source of the Fusarium mycotoxins in Central Europe. This is the first reported study dealing with zearalenone and trichothecene contamination of poultry mixed feeds from Slovakia.

Martins *et al.* (2006) carried out a study to investigate the co-occurrence of zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FB1 and FB2) in 52 samples of mixed-feed for poultry contaminated with Fusarium verticillioides. The zearalenone and deoxynivalenol were checked using immunoaffinity column and the extraction of fumonisin was performed by strong anion exchange (SAX) solid phase column. Detection and quantification were determined by high performance liquid chromatography (HPLC). The limit of detection was 5 µg/kg for ZEN, 100 µg/kg for DON and 50 and 100 µg/kg for FB1 and FB2 respectively.Fusarium toxins were detected in 20 samples. Sixteen samples were positive for ZEN (30.7%) presenting levels that ranged from 7.4 µg/kg to 61.4 µg/kg (mean=27.0 µg/kg). 13.5% of the samples presented contaminations of DON, with levels ranging from 100.0 µg/kg to 253 µg/kg (mean=l18.07 µg/kg). FB1 was detected in 19.2% of samples, with levels ranging from 50.0 µg/kg to 110.0 µg/kg (mean=73.6 µg/kg). FB2 was not detected in any sample. In positive samples simultaneously contamination with two or three mycotoxins were detected in 9 of them (17.3%).

Oliveira *et al.* (2006) evaluated the natural occurrence of aflatoxin B(1), fumonisin B(1) and zearalenone in poultry feed samples. Fungal counts were similar between all culture media tested (10(3)CFU g(-1)). The most frequent genus isolated was Penicillium spp. (41.26%) followed by Aspergillus spp. (33.33%) and Fusarium spp. (20.63%). High precision liquid chromatography was applied to quantify aflatoxin B(1) and fumonisin B(1). Thin layer chromatography was used to determine zearalenone levels. Aflatoxin B(1)values ranged between 1.2 and 17.5 microg kg(-1). Fumonisin B(1) levels ranged between 1.5 and 5.5 microg g(-1). Zearalenone levels ranged between 0.1 and 7 microg g(-1). The present study shows the simultaneous occurrence of two carcinogenic mycotoxins, aflatoxin B(1) and fumonisin B(1), together with another Fusarium mycotoxin (zearalenone) in feed intended for poultry consumption. Many samples contained AFB(1)levels near the permissible maximum and it could affect young animals. A synergistic toxic response is possible in animals under simultaneous exposure

Yegani *et al.* (2006) conducted a study to investigate the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of broiler breeders. Forty-two 26-wk-old broiler breeder hens and nine 26-wk-old roosters were fed the following diets: (1) control, (2) contaminated grains, and (3) contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GMA) for 12 wk. The major contaminant was **deoxynivalenol** (12.6 mg/kg of feed), with lesser amounts of **zearalenone** and 15-acetyl-deoxynivalenol. Feed consumption and BW were not affected by diet. The feeding of contaminated grains did not significantly affect egg production. Decreased eggshell thickness was seen, however, at the end of wk 4, and dietary supplementation with GMA prevented this effect. There was no effect of diet on other egg parameters measured. There was a significant increase in early (1 to 7 d) embryonic mortality in eggs from birds fed contaminated grains at wk 4, but mid- (8 to 14 d) and late- (15 to 21 d) embryonic mortalities were not affected by diet. There were no differences in newly hatched chick weights or viability. The ratio of chick weight to egg weight was not affected by the feeding of contaminated grains. Weight gains of chicks fed a standard broiler starter diet at 7, 14, and 21 d of age were not significantly affected by previous dietary treatments for the dam. It was found that rooster semen volume and sperm concentration, viability, and motility were not affected by the feeding of contaminated diets. There was no effect of diet on the relative weights of liver, spleen, kidney, and testes. The feeding of contaminated

grains decreased antibody titers against infectious bronchitis virus at the end of wk 12, and this was prevented by dietary supplementation with GMA. There was no effect of the diet on serum antibody titers against Newcastle disease virus. It was concluded that the feeding of blends of grains contaminated with *Fusarium* mycotoxins could affect performance and immunity in broiler breeder hens.

Borutova *et al.* (2008) investigated the effects of dietary contamination with various levels of deoxynivalenol (DON) and zearalenone (ZEA) on Ross 308 hybrid broilers of both sexes. After hatching, all chickens were fed an identical control diet for two weeks. Then chickens of Group 1 received a diet contaminated with DON and ZEA, both being 3.4 mg kg(-1), while Group 2 received DON and ZEA at 8.2 and 8.3 mg kg(-1), respectively. The diet of the control group contained background levels of mycotoxins. Samples of blood and tissues were collected after two weeks. Intake of both contaminated diets resulted in a significantly decreased activity of glutathione peroxidase (GPx) and increased level of malondialdehyde (MDA) in liver tissue, while in kidneys the concentration of MDA was significantly increased only in Group 1. On the other hand, activities of blood GPx and plasma gamma-glutamyltransferase (GGT) were elevated in Group 2 only. Activities of thioredoxin reductase in liver and GPx in duodenal mucosa tissues, superoxide dismutase (SOD) in erythrocytes as well as levels of MDA in duodenal mucosa and alpha-tocopherol in plasma were not affected by dietary mycotoxins. Blood phagocytic activity was significantly depressed in Group 1 and 2. These results demonstrate that diets contaminated with DON and ZEA at medium levels are already able to induce oxidative stress and compromise the blood phagocytic activity in fattening chickens.

Kolf-Clauw *et al.* (2008) compared ZEA activation in avian food species. ZEA and its reduced metabolites were quantified in subcellular fractions of six avian species and rat livers. The alpha-ZOL/beta-ZOL ratio in rats was 19. The various avian food species cannot be considered to be equivalent in terms of ZEA reduction ($P<0.001$). Quails represented high "beta reducers", with alpha-ZOL/beta-ZOL ratio less than two. Weak "beta reducers" included on one part ducks and chickens showing alpha-ZOL/beta-ZOL ratio greater than 3 and up to 5.6 and on a second part geese, showing a lower production of alpha-ZOL than other poultry. Comparisons of enzyme kinetics in ducks and in quails show that these variations can be explained by the action of various isoforms of dehydrogenases. These results are relevant to food safety, in the context of frequently inevitable contamination of animal feed.

Yunus *et al.* (2012) investigated the effects of deoxynivalenol (DON), a type-B trichothecene, on broilers. Male broilers at 7 d of age were fed either a basal diet (0.265 ± 0.048 mg of DON; 0.013 ± 0.001 mg of zearalenone/kg), a low DON diet (1.68 mg of DON/kg; 0.145 ± 0.007 mg of zearalenone/kg), or a high DON diet (12.209 ± 1.149 mg of DON/kg; 1.094 ± 0.244 mg of zearalenone/kg). Increasing levels of DON decreased the weekly weight gain linearly ($P \leq 0.041$) during the first 3 wk of exposure; there were no significant differences in the weight gain of the birds after wk 3. With increasing levels of DON, the titers against Newcastle disease virus increased linearly during wk 2 ($P = 0.022$) and wk 4 ($P = 0.033$) of exposure, whereas the titers against infectious bronchitis virus decreased linearly ($P = 0.006$) during wk 5 of exposure. The serum protein concentration increased linearly ($P = 0.017$) during wk 2 and quadratically ($P = 0.002$) during wk 4 of exposure. Under these experimental conditions, the performance and vaccine response of the broilers were

modulated to varying degrees at concentrations of DON that are currently permitted (up to 5 mg/kg of diet) in many countries. Further studies are therefore required to clarify the implications of these results on the welfare of chickens.

Wang *et al.* (2012) studied the in vitro effects of the treatment of ConA-stimulated splenic lymphocytes with ZEN (0–25 μg/mL). ZEN modulates the expression of IL-2, IL-6, and IFN-γ. The IL-2 levels were up to fourfold higher ($P < 0.05$) compared with the levels in the control at toxin concentrations of 25 μg/mL after 48 h of treatment. The IL-6 levels were critically suppressed at this concentration; these changes were very statistically significant ($P < 0.05$). At lower ZEN concentrations (0.1, 0.4 and 1.6 μg/mL), the IFN-γ levels changed slightly; however at 6.25 and 25 μg/mL, the IFN-γ results reached statistical significance compared with the control levels ($P < 0.05$). These data suggest that ZEN has potent effects on the expression of chicken splenic lymphocytes cytokines at the mRNA level.

Osselaere (2013) studied the absolute oral bioavailability and the toxicokinetic parameters of deoxynivalenol, T-2 and zearalenone in broilers. Toxins were administered intravenously and orally in a two-way cross-over design. For deoxynivalenol a bolus of 0.75 mg/kg BW was administered, for T-2 toxin 0.02 mg/kg BW and for zearalenone 0.3 mg/kg BW. Blood was collected at several time points. Plasma levels of the mycotoxins and their metabolite(s) were quantified using LC-MS/MS methods and toxicokinetic parameters were analyzed. Deoxynivalenol has a low absolute oral bioavailability (19.3%). For zearalenone and T-2 no plasma levels above the limit of quantification were observed after an oral bolus. Volumes of distribution were recorded, i.e. 4.99 L/kg, 0.14 L/kg and 22.26 L/kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. Total body clearance was 0.12 L/min.kg, 0.03 L/min.kg and 0.48 L/min.kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. After IV administration, T-2 toxin had the shortest elimination half-life (3.9 min), followed by deoxynivalenol (27.9 min) and zearalenone (31.8 min)

Osselaere *et al.* (2013) investigated the effects of the mycotoxin T-2 on hepatic and intestinal drug-metabolizing enzymes (cytochrome P450) and drug transporter systems (MDR1 and MRP2) in poultry during this study. Broiler chickens received either uncontaminated feed, feed contaminated with 68µg/kg or 752µg/kg T-2 toxin. After 3weeks, the animals were euthanized and MDR1, MRP2, CYP1A4, CYP1A5 and CYP3A37 mRNA expression were analyzed using qRT-PCR. Along the entire length of the small intestine no significant differences were observed. In the liver, genes coding for CYP1A4, CYP1A5 and CYP3A37 were significantly down-regulated in the group exposed to 752µg/kg T-2. For CYP1A4, even a contamination level of 68µg/kg T-2 caused a significant decrease in mRNA expression. Expression of MDR1 was not significantly decreased in the liver. In contrast, hepatic MRP2 expression was significantly down-regulated after exposure to 752µg/kg T-2. Hepatic and intestinal microsomes were prepared to test the enzymatic activity of CYP3A. In the ileum and liver CYP3A activity was significantly increased in the group receiving 752µg/kg T-2 compared to the control group. The results of this study show that drug metabolizing enzymes and drug transporter mechanisms can be influenced due to prolonged exposure to relevant doses of T-2.

Osselaere (2013) studied the absolute oral bioavailability and the toxicokinetic parameters of deoxynivalenol, T-2 and zearalenone in broilers. Toxins were administered intravenously and orally in a two-way cross-over design. For

deoxynivalenol a bolus of 0.75 mg/kg BW was administered, for T-2 toxin 0.02 mg/kg BW and for zearalenone 0.3 mg/kg BW. Blood was collected at several time points. Plasma levels of the mycotoxins and their metabolite(s) were quantified using LC-MS/MS methods and toxicokinetic parameters were analyzed. Deoxynivalenol has a low absolute oral bioavailability (19.3%). For zearalenone and T-2 no plasma levels above the limit of quantification were observed after an oral bolus. Volumes of distribution were recorded, i.e. 4.99 L/kg, 0.14 L/kg and 22.26 L/kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. Total body clearance was 0.12 L/min.kg, 0.03 L/min.kg and 0.48 L/min.kg for deoxynivalenol, T-2 toxin and zearalenone, respectively. After IV administration, T-2 toxin had the shortest elimination half-life (3.9 min), followed by deoxynivalenol (27.9 min) and zearalenone (31.8 min)

Iqbal *et al.* (2014) analyzed aflatoxins (AFs), ochratoxin A (OTA) and zearalenone (ZEN) in 115 chicken meat and 80 eggs samples, collected from central areas of Punjab, Pakistan. The study was carried out using reverse phase HPLC, equipped with fluorescence detector. The results revealed that 35% samples of chicken and 28% samples of eggs were found contaminated with AFs, and maximum level of AFB1 and total AFs was found in the liver part of chicken (layer) 7.86 and 8.01 mg/kg, respectively. Furthermore, 41% samples of chicken and 35% sample of eggs were found contaminated with OTA and maximum level 4.70 mg/kg was found in the liver part of chicken meat. However, 52% samples of meat and 32% samples of eggs were found contaminated with ZEN and maximum level 5.10 mg/kg was found in the liver part of chicken meat. The occurrence and incidence of AFs, OTA and ZEN in chicken meat and eggs are alarming and it may produce health hazards and urged the need of continuous monitoring for these toxins in chicken meat and eggs.

Guerre (2015) mentioned that , despite the fact avian species are highly exposed to fusariotoxins, the avian species are considered as resistant to their toxic effects, partly because of low absorption and rapid elimination, thereby reducing the risk of persistence of residues in tissues destined for human consumption. This review focuses on the main fusariotoxins deoxynivalenol, T-2 and HT-2 toxins, zearalenone and fumonisin B1 and B2. The key parameters used in the toxicokinetic studies are presented along with the factors responsible for their variations. Then, each toxin is analyzed separately. Results of studies conducted with radiolabelled toxins are compared with the more recent data obtained with HPLC/MS-MS detection. The metabolic pathways of deoxynivalenol, T-2 toxin, and zearalenone are described, with attention paid to the differences among the avian species. Although no metabolite of fumonisins has been reported in avian species, some differences in toxicokinetics have been observed. All the data reviewed suggest that the toxicokinetics of fusariotoxins in avian species differs from those in mammals, and that variations among the avian species themselves should be assessed.

Liu *et al.* (2016) conducted a survey to determine whether mycotoxins present in the foods consumed by red-crowned cranes (*Grus japonensis*) in the Yancheng Biosphere Reserve, China, collected in the reserve’s core, buffer, and experimental zones during overwintering periods of 2013 to 2015, a total of 113 food samples were analyzed for aflatoxin B_1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A using high performance liquid chromatography (HPLC). The contamination incidences vary among different zones and the mycotoxins levels of different food samples also presented disparity. Average mycotoxin concentration from rice grain was greater

than that from other food types. Among mycotoxin-positive samples, 59.3% were simultaneously contaminated with more than one toxin. This study demonstrated for the first time that red-crowned cranes were exposed to mycotoxins in the Yancheng Biosphere Reserve and suggested that artificial wetlands could not be considered good habitats for the birds in this reserve, especially rice fields.

References:

1. Allen NK, Mirocha CJ, Aakhus-Allen S, Bitgood JJ, Weaver G, Bates F. Effect of dietary zearalenone on reproduction of chickens. Poult Sci. 1981 Jun;60(6):1165-74
2. Allen NK, Peguri A, Mirocha CJ, Newman JA. Effects of fusarium cultures, T-2 toxin, and zearalenone on reproduction of turkey females. Poult Sci. 1983 Feb;62(2):282-9.
3. Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*,*16*(3), 497–516. http://doi.org/10.1128/CMR.16.3.497-516.2003 Borutova R, Faix S, Plachaa I, Gresakovaa L, Cobanovaa K, et al. (2008) Effects of deoxynivalenol and zearalenone on oxidative stress and blood phagocytic activity in broilers. Arch Anim Nutr 62: 303–312
4. Branton, S.L.; Deaton, J.W.; Hagler, W.M.; Maslin, W.R., Jr.; Hardin, J.M. Decreased egg production in commercial laying hens fed zearalenone- and deoxynivalenol-contaminated grain sorghum. *Avian Dis.* 1989, *33*, 804–808
5. Chi, M. S., Mirocha, C. J., Weaver, G. A., & Kurtz, H. J. (1980). Effect of zearalenone on female White Leghorn chickens. *Applied and Environmental Microbiology*, *39*(5), 1026–1030
6. CHOWDHURY, S.R. and T.K. SMITH (2005): Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on hepatic fractional protein synthesis rates of laying hens and the efficacy of a polymeric glucomannan mycotoxin adsorbent. Poultry Science. 84, 1671-1674
7. Dailey R.E., Reese R.E., Brouwer E.A. Metabolism of [14C]zearalenone in laying hens. J. Agric. Food Chem. 1980;28:286–291
8. Dänicke S., Ueberschär K.H., Halle I., Valenta H., Flachowsky G. Excretion kinetics and metabolism of zearalenone in broilers in dependence on a detoxifying agent. Arch. Tierernahr. 2001;55:299–313.
9. Dänicke S., Ueberschär K.H., Halle I., Matthes S., Valenta H., Flachowsky G. Effect of addition of a detoxifying agent to laying hen diets containing uncontaminated or Fusarium toxin-contaminated maize on performance of hens and on carryover of zearalenone. Poult. Sci. 2002;81:1671–1680
10. Dänicke S., Matthes S., Halle I., Ueberschär K.H., Döll S., Valenta H. Effects of graded levels of Fusarium toxin-contaminated wheat and of a detoxifying agent in broiler diets on performance, nutrient digestibility and blood chemical parameters. Br. Poult. Sci. 2003;44:113–126.
11. Dänicke S., Ueberschär K.H., Valenta H., Matthes S., Matthäus K., Halle I. Effects of graded levels of Fusarium-toxin-contaminated wheat in Pekin duck diets on performance, health and metabolism of deoxynivalenol and zearalenone. Br. Poult. Sci. 2004
12. Gromadzka, K., A. Waskiewicz, J. Chelkowski and. Golinski.. Zearalenone and its metabolites: occurrence, detection, toxicity and guidelines. *World Mycotoxin Journal, May 2008; 1(2): 209-220*

13. Guerre.Ph. Fusariotoxins in Avian Species: Toxicokinetics, Metabolism and Persistence in Tissues. Toxins (Basel). 2015 Jun; 7(6): 2289–2305.
14. Iqbal Shahzad Zafar, Sonia Nisar , Muhammad Rafique Asi c , S. Jinap. Natural incidence of aflatoxins, ochratoxin A and zearalenone in chicken meat and eggs.Food Control 43 (2014) 98e103
15. Keshavarz, K. (1993) Corn Contaminated with Deoxynivalenol: Effects on Performance of PoultryJ Appl Poult Res (1993) 2 (1): 43-50
16. Kolf-Clauw M., Ayouni F., Tardieu D., Guerre P. Variations in zearalenone activation in avian food species. Food Chem. Toxicol. 2008;46:1467–1473
17. **Kuiper-Goodman, T., P. M. Scott, and H. Watanabe.** 1987. Risk assessment of the mycotoxin zearalenone. Regul. Toxicol. Pharmacol. 7**:**253-306.
18. Labuda, R.; Parich, A.; Berthiller, F.; Tančinová, D. Incidence of trichothecenes and zearalenonein poultry feed mixtures from Slovakia. *Int. J. Food Microbiol.* 2005, *105*, 19–25.
19. Liu, Da-wei , Hong-yi Liu, Hai-bin Zhang, Ming-chang Cao, Yong Sun, Wen-da Wuand Chang-hu Lu. Potential natural exposure of endangered red-crowned crane (*Grus japonensis*) to mycotoxins aflatoxin B_1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A* J Zhejiang Univ Sci B. 2016 Feb; 17(2): 158–168
20. Martins, H. M., M. M. Guerra and F. Bernardo. Zearalenone, deoxynivalenol and fumonisins in mixed-feed for laying hens, Mycotoxin Research Vol. 22, No. 4 (2006), 206-210
21. Maryamma K.I., Manomohan C.B., Nair M.G., Ismail P.K., Sreekumaran T., Rajan A. Pathology of zearalenone toxicosis in chicken and evaluation of zearalenone residues in tissues. Ind. J. Anim. Sci.1992;62:105–107
22. Mirocha C.J., Robison T.S., Pawlosky R.J., Allen N.K. Distribution and residue determination of [3H]zearalenone in broilers. Toxicol. Appl. Pharmacol. 1982;66:77–87
23. Oliveira, G.R., Ribeiro, J.M., Fraga, M.E., Cavaglieri, L.R., Direito, G.M., Keller, K.M, Dalcero, A.M. and Rosa, C.A., 2006. Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia 162: 355-362Olsen M., Mirocha C.J., Abbas H.K., Johansson B. Metabolism of high concentrations of dietary zearalenone by young male turkey poults. Poult. Sci. 1986;65:1905–1910.
24. Osselaere A., Li S.J., de Bock L., Devreese M., Goossens J., Vandenbroucke V., van Bocxlaer J., Boussery K., Pasmans F., Martel A., et al. Toxic effects of dietary exposure to T-2 toxin on intestinal and hepatic biotransformation enzymes and drug transporter systems in broiler chickens. Food Chem. Toxicol.2013;55:150–155
25. Osselaere A., Devreese M., Goossens J., Vandenbroucke V., De Baere S., De Backer P., Croubels S. Toxicokinetic study and absolute oral bioavailability of deoxynivalenol, T-2 toxin and zearalenone in broiler chickens. Food Chem. Toxicol. 2013;51:350–355
26. Shier, W.T., Shier, A.C., Xie, W. and Mirocha, C.J., 2001. Structure - activity relationships for human estrog
27. Swamy HV, Smith TK, MacDonald EJ, Boermans HJ, Squires EJ. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J Anim Sci. 2002 Dec;80(12):3257-67.
28. SWAMY, H.V.L.N., T.K. SMITH, N.A. KARROW, and H.J. BOERMANS (2004): Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins

on growth and immunological parameters of broiler chickens. Poultry Science. 83, 533-543

29. Sypecka, Zuzana , Mitchell Kelly , and Paul Brereton, Deoxynivalenol and Zearalenone Residues in Eggs of Laying Hens Fed with a Naturally Contaminated Diet: Effects on Egg Production and Estimation of Transmission Rates from Feed to Eggs *J. Agric. Food Chem.*, 2004, 52 (17), pp 5463–5471
30. Urry, W.H., Wehrmeister, H.L., Hodge, E.B. and Hidy, P.H., 1966. The structure of zearalenone. Tetrahedron Letters 27: 3109-3114.
31. Wang, Y. C., Deng, J. L., Xu, S. W., Peng, X., Zuo, Z. C., Cui, H. M., … Ren, Z. H. (2012). Effects of Zearalenone on IL-2, IL-6, and IFN-γ mRNA Levels in the Splenic Lymphocytes of Chickens. *The Scientific World Journal*, *2012*, 567327. http://doi.org/10.1100/2012/567327
32. Yegani *et al.* (2006Yunus AW, Ghareeb K, Twaruzek M, Grajewski J, Böhm J (2012) Deoxynivalenol as a contaminant of broiler feed: effects on bird performance and response to common vaccines. Poult Sci 91: 844–851

4.7.6. Fusaric acid (FA)

Fusaric Acid (FA) is one of several mycotoxins produced by the fungus Fusarium verticillioides (formerly moniliforme) that is ubiquitous on corn throughout the world (**Burmeister *et al.,* 1985).**

Fusaric acid is a hypotensive agent and is moderately toxic when compared to other Fusarium mycotoxins (**Smith and Sousadias, 1993).**

Aspergillus tubingensis isolate CDRAt01 grown with the addition of FA indicated the formation of a metabolite over time that was associated with a decrease of fusaric acid as a result of its conversion to fusarinol.. Fusarinol was significantly less toxic than FA. Therefore, the A. tubingensis strain provides a novel detoxification mechanism against fusaric acid (**Crutcher *et al*., 2014)**

Fusaric Acid

N O OH

Fusarinol

N OH

Structures of fusaric acid and fusarinol

- Fusaric acid (FA) is a naturally occurring metabolite of *Fusarium moniliforme* that has a low toxicity in animals when compared to other *Fusarium* mycotoxins (Smith and Sousadias, 1993).
- Fusaric acid has been reported to occur naturally at levels up to 250 mg/kg (Dowd, 1988).
- This compound is only moderately toxic to animals, but has antibiotic, pharmacological, and insecticidal properties (Burmeister *et al*., 1985), which has resulted in its being classified as a phytotoxin, rather than a mycotoxin (Matsuo, 1983).
- Fusaric acid reported to synergize the toxicity of some trichothecenes in a bioassay with caterpillars **(Dowd, 1988**)
- Fusaric acid reported to synergize the toxicity of fumonisin B1 in a fertile chicken egg embryo bioassay (**Bacon et al., 1995**).
- Fusaric acid acts synergistically with DON to magnify the negative effects of DON.
- The cumulative effects of all mycotoxins present can lower performance, lower immune response, raise health costs due to ineffective treatments, reduce the value of nutritional inputs, and decrease the profitability and efficiency of the flock.

Effects of FA in poultry

- **Chu *et al.* (1993)** found that levels up to 150 mg FA/kg diet did not negatively affect chick performance, but did suppress cell mediated immunity in chicks.
- **Fairchild *et al.* (2005)** reported no differences in growth performance of poults fed up to 300 mg FA/kg diet for 18 days, whereas
- **Ogunbo et al. (2005)** reported no differences in growth performance in chicks and poults fed up to 400 mg FA/kg diet from hatch to 21 days.
- **Chu *et al.* (1993)** fed chicks FA at levels up to 150 mg/kg diet and observed no deleterious effects on chick performance. Therefore, the objective of this research was to determine the effects of FA in the diets of young broiler chicks and turkey poults, at levels below and above the highest reported naturally occurring level of250 mg FA/kg diet.
- **Fairchild *et al.* (2005)** reported significant reduction in relative intestinal weight and jejunal serosa thickness in turkey poults fed 300 mg of purified FA/kg of feed for 18 d. Feeding 4 mg of DAS/kg of feed to turkey poults did not affect the weight of intestine; however, feeding both FA and DAS to poults decreased enterocyte height at midvillus by 59%. This decrease, however, is indicative of *Fusarium* mycotoxins altering digestive and absorptive function.

Fusaric acid producing Fusarium species

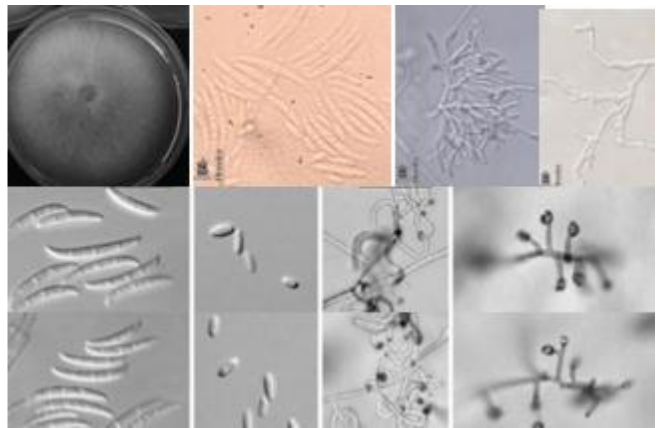
1. *Fusarium circinatum*
2. *Fusarium fujikuroi*
3. *Fusarium heterosporum*
4. *Fusarium napiforme*
5. *Fusarium nygamai*
6. *Fusarium oxysporum*
7. *Fusarium proliferatum*
8. *Fusarium sambucinum*

9. *Fusarium subglutinans*
10. *Fusarium thapsinum*
11. *Fusarium redolens*
12. *Fusarium sacchari*

Description of fusaric acid producing species

1. Fusarium circinatum **Nirenberg & O'Donnell, Mycologia 90: 446 (1998)**

Colonies on PDA with entire margin. Aerial mycelium almost white, hairy to lanose-funiculose. Pigmentation in reverse greyish white to grey to dark violet at the center of the colom. Conidiophores of the aerial mycelium erect. strongly branched, branches terminating mostly in I or 2 phialides. Sporodochial conidiophores verticillately branched. Phialides of the aerial conidiophores cylindrical, mono- and polyphialidic. Micrconidia borne in the aerial mycelium mostly obovoid, occasionally oval to allantoid, mostly 0-1 septate, occasionally l-septate. Macronidia borne in sporodochia slender, cylindrical, mostly 3-septate. Chlamydospores absent.

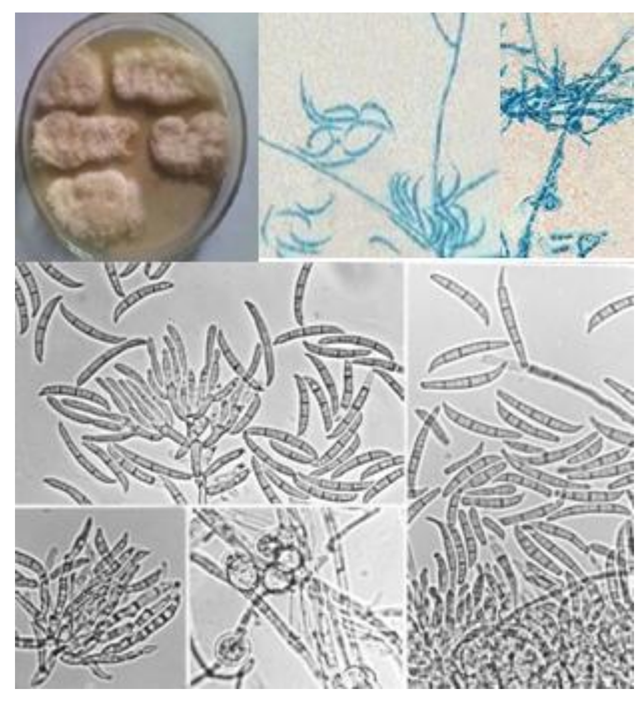


F. circinatum www.scielo. Circinus Macro and Microconidias Mono and Poly-Phialides, www.efa-dip.org, John F. Leslie and Brett A. Summerell

2. Fusarium heterosporum **Nees, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur.: 135 (1817)**

Fusisporium lolii Wm.G. Sm., Diseases of field and garden crops, chiefly as are caused by fungi: 213 (1884))

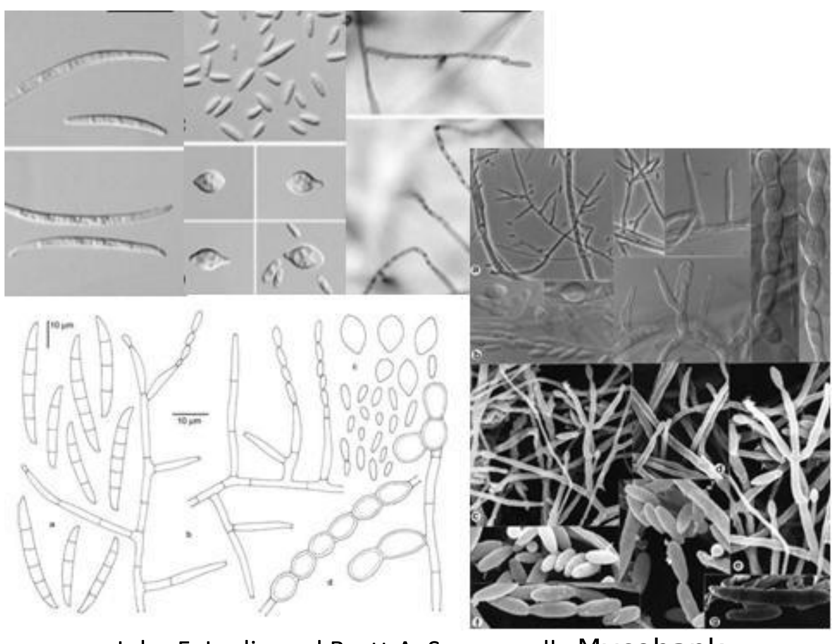
Macroconidia: abundant, 3-5 septa, thin-walled, slender to straight . Apical cell tapering, basal cell foot-shaped. Sporodochia: abundant, bright orange. Microconidia: absent. Chlamydospres: absent



Jandial and Sumbali, 2012 G. Hagedorn, M. Burhenne & H. I. Nirenberg

3. **Fusarium napiforme** Marasas, P.E. Nelson & Rabie, Mycologia 79 (6): 910 (1987)

Macroconidia: abundant in sporodochia, 3-5 septa, moderately long, falcate, apical cell curved and tapering, basal cell foot-shaped. Sporodochia: bright orange. Microconidia:lemon-shaped and napiform, 0-1 septa, long chains. Chlamydospores: produced slowly

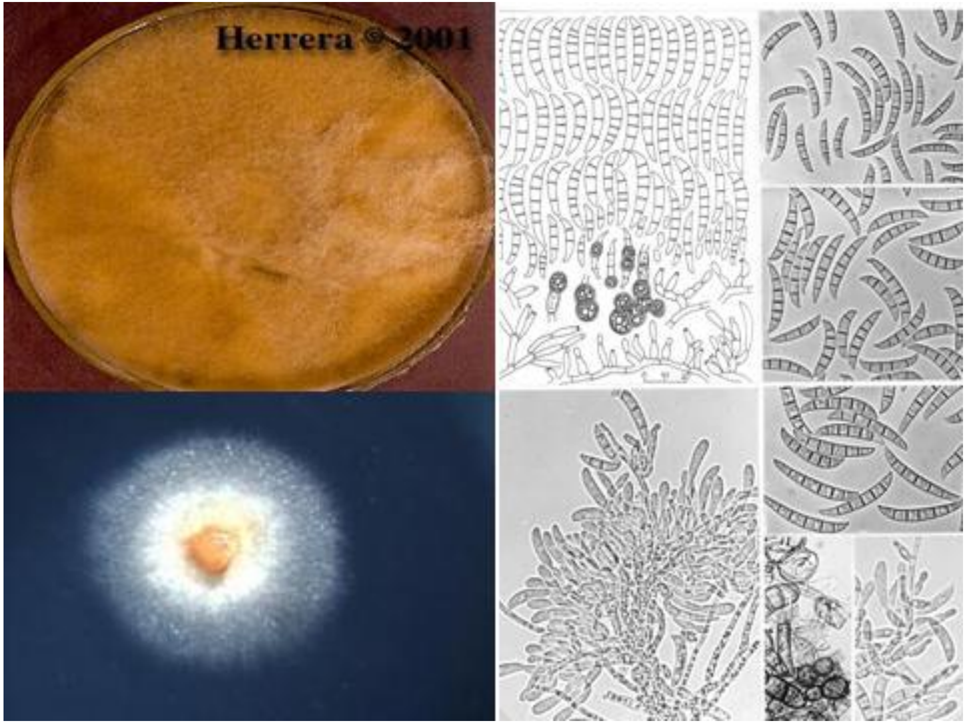


John F. Leslie and Brett A. Summerell , Mycobank

4. Fusarium sambucinum **Fuckel, Hedwigia 2 (15): 135, Fung. Rhen. no 211 (1863)**

=Fusarium roseum Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3: 10, t. 1:10 (1809)
=Fusarium sulphureum Schltdl., Flora Berolinensis, Pars secunda: Cryptogamia: 139 (1824) =Fusarium sambucinum var. sambucinum , Jahrbücher des Nassauischen Vereins für Naturkunde 23-24: 167 (1870) [
=Fusarium trichothecioides Wollenw., Journal of the Washington Academy of Sciences 2: 147 (1912)
=Fusarium sambucinum var. minus Wollenw., Fusaria Autographice Delineata 3: (1930)
=Fusarium sambucinum f. 2 Wollenw., Fusaria Autographice Delineata 3: 942 (1930)
=Fusarium sambucinum var. medium Wollenw., Zeitschrift für Parasitenkunde 3: 358 (1931)
=Fusarium sambucinum f. 6 Wollenw., Zeitschrift für Parasitenkunde 3: 358 (1931)

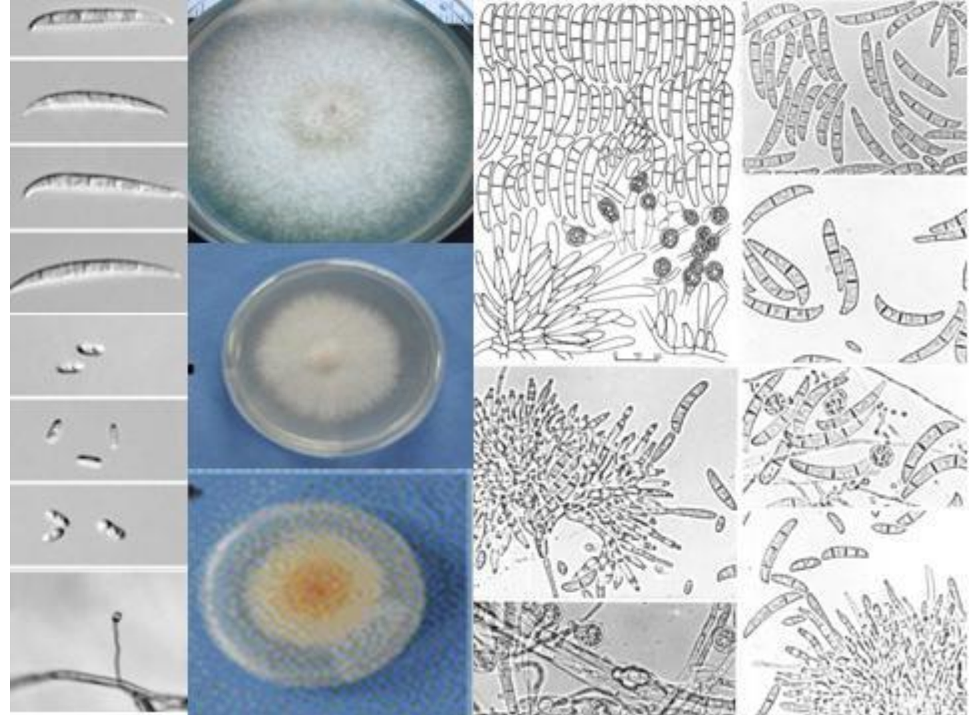
Macroconidia: abundant in sporodochia, 3-5 septa, falcate, slender, short, apical cell pointed, basal cell foot-shaped. Sporodochia: orange, common. Microconidia: oval, 0-1 septa. Chlamydospores: in chains or clusters



ddis.ifas.ufl.edu G. Hagedorn, M. Burhenne & H. I. Nirenberg

5. Fusarium redolens **Wollenw., Phytopathology 3 (1): 29 (1913)** ≡Fusarium oxysporum var. redolens (Wollenw.) W.L. Gordon, Canadian Journal of Botany 30 (2): 238 (1952)

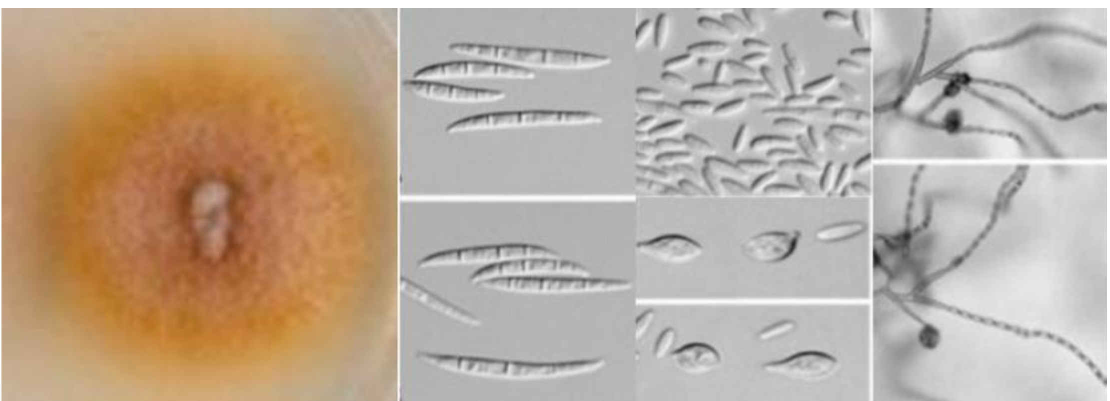
Macroconidia: abundant, 3-5 septa, thick-walled, upper third wide, apical cell hooked, basal cell foot-shaped. Sporodochia: sparse, pale brown. Microconidia: common in the aerial mycelia, oval to cylidrical, 0-1 septa. Chlamydospores: absent



John F. Leslie and Brett A. Summerell ,Truman Univ. Feng Pan,et al.,2015 G. Hagedorn, M. Burhenne & H. I. Nirenberg

6. Fusarium thapsinum Klittich, J.F. Leslie, P.E. Nelson & Marasas, Mycologia 89: 644 (1997)

Colonies produce white mycelium, violete pigments with age. Sporodochia rare, pale orange. Macroconidia rare, slender, falcate or straight, thin-walled, 3-5-septate, apical cell curved and tapering, foot cell poorly-shaped, Microconidia abundant, on monophiliedes, club-shaped, 0-septate. Chlamydospores absent



J.F. Leslie, P.E. Nelson & Marasas

Reports:

Burmeister *et al.* (1985) reported that Fusarium moniliforme NRRL 13,163 produced two new fusaric acid analogs, a 10,11-dihydroxyfusaric acid and a diacid of fusaric acid in which the C-11 methyl was oxidized to a carboxyl. Several hundred milligrams of the 10,11-dihydroxyfusaric acid were routinely recovered from a kilogram of corn grit medium. It crystallized as white, irregularly shaped rectangles that melted at 153 to 154 degrees C. The

diacid analog of fusaric acid crystallized as white rods that melted at 210 to 211 degrees C. Unlike the consistent recovery experienced with the 10,11-dihydroxyfusaric acid, the diacid analog proved difficult to purify after the initial discovery and was detectable in subsequent fermentations only by mass spectrometry.

Bacon et al. (1995) studied toxic interactions of fusaric acid and fumonisin B1, two mycotoxins produced by Fusarium moniliforme, in the chicken embryo. The yolk sacs of fertile White Leghorn eggs were injected before incubation with separate and combined solutions of either fusaric acid and or fumonisin B1. The toxins were administered in either a sterile 10 mM buffered phosphate solution, pH 6.90, which produced a final pH of 6.6 +/- 0.2, or sterile distilled water. Toxicity was based on absence of egg pip at the end of the 21-day incubation period. Toxins administered in the phosphate buffer solution were more toxic than those administered in distilled water. When both toxins were combined in equal concentrations and injected into eggs, increased toxicity resulted. Fusaric acid was shown to be a mild toxin to the eggs and when a relatively nontoxic concentration of it was combined with graded doses of fumonisin B1, a synergistic toxic response was obtained. Fusaric acid is only moderately toxic to the chicken egg, however its co-occurrence with other fusaria toxins found on corn and other cereals might present possible antagonisms or synergisms. The results of this egg model suggest that fusaric acid might play a role in enhanced and unpredicted toxicity in mammalian systems if it is consumed with other mycotoxins.

Swamy *et al.* (2002) fed three hundred sixty, 1-d-old male broiler chicks, diets containing grains naturally contaminated with Fusarium mycotoxins for 56 d. The four diets included control (0.14 mg/kg deoxynivalenol, 18 mg/ kg fusaric acid, < 0.1 mg/kg zearalenone), low level of contaminated grains (4.7 mg/kg deoxynivalenol, 20.6 mg/kg fusaric acid, 0.2 mg/kg zearalenone), and high level of contaminated grains without (8.2 mg/kg deoxynivalenol, 20.3 mg/kg fusaric acid, 0.56 mg/kg zearalenone) and with (9.7 mg/kg deoxynivalenol, 21.6 mg/kg fusaric acid, 0.8 mg/kg zearalenone) 0.2% esterified-glucomannan polymer derived from Saccharomyces cerevisiae1026 (E-GM). Body weight gain and feed consumption responded in a significant quadratic fashion to the inclusion of contaminated grains during the finisher period. Efficiency of feed utilization, however, was not affected by diets. The feeding of contaminated grains in the finisher period also caused significant linear increases in blood erythrocyte count and serum uric acid concentration and a significant linear decline in the serum lipase activity. Dietary inclusion of contaminated grains resulted in a significant quadratic effect on serum albumin and y-glutamyltransferase activity. Blood hemoglobin and biliary IgA concentrations, however, responded in significant linear and quadratic fashions. Supplementation of E-GM counteracted most of the blood parameter alterations caused by the Fusarium mycotoxin-contaminated grains and reduced breast muscle redness. It was concluded that broiler chickens may be susceptible to Fusarium mycotoxicoses when naturally contaminated grains are fed containing a combination of mycotoxins.

Swamy *et al.* (2004) conducted an experiment to investigate the effects of feeding grains naturally contaminated with Fusarium mycotoxins on growth and immunological parameters of broiler chickens. Three hundred sixty, 1- d-old male broiler chicks were fed 1 of 4 diets containing grains naturally contaminated with Fusarium mycotoxins for 56 d. The diets included (1) control; (2) low level of contaminated grains (5.9 mg/kg d**eoxynivalenol** (DON), 19.1 mg/kg **fusaric acid**

(FA), 0.4 mg/kg **zearalenone**, and 0.3 mg/kg 15-acetyldeoxynivalenol; (3) high level of contaminated grains (9.5 mg/kg DON, 21.4 mg/kg FA, 0.7 mg/kg zearalenone, and 0.5 mg/kg 15-acetyldeoxynivalenol); and (4) high level of contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GM polymer). Body weight gains and feed consumption of chickens fed contaminated grains decreased linearly with the inclusion of contaminated grains during the grower phase (d 21 to 42). Efficiency of feed utilization, however, was not affected by diet. Production parameters were not significantly affected by the supplementation of GM polymer to the contaminated grains. Peripheral blood monocytes decreased linearly in birds fed contaminated grains. The feeding of contaminated diets linearly reduced the B-cell count at the end of the experiment, whereas the T-cell count on d 28 responded quadratically to the contaminated diets. The feeding of contaminated diets did not significantly alter serum or bile immunoglobulin concentrations, contact hypersensitivity to dinitrochlorobenzene, or antibody response to SRBC. Supplementation with GM polymer in the contaminated diet nonspecifically increased white blood cell count and lymphocyte count, while preventing mycotoxin-induced decreases in B-cell counts. It was concluded that broiler chickens are susceptible during extended feeding of grains naturally contaminated with Fusarium mycotoxins.

Fairchild *et al.* (2005) studied the effect of diacetoxyscirpenol and fusaric acid on poults: Individual and combined effects of dietary diacetoxyscirpenol and fusaric acid on turkey poult performance. Turkey poults were randomly placed in batteries and fed one of four dietary treatments: control (C); control plus 4ppm diacetoxyscirpenol (DAS); control plus 300 ppm (FA); and control plus 4ppm DAS and 300ppm FA (FD). There were 10 poults per pen with 6 replicate pens per treatment. Individual BW, BW gains (BWG) and feed consumption by pen was determined at d6, d12, and d18. Period and cumulative feed to gain was calculated. Mouth lesions were scored for treatments at d18. On d18 poults were euthanized for determination of organ weights and jejunal histomorphometrics. FA had no effect on BW or BWG at any period compared to C. Poults fed FD had reduced BW and BWG compared to C, while poults fed DAS had lower BW than all treatments at every period. Poults fed FA or C had better feed to gain ($P<0.05$) than poults fed DAS or FD at d6. There were no differences among the treatments at d12 or d18. Poults fed FA had significantly lower relative intestine wt than poults fed other diets, and significantly higher relative bursa wt at d18 when compared to poults fed DAS or FD. DAS, FA and FD altered intestinal architecture. Poults fed DAS or FD had higher mouth lesion scores than poults fed FA or C, but mouth lesion scores in DAS and FD poults were not different from each other. Dietary DAS resulted in decreased poult performance, while dietary FA had little or no effect. Fusaric acid fed in combination with DAS resulted in some protective effect towards DAS.

Ogunbo *et al.* (2007) conducted two experiments to evaluate the individual and combined effects of fusaric acid (FA) and T-2 toxin (T-2) in broiler chicks and turkey poults. In each experiment, 80 day-old birds were allotted randomly to a 2×2 factorial arrangement with treatments of 0 and 250 mg FA/kg feed and 0 and 4 mg T-2/kg feed. Diets were fed to 4 pen replicates of 5 birds each for 21 days. Feed intake and body weight gain of poults were reduced by the T-2 and the FA\T-2 combination diets. Poults fed T-2 and the FA\T-2 combination diets were also less efficient in converting feed to gain. There were no treatment effects on performance of broilers. Poults fed FA and the FA\T-2 combination diets had increased heart weights, whereas

chicks fed FA and the FA\T-2 combination diets had increased kidney weights. Poults fed the combination FA\T-2 diet had higher serum Mg. Uric acid concentrations were higher in chicks fed the FA and FA\T-2 combination diets. Oral lesions were present in chicks (68%) and poults (100%) fed T-2 with or without FA. Data indicate no toxic synergy when FA and T-2 were fed simultaneously to broilers and turkeys at these dietary concentrations.

Che *et al.* (2011) conducted an experiment to determine the effects of different mycotoxin adsorbents including esterified glucomannan (EGM), hydrated sodium calcium aluminosilicate (HSCAS) and compound mycotoxin adsorbent (CMA) on performance, blood parameters, and liver pathological changes in broilers fed mold-contaminated feed. Two hundred and forty 10-day-old broilers were randomly assigned to one of the five dietary treatments including: i) control diet; ii) mold-contaminated diet; iii) mold contaminated diet+0.05% EGM; iv) mold-contaminated diet+0.2% HSCAS; v) mold-contaminated diet+0.1% CMA. At 35-days-old, blood and liver tissue samples were collected for analysis. 0.1% CMA improved ADG and ADFI during 10-42 d compared to the mold contaminated group ($p<0.05$). The mold-contaminated diet increased total white blood cell (WBC) number, haemoglobin (Hgb) concentration, hematocrit (Hct) level, serum aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) activities, and decreased red blood cell (RBC) number and serum globulin (GLB) and urea nitrogen (BUN) concentrations ($p<0.05$). The three mycotoxin adsorbents alleviated the alteration of RBC, WBC, Hgb and AST caused by the mold-contaminated diet. Furthermore, 0.1% CMA increased GLB concentration and decreased Hct level and GGT activity ($p<0.05$). Liver superoxide dismutase (SOD) activity was reduced, and myeloperoxidase (MPO) activity was increased by the mold-contaminated diet ($p<0.05$). Both EGM and HSCAS prevented the increase of MPO activity ($p<0.05$). Liver lesion, including severe vacuolar degeneration of hepatocytes, was observed in chicks fed the mold-contaminated diet. 0.05% EGM prevented these effects except for biliary hyperplasia and mild vacuolar degeneration. 0.2% HSCAS showed medium vacuolar degeneration of hepatocytes. Liver of broilers fed 0.1% CMA revealed a mild vacuolar degeneration. These results indicated that a mold-contaminated diet results in adverse effects on blood parameters and liver morphology. 0.05% EGM and 0.2% HSCAS partially alleviated the adverse effects. However, 0.1% CMA almost completely ameliorated the adverse effects.
Robert *et al.* (2011) investigated the biosynthesis of fusaric acid using^{13}C-labeled substrates including [1,2-$^{13}C_2$]acetate as well as ^{13}C- and ^{15}N-labeled aspartate and [^{15}N]glutamine. The incorporation of labeled substrates is consistent with the biosynthesis of fusaric acid from three acetate units at C5–C6, C7–C8, and C9–C10, with the remaining carbons being derived from aspartate via oxaloacetate and the TCA cycle; the oxaloacetate originates in part by transamination of aspartate, but most of the oxaloacetate is derived by deamination of aspartate to fumarate by aspartase. The nitrogen from glutamine is more readily incorporated into fusaric acid than that from aspartate.

References:

1. Bacon, C.W., J.K. Porter and W.P. Norred, 1995. Toxic interaction of fumonisin B1 and fusaric acid measured by injection into fertile chicken eggs. Mycopathologia. 1995;129(1):29-35.

2. Burmeister, H. R., Grove, M. D., Peterson, R. E., Weisleder, D., & Plattner, R. D. (1985). Isolation and characterization of two new fusaric acid analogs from Fusarium moniliforme NRRL 13,163. *Applied and Environmental Microbiology*,*50*(2), 311–314.
3. Che, Zhengquan, Yulan Liu, Huirong Wang, Huiling Zhu, Yongqing Hou, Binying Ding, The Protective Effects of Different Mycotoxin Adsorbents against Blood and Liver Pathological Changes Induced by Mold-contaminated Feed in Broilers, *Asian-Australasian Journal of Animal Sciences*, 2011, 24, 2, 250
4. Chu, Q., W. Wu and E.B. Smalley, 1993. Decreased cell mediated immunity and lack of skeletal problems in broiler chickens consuming diets amended with fusaric acid. Avian Dis., 37: 863-867
5. Crutcher FK, Liu J, Puckhaber LS, Stipanovic RD, Duke SE, Bell AA, Williams HJ, Nichols RL. Conversion of fusaric acid to Fusarinol by Aspergillus tubingensis: a detoxification reaction. J Chem Ecol. 2014 Jan;40(1):84-9..
6. Dowd, P.F., 1988. Toxicological and biochemical interactions of the fungal metabolites fusaric acid and kojic acid with xenobiotics in Heliothis zea (F.) and Spodoptera frugiperda (J.E. Smith). Pestic.
7. Fairchild, A.S., J.L. Grimes, J.K. Porter, W.J. Croom, Jr., L.R. Daniel and W.M. Hagler, Jr., 2005. Effects of diacetoxyscirpenol and fusaric acid on poults: Individual and combined effects of dietary diacetoxyscirpenol and fusaric acid on turkey poult performance. Int. J. Poult. Sci., 4: 350-355
8. Jayaramu, G. M., M. L. Satyanarayana, P. Ravikumar, V. T. Shilpa, H. D. Narayanaswamy & Suguna Rao. OCHRATOXIN A AND CITRININ INDUCED PATHOMORPHOLOGICAL CHANGES IN BROILER CHICKEN. G.J.B.B., VOL.2 (2) 2013: 230-234
9. Ogunbo, S.O., D.R. Ledoux, J.N. Broomhead, A.J Bermudez and G.E. Rottinghaus, 2005. Effects of fusaric acid in broiler chicks and turkey poults. Int. J. Poult. Sci., 4: 356-359
10. Porter, J. K., Bacon, C. W., Wray, E. M. and Hagler, W. M. (1995), Fusaric acid in *Fusarium moniliforme* cultures, corn, and feeds toxic to livestock and the neurochemical effects in the brain and pineal gland of rats. Nat. Toxins, 3: 91–100.
11. Robert D. Stipanovic, Michael H. Wheeler, Lorraine S. Puckhaber, Jinggao Liu, Alois A. Bell, Howard J. Williams, Nuclear Magnetic Resonance (NMR) Studies on the Biosynthesis of Fusaric Acid from Fusarium oxysporum f. sp.vasinfectum, *Journal of Agricultural and Food Chemistry*, 2011, 59, 10, 5351
12. Swamy HV, Smith TK, MacDonald EJ, Boermans HJ, Squires EJ. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J Anim Sci. 2002 Dec;80(12):3257-67.
13. SWAMY, H.V.L.N., T.K. SMITH, N.A. KARROW, and H.J. BOERMANS (2004): Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on growth and immunological parameters of broiler chickens. Poultry Science. 83, 533-543

4.8. Oosporein Toxicosis (Avian gout)

Oosporein, a toxic pigment produced by Chaetomium trilaterale, C. aureum and several other species of filamentous fungi, is considered to be primarily a renal toxin.

The importance of oosporein is that the toxic isolates have been found in various agricultural commodities such as animal feeds, cereal grains and food products.

- Mouldy corn in particular, growing C. trilaterale, may yield high concentrations of oosporein toxin.
- In studied young broiler chickens and turkey poults, oosporein toxicosis is dose-dependent and can cause dehydration, stunted growth, pale nephromegaly and death, and appears to severely affect uric acid secretion leading to hyperuricemia and visceral and articular gout.
- turkey poults seemed to tolerate higher doses of oosporein before toxicosis was apparent than did broilers, bringing up the issue of physiological differences between these two species.

Chemical properties

Oosporein is 3,3',6,6'-tetrahydroxy-4,4'-dimethyl-1,1'-bi(cyclohexa-3,6-diene)-2,2',5,5'-tetraone],

Chemical formula: $C_{14}H_{10}O_8$,

The molecule has 2 symmetry, with the mid-point of the C-C bond linking the cyclo-hexadienedione rings located on a twofold rotation axis.

In the molecule, the ring is approximately planar, with an r.m.s. deviation of 0.0093 Å, and the two rings make a dihedral angle of 67.89 (5)°.

Intermolecular O-H...O hydrogen bonding occurs in the crystal structure.

Crystal Structures of the Fungal Metabolite Oosporein

- Oosporein is a symmetrical red coloured 2,5-dihydroxybenzoquinone derivative biosynthesized by a broad variety of soil borne fungi.
- The compound, being known for almost six decades, is the major secondary metabolite of the entomopathogenic fungi Beauveria brongniartii which is successfully applied as a biological control agent against the European cockchafer Melolontha melolontha.
- In the course of isolating and purifying pure oosporein from biological cultures a dioxane solvate and a non-solvated were obtained.

- The molecular geometry of oosporein is x-shaped with a dihedral angle of 67.8 and 79.9° in the non-solvated form and the dioxane solvate respectively. Surprisingly the two forms crystallize in the same space group (monoclinic, C2/c) showing a similar O-H...O network. The non-solvated form shows two dimensional O-H...O tetrameric layers which are off stacked leading to a densely packed structure. In the dioxane solvate one solvent molecule is involved in the O-H...O hydrogen bond network resembling the overall network of the anhydrous form. This pseudo-tetrameric arrangement results in a large channel along the c-axis which is occupied by highly disordered dioxane molecules.]

Oosporein producing fungi

Oosporein has been reported ((Taniguchi et al., 1984; Nagaoka et al., 2004; Mao et al., 2010; He et al., 2012; Sahab, 2012). to be produced by:

1. *Chaetomium aureum*,
2. *Chaetomium cupreum*,
3. *Beauveria bassiana*,
4. *Tremella fuciformis*,
5. *Phlebia mellea*,
6. *Verticillium psalliotae*
7. *Oospora colorans*

Oosporein natural occurrence:

- Oosporein occurs naturally worldwide in a variety of food grains intended for human and animal consumption and potentially high concentrations are encountered as contaminants in many important crops (Mao et al., 2010**).**
- The main route of animal exposure to oosporein is through ingestion of contaminated food stuff such as maize, wheat, and other cereals (Manning and Wyatt, 1984**).**
- Oosporein leads to adverse health effects ranging from acute lesions to chronic nephrites in livestock, poultry, and human (Cole et al., 1974**;**Brown et al., 1987**;** Ross et al., 1989**).**

Cytotoxicity

- Preliminary reports based on feeding experiments, described the nephrotoxic potential of Oosporein to Cockerels and broiler chicks (Cole et al., 1974**;** Brown et al., 1987**).**
- Oosporein was reported to be toxic to 1 day old chickens (Manning and Wyatt, 1984**).**
- Toxicity studies of oosporein in mice and hamsters indicated an LD_{50} value of 0.5 mg kg^{-1} body weight, when injected intraperitoneally (Wainwright et al., 1986**).**

- Oosporein inhibits ATPase activity, but the mechanism has not been studied, it is assumed to be a consequence of membrane disruption, since it alters erythrocyte morphology to promote cell lysis (Jeffs and Khachatourians, 1997**).**
- Contradictorily few reports have shown no such toxic effect of Oosporein when studied in cells, such as hamster tumor cells, baby hamster kidney cells and invertebrate models like *Artemia salina* and *Daphnia magna* (Abendstein and Strasser, 2000**;** Favilla et al., 2006**).**
- Aleo et al. (1991) studied the nephrotoxic effects of oosporein on rat renal proximal tubules which confered that the proximal tubule viability was altered. However, there was no evidence to support a direct inhibitory effect on mitochondrial respiration at a maximum oosporein concentration of 306 µg mL^{-1}.
- Mao et al. (2010) reported antitumor activity of oosporein on HL-60 and A549 cell lines with an IC_{50} of 28 µM. About cytotoxicity of oosporein, the reports are not consistent and there are no reports on mechanism of oosporein incited cytotoxicity.

.

Reports:

PEGRAM and WYATT (1981) fed diets containing oosporein at graded concentrations from 0 to 600 µg/g to male broiler chicks from hatching to 3 weeks of age. At dietary toxin levels of 100 µg/g and below, no detrimental effects were observed. Dietary oosporein concentrations of 200 µg/g and above elicited dose-related mortality resulting from severe visceral and articular gout. Three-week cumulative mortality percentages were 0, 13, 30, 57, and 95% for the 0, 200, 300, 400, and 600 µg/g levels, respectively. Upon necropsy, the prominent lesions observed were massive urate deposits in various tissues, swollen and pale kidneys, dehydration, proventricular enlargement with mucosal necrosis, and a green discoloration of the gizzard lining. The effects on the proventriculus and gizzard occurred at doses as low as 200 µg/g and were the most sensitive indicators of oosporein-toxicosis. In addition to the proventriculus, the relative weights of the kidney and liver were significantly increased in a dose-related fashion. A significant reduction in 3-week body weight at 400 µg/g apparently resulted from the lower feed consumption concomitantly observed at this level of dietary toxin. Oosporein also caused an increase in water consumption at 400 and 600 µg/g. Blood analyses indicated no toxin-related effect on plasma glucose, plasma protein, packed red blood cell volume, hemoglobin, and prothrombin times. The plasma concentration of uric acid was significantly elevated at 400 µg/g. These data and mechanistic considerations suggest that oosporein should be classified as a nephrotoxin in the broiler chicken.

Brown *et al.* (1987) examined Kidneys from broiler chicks receiving 300 micrograms of oosporein K salt per gram of feed continuously from 0 to 21 days of age by light and electron microscopy. Chicks that died at 3 days had nephrosis of initial proximal tubular segments with an early pyogranulomatous interstitial response. Macula densa cells had cytoplasmic accumulations of periodic-acid-Schiff-positive granules. Kidneys from chicks surviving 21 days had hypercellular or atrophic glomeruli and hyperplastic dilated proximal tubules. Centrilobular distal tubules were dilated and

filled with hyaline basophilic casts. Interstitial fibrosis was prominent in cortical and medullary zones. These findings indicate that oral oosporein is a severe nephrotoxin which can cause visceral urate deposition and severe nephrosis of initial proximal tubular segments. The histopathology of this mycotoxicosis was compared with those of infectious-bronchitis-induced nephrosis and avian urolithiasis syndrome.

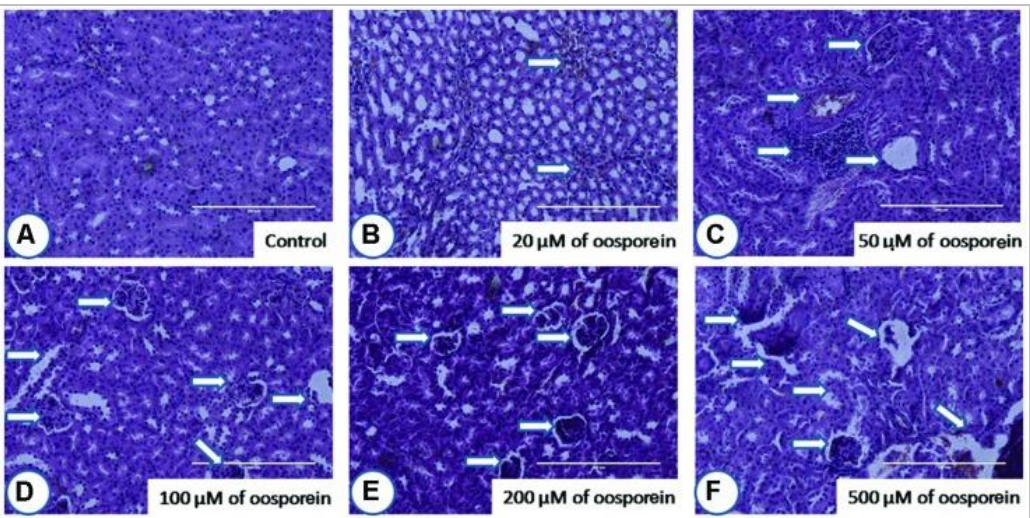
Ross *et al.* (1989) reported on chemical ionization (CI) mass spectral investigations of oosporein for the purpose of confirming TLC tests for the presence of oosporein in samples of poultry rations.

Strasser, *et al.* (2000) reported that Oosporein was the only major secondary metabolite produced by three commercial isolates of the entomopathogenic fungus *Beauveria brongniartii* in submerged cultures and on sterilised barley kernels. None of the other toxins (bassianin, beauvericin and tenellin) normally produced by *Beauveria* species were detected by sensitive HPLC and MS techniques. The maximum amount of oosporein produced in batch reactors was 270 mg l^{-1}, after 4 days incubation, while that produced on sterilised barley kernels ranged between 2.0 and 3.2 mg kg^{-1}, after 14 days incubation. The mean amount of oosporein detected in cockchafer larvae infected with *B. brongniartii* was 0.23 mg. Melocont®-Pilzgerste, a commercial product based on *B. brongniartii*, was not phytotoxic to *Lepidium sativum* and *Phleum pratense* nor were fungal metabolites detected in these indicator plants. No systemic effects of oosporein were observed in treated pasture turf maintained for several months in the greenhouse.

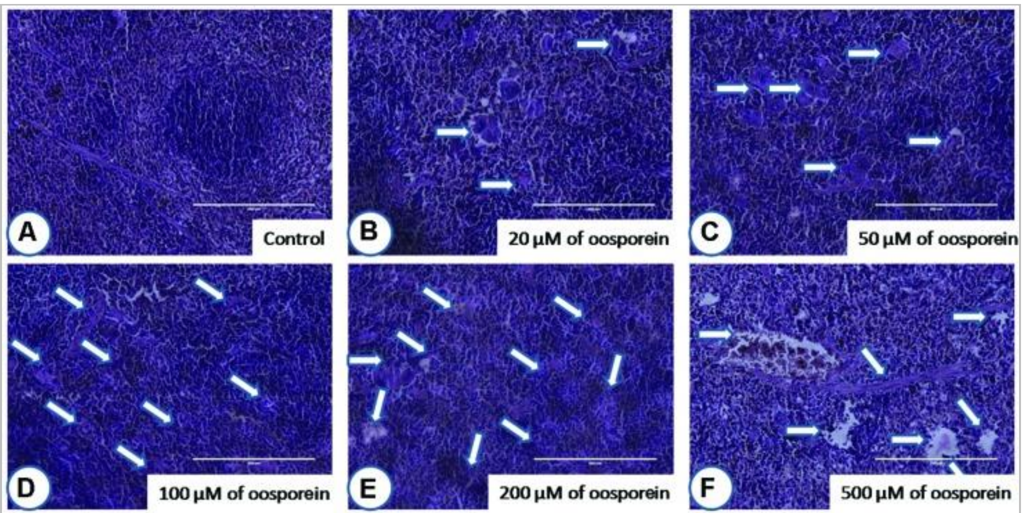
Manning and Wyatt (1984). compared the toxicity to broiler chicks of Chaetomium contaminated corn and various chemical forms of oosporein by feeding diets containing 60% Chaetomium contaminated corn (300 micrograms oosporein/g diet), and 300 or 150 micrograms/g of purified oosporein in either the K salt, Na salt, or organic acid form from hatching to 3 weeks of age. The Chaetomium contaminated corn diet caused 100% mortality during the first week of feeding. Necropsies revealed extensive visceral and articular gout, enlarged pale kidneys, dehydration, proventricular enlargement with mucosal necrosis, and a dark green discoloration of the gizzard lining. When the mortality percentages of the two experiments conducted were considered collectively, the K and Na salts of oosporein caused significantly higher mortality than the organic acid form of oosporein. The K salt caused the most severe lesions and the organic acid caused the least severe lesions. No mortality occurred at the 150 micrograms/g K salt or 150 micrograms/g organic acid levels. Relative kidney weights were increased by all forms of oosporein at 300 micrograms/g, but at 150 micrograms/g only the K salt caused an increase in kidney weight. The LD50 values, based on mortality from 1 to 10 days, were 5.77, 5.00, and 4.56 mg/kg for oosporein acid, oosporein Na salt, and oosporein K salt, respectively. These results suggest that the salts of oosporein (particularly the K salt) are more toxic than the organic acid, and the natural occurrence of oosporein in a salt form could contribute to the increased toxicity of the Chaetomium contaminated corn.

Ramesha et al. (2015) isolated oosporein from fungus *Cochliobolus kusanoi* of *Nerium oleander* L. Toxic effects of oosporein and the possible mechanisms of cytotoxicity as well as the role of oxidative stress in cytotoxicity to kidney cells and splene cells were evaluated *in vitro*. Also to know the possible *in vivo* toxic effects of oosporein on kidney and spleen, Balb/C mouse were treated with different concentrations of oosporein ranging from 20 to 200 μM). After 24 h of

exposure histopathological observations were made to know the effects of oosporein on target organs. Oosporein induced elevated levels of reactive oxygen species (ROS) generation and high levels of malondialdehyde, loss of mitochondrial membrane potential, induced glutathione hydroxylase (GSH) production was observed in a dose depended manner. Effects oosporein on chromosomal DNA damage was assessed by Comet assay, and increase in DNA damage were observed in both the studied cell lines by increasing the oosporein concentration. Further, oosporein treatment to studied cell lines indicated significant suppression of oxidative stress related gene (*Superoxide dismutase1* and *Catalase*) expression, and increased levels of mRNA expression in apoptosis or oxidative stress inducing genes *HSP70, Caspase3, Caspase6*, and *Caspase9* as measured by quantitative real time-PCR assay. Histopathological examination of oosporein treated mouse kidney and splenocytes further revealed that, oosporein treated target mouse tissues were significantly damaged with that of untreated sam control mice and these effects were in directly proportional to the the toxin dose. Results of the present study reveals that, ROS is the principle event prompting increased oosporein toxicity in studied *in vivio* and *in vitro* animal models. The high previlance of these fungi in temperate climates further warrants the need of safe food grain storage and processing practices to control the toxic effects of oosporein to humans and live stock.



Histopathological observations of kidney upon treatment with different concentrations of oosporein. Histopathology of kidney stained with hematoxylin-eosin-methylene blue. **(A)** Control kidney showed no histopathological damage, whereas oosporein exposed kidney showed clear evidence of histopathological damage with cortical tubular dilation with epithelial vacuolation and necrosis as shown as arrow marks. Damage was graded as **(B)** mild, **(C)** mild, **(D)** moderate, **(E)** moderate, and **(F)** severe extreme.



Histopathological observations of spleen upon treatment with different concentrations of oosporein. Histopathology of spleen stained with hematoxylin-eosin-methylene blue. **(A)** Control spleen showed no histopathological damage, whereas oosporein exposed spleen showed clear evidence of histopathological damage with splenic granulomas, macrophage infiltration and splenomegaly as shown as arrow marks. Damage was graded as **(B)** mild, **(C)** mild, **(D)** moderate, **(E)** moderate, and **(F)** severe extreme.

References:

1. Abendstein D., Strasser H. (2000). "Consideration on toxic metabolites produced by *Beauveria brongniartii*," in *Integrated Control of Soil Pests Subgroup "Melolontha"*, ed. Kelley S., editor. (Dijon: IOBC/WRP Bulletin;), 99–105.
2. Aleo, M. D., Wyatt, R. D., and Schnellmann, R. G. (1991). Mitochondrial dysfunction is an early event in ochratoxin A but not oosporein toxicity to rat renal proximal tubules. *Toxicol. Appl. Pharm.* 107, 73–80.
3. Brown, T. P., Fletcher, O. J., Osuna, O., and Wyatt, R. D. (1987). Microscopic and ultrastructural renal pathology of oosporein-induced toxicosis in broiler chicks. *Avian. Dis* 31, 868–877.
4. Cole, R. J., Kirksey, J. W., Cutler, H. G., and Davis, E. E. (1974). Toxic effects of oosporein from *Chaetomium trilaterale*. *J. Agr. Food Chem.* 22, 517–520.
5. Favilla M., Macchia L., Gallo A., Altomare C. (2006). Toxicity assessment of metabolites of fungal biocontrol agents using two different (*Artemia salina* and *Daphnia magna*) invertebrate bioassays.*Food Chem. Toxicol.* 44 1922–1931.
6. He, G., Yan, J., Wu, X. Y., Gou, X. J., and Li, W. C. (2012). Oosporein from *Tremella fuciformis*. *Acta Cryst. Sect. E Struct. Rep.* 68, (pt 4):01231. doi: 10.1107/S1600536812012950
7. Jeffs L. B., Khachatourians G. G. (1997). Toxic properties of *Beauveria* pigments on erythrocyte membranes. *Toxicon* 35 1351–1356.
8. Manning, R. O., and Wyatt, R. D. (1984). Comparative toxicity of Chaetomium contaminated corn and various chemical forms of oosporein in broiler chicks. *Poultry Sci.* 63, 251–259. doi: 10.3382/ps.0630251
9. Mao B. Z., Huang C., Yang G. M., Chen Y. Z., Chen S. Y. (2010). Separation and determination of the bioactivity of oosporein from *Chaetomium cupreum*. *Afr. J. Biotechnol.* 9 5955–5961.
10. Pegram RA, Wyatt RD: Avian gout caused by oosporein, a mycotoxin produced by Chaetomium trilaterale. Poult Sci 60:2429- 2440, 1981

11. Pegram RA, Wyatt RD, Smith TL Oosporein-toxicosis in the turkey poult. Avian Dis 26:47-59, 1982.
12. Ramesha, A., Venkataramana, M., Nirmaladevi, D., Gupta, V. K., Chandranayaka, S., & Srinivas, C. (2015). Cytotoxic effects of oosporein isolated from endophytic fungus *Cochliobolus kusanoi*. *Frontiers in Microbiology*, *6*, 870. http://doi.org/10.3389/fmicb.2015.00870
13. Ross P. F., David L. O., George E. R. Mass spectral confirmation of oosporein in poultry rations. *J. Vet. Diagn. Invest.* 1 271–272. (1989).
14. Strasser, H., Abendstein, D., Stuppner, H., and Butt, T. M. (2000). Monitoring the distribution of secondary metabolites produced by the entomogenous fungus *Beauveria brongniartii* with particular reference to oosporein. *Mycol. Res.* 104, 1227–1233. doi: 10.1017/S0953756200002963
15. Wainwright M., Betts R. P., Teale D. M. (1986). Antibiotic activity of oosporein from *Verticillium psalliotae*. *T. Brit. Mycol. Soc.* 86 168–170.

4.9. Avian ergotism

Ergotism is the effect of long term ergot poisoning, due to the ingestion of the alkaloids produced by the Claviceps fungus that infects cereals. The term ergot is a common name given to the sclerotia (fruiting bodies) of species of Claviceps naturally occurring in rye, wheat, barley, sorghum and some related crops. The fungus infects the plants at flowering and the infected florets eventually host the fungus as it develops hard sclerotia (ergots) that contain toxic alkaloids

Ergotism is the symptom that develops in humans and animals after eating food or feed with ergot contamination. Ergotism in humans is now rare because of the strict guidelines for allowable ergot bodies in grain. Ergot poisoning from eating contaminated rye flour led to deaths in the Middle Ages. Symptoms include impaired blood circulation, causing alternating burning and freezing sensations, followed by gangrene of extremities. This symptom was referred to as St. Anthony's Fire. Nervous convulsions can also occur and lead to eventual death. Commercially produced flour and grain products are at very little risk of contamination, but home-grown grain should not be used unless checked thoroughly to ensure it is free of ergot.

Ergotism can still be common in livestock when fed contaminated grain at the farm level. Symptoms may include lameness, loss of body parts from gangrene, abortions in pregnant animals, seizures, and eventually death. Consumption of contaminated feeds with sub-lethal doses may still lead to problems of poor growth and performance, loss of milk production in lactating animals, and animals going "off feed." Animals will recover from these milder symptoms when contaminated feed is removed. Animals differ in their susceptibility to ergot poisoning. Young or pregnant animals are considered highly susceptible.

1. **Rye ergotism** (Claviceps purpurea) also known as "St. Anthony's Fire", was one of the first mycotoxicoses to be recognized in the world (**van Rensburg and Altenkirk, 1974).**

Ergot of Rye is a plant disease that is caused by the fungus *Claviceps purpurea*. The so-called **ergot** that replaces the grain of the rye is a dark, purplish**sclerotium**, from which the sexual stage, of the lifecycle will form after over wintering. The sexual stage consists of stroma in which the asci and ascospores are produced

Ergot (sclerotia) on rye   Ergot (sclerotia) , sclerotia producing stroma with e asci and ascospores.

https://scottnevinssuicide.wordpress.com Rye grass seed and rye grain

2. **Sorghum ergot** (Claviceps africana) is widespread in Africa and Asia and has recently been introduced into Australia (**Ryley *et al*. 1996**).

Sorghum ergot is a disease caused by a fungus (Claviceps africana) that infects the ovaries of sorghum flowers and often converts them into a white, fungal mass (sphacelia). The most obvious external symptom of infection is the abundant exudation from infected flowers of an amber-colored, sticky fluid, or "honeydew," which often drips onto the leaves and soil. Spores of the fungus are contained within the honeydew, and when these germinate they produce secondary spores on the surface of the honeydew, giving it a white-scum to powdery appearance. Wind rapidly spreads these secondary spores over long distances. The fungus also can be spread by seed contaminated with sphacelia or honeydew. Under certain conditions, Claviceps africana produces very durable, compact fungal structures called sclerotia.

Newly formed honeydew dripping from an infected panicle. White secondary sporulation of the ergot fungus on the surface of honeydew. Joseph Krausz and Thomas Isakeit, Texas A&**M Univ**

3. **Wheat ergot:** is caused by the fungus Claviceps purpurea. In an infected plant, kernels are replaced by ergot bodies or sclerotia. These are black or dark purple and hard. Because they grow in place of a wheat kernel, these bodies can be almost the same size and shape as a wheat kernel. You may also see ergot bodies that are much larger than wheat kernels.

on the wheat head (left) an ergot body replaces the affected kernel as it grows, resulting in an ergot body that is similar in size and shape to the kernel it replaced.http://www.grainscanada.gc.ca/

The life cycle of ergot

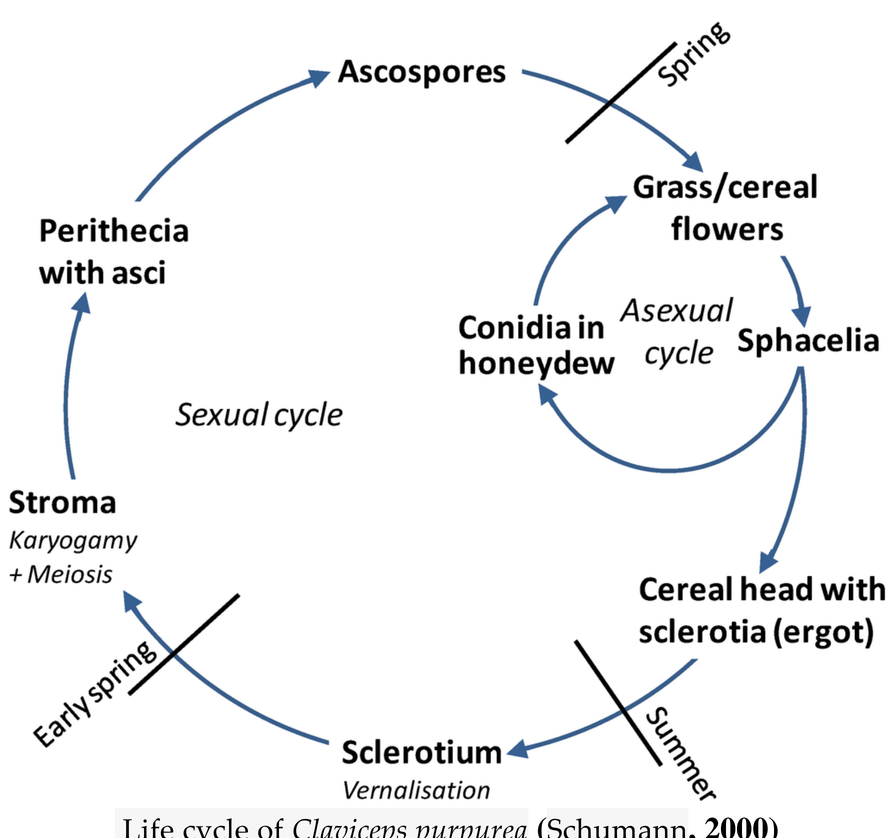
- There are two stages to the ergot disease cycle. The first stage occurs in the spring when ergot bodies germinate to produce tiny drumstick-shaped fruiting structures. Ergot bodies may be present in a field from a previous cereal crop, or from grasses along roadsides or neighbouring pastures. Ergot bodies may also be introduced into a field with planted seed.

- The drumstick-type structures produce spores called ascospores that become wind-borne. Ascospores land on florets and penetrate the ovaries of early

flowering plants such as wild grasses, fall-sown cereals, or early-sown spring crops. Within five days of the floret being infected by an ascospore, the second stage in the disease cycle occurs. This stage is known as the "honeydew stage."

- During the honeydew stage, the florets exude a sticky ooze of spores (conidia). Conidia are spread by insects and rain-splash to other florets. These spores can be disseminated for as long as flowering occurs. The honeydew stage declines once the infected ovary enlarges and becomes replaced by the hardened ergot body.

- Ergot bodies fall from the head to the soil before or during harvest, or may be harvested with the seed. Ergot bodies rarely survive for more than one year in the soil.



Life cycle of *Claviceps purpurea* (Schumann**, 2000)**

Claviceps

Claviceps includes about 50 known species, mostly in the tropical regions. Economically significant species include

1. *C. purpurea* (parasitic on grasses and cereals),
2. *C. fusiformis* (on pearl millet, buffel grass),

3. *C. paspali* (on dallis grass),
4. *C. africana* (on sorghum),
5. C. Lutea *(on paspalum).*

1. *C. zizaniae*,
2. *C. grohii*,
3. *C. sulcata*,
4. *C. purpurea*
5. *C. citrina*,
6. *C. phalaridis*,
7. *C. sorghicola*,
8. *C. gigantea*,
9. *C. sorghi*,
10. *C. viridis*,
11. *C. pusilla*.

.

Alkaloids

Ergot alkaloids are produced by a number of Claviceps species that infect cereal grains such as rye, wheat, triticale, barley, oats, sorghum, corn, rice, and several grass species **(Lorenz, 1979).**

The ergot alkaloids have a high biological activity and a broad spectrum of pharmacological effects, hence they are of considerable importance to medicine. They have adrenoblocking, antiserotonin and dopaminomimetic properties. Ergot alkaloids have a therapeutic effect on some forms of migraine, post-partum haemorrhages, mastopathy, and a sedative effect on the central nervous system. These compounds are now obtained both by methods of artificial parasitic cultivation on rye and by techniques using *in vitro* culture .

Naturally occurring ergot consists of two types of alkaloids. The first, the clavine-type alkaloids, are derivatives of 6,8-dimethylergoline. The second type comprises the lysergic acid derivatives, which are peptide alkaloids. All ergot alkaloids can be considered as derivatives of the tetracyclic compound 6-methylergoline. It is the lysergic acid derivatives, or peptide alkaloids, that are the pharmacologically active alkaloids. Each active alkaloid occurs with an inactive isomer involving isolysergic acid. These alkaloids have been studied over many years and were not easy to characterize. Six pairs of alkaloids predominate in the sclerotium and fall into the water-soluble ergometrine group or the water-insoluble ergotamine and ergotoxine groups. Alkaloids of groups 2 and 3 are polypeptides in which lysergic acid or isolysergic acid is linked to other amino acids. In the ergometrine alkaloids lysergic acid or its isomer is linked to an amino alcohol.

Structure of lysergic acid amines, representing the basic structure of ergot alkaloids.
Structure of peptide alkaloids: in ergotamine, for example, R1 is a methyl group and R2 is methylbenzene.

Alkaloids of the ergot sclerotium			
Group	Alkaloid	Formula	Discovered
1.Ergometrine group	Ergotmetrine	$C_{19}H_{22}O_2N_3$	Dudley & Moir (1935)
	Ergotmetrinine		
2.Ergotamine group	Ergotamine	$C_{33}H_{35}O_5N_5$	Spiro & Stoll (1920)
	Ergotaminine		
	Ergosine	$C_{30}H_{37}O_5N_5$	Smith & Timmis (1937)
	Ergosinine		
	Ergocristine	$C_{35}H_{39}O_5N_5$	Stoll & Burckhardt (1937)
3.Ergotoxine group	Ergocristinine		
	Ergocryptine	$C_{32}H_{41}O_5N_5$	Stoll & Hofmann (1938,1943)
	Ergocryptinine		
	Ergocornine	$C_{31}H_{39}O_5N_5$	
	Ergocorninine		

Claviceps purpurea. produces several toxic alkaloids including ergocornine, ergocristine, ergokryptine, and ergotamine **(Lacey, 1991**).

- Ergot alkaloids such as ergotamine have been reported to cause arterial and venous vasoconstriction, increased blood pressure, and decreased blood flow to the extremities (**Osweiller et al., 1985**).
- The major alkaloids produced by sorghum ergot are dihydroergosine (DHES) which usually represents >80% of the total, festuclavine and dihydroelymoclavine.
- In general, the ergot alkaloids have toxicological properties relating to their pharmacological activity, which includes
    - effects on the central nervous system,
    - action on smooth muscle, and adrenaline, serotonin and dopamine antagonism.
- The dihydro-alkaloids are considered much less active in regard to vasoconstriction and endothelial damage than the parent alkaloids (**Goodman and Gillman 1970).**

- Chickens are more tolerant than other livestock species to sorghum ergot, but high concentrations of rye ergot (5-10%) in diets can produce gangrene of the comb.

The effect of ergot on poultry production

- Poultry appear to tolerate higher levels of these toxins in feedstuffs than do ruminants, horses or swine.
- Characteristic signs of increased ergot in broilers are reduced feed consumption, depressed growth, incoordination, poor feathering and vasoconstriction resulting in elevated blood pressure, restricted blood flow and subsequent necrosis of toes, beak and skin (**Young and Marquardt, 1982 ; Rotter et al., 1985a,b).**
- Similar results were found by **Mannion and Blaney (1998) and Deo (2000)** where poultry showed:
    - depression in growth and poor feed conversion ratio when fed ergot contaminated sorghum in Queensland.
    - Higher dietary ergot levels (0.4 to 9.0 %) resulted in a depression in growth and increased chick mortality.
- Laying hens were shown to be more tolerant to dietary ergot (9%) than chicks, but egg production was adversely affected at higher levels of ergot alkaloids (**Bandyopadhyay et al., 1998).**
- The discovery and negative impact of sorghum ergot alkaloids (SEA) in poultry have been recently reported in the USA (Bailey and Fazzino, 1998) and in Australia (**Blaney et al; 1998**).Sorghum ergot alkaloid has significant adverse effects on overall performance. It has caused significant depression in growth, poor FCR, a reduction in dietary ME and increased diarrhoea and

death preceded by apparent gasping for breath but no difference in feed intake in hens

- **Rotter et al. (1985)** reported that in growing chicks, a concentration of 3.1 mgkg-1 total rye ergot alkaloid produced a statistically significant reduction in weight gain, and feed efficiency, progressing to an 80% decline in weight when fed 24.6 mgkg-1 ergot alkaloids.

Effect of feeding sorghum ergot (Claviceps africana) on poultry production

Three experiments were conducted to study the effect of sorghum ergot (Claviceps africana) alkaloid (dihydroergosine, DHES) on poultry (**BAILEY *et al.*, 1999)**

1. **Effect of sorghum ergot (Claviceps africana) alkaloid (dihydroergosine, DHES) on the production of laying hens.**

A total of 96 commercial ISA Brown laying hens were paired caged in a semi-controlled environment room, and fed different levels of ergot contaminated sorghum.

The basal diets were :(1) 24 mgkg-1 DHES, (2) 12 mgkg-1 DHES, (3) 6 mgkg-1 DHES and (4) zero DHES (normal sorghum). Mycosorb was added to half of each basal diet, making 8 diets in total.

The diets were fed and egg production, feed intake and egg weight were measured, and feed conversion ratio (FCR) was calculated for a period of 6 weeks.

- Egg production from the diet containing 24 mgkg-1 DHES was significantly less than that produced from the other diets.
- The addition of Mycosorb was beneficial.
- Egg weight at week four was significantly decreased by 24 mgkg-1 DHES.
- Egg mass decreased as the level of DHES in the diets increased but Mycosorb addition increased egg mass.
- Neither DHES nor Mycosorb had any significant effect on feed intake or feed conversion ratio overall.

This work showed that if the maximum limit of sorghum ergot was increased from the present level of 0.3% to 1% (i.e. from approximately 1 mgkg-1 to 5 mgkg-1 DHES) it would not significantly affect the production or efficiency of laying hens.

2. **Effect of DHES on dry matter digestibility in laying hens.**

A total of 32 commercial Brown laying hens 66 weeks old were pair caged in a semicontrolled environment room, and fed different levels of ergot contaminated sorghum.

- There was no significant (P>0.05) effect of DHES or Mycosorb addition on dry matter digestibility in laying hens.
- There was a trend of decreasing digestibility with increasing level of DHES in the diet.
- It was concluded that increasing the maximum allowable concentration of ergot in layer diets from 0.3% to 1% (from 1 to 5 mg DHES/kg diet) would not significantly affect the digestibility of the diet.

3. **Effect of DHES on alkaloid residues in eggs**.

Hens were fed diets containing up to 24 mg DHES/kg for several weeks and eggs were collected daily. Over 80 eggs from ergot-fed birds and 80 from control birds were blended and assayed by an ELISA that is very specific for DHES. The ELISA had a detection limit of 0.005 mg/kg DHES, but DHES was not detected in any egg. Over 40 eggs from the ergot-fed birds were also assayed by HPLC with fluorescence detection, also with negative results

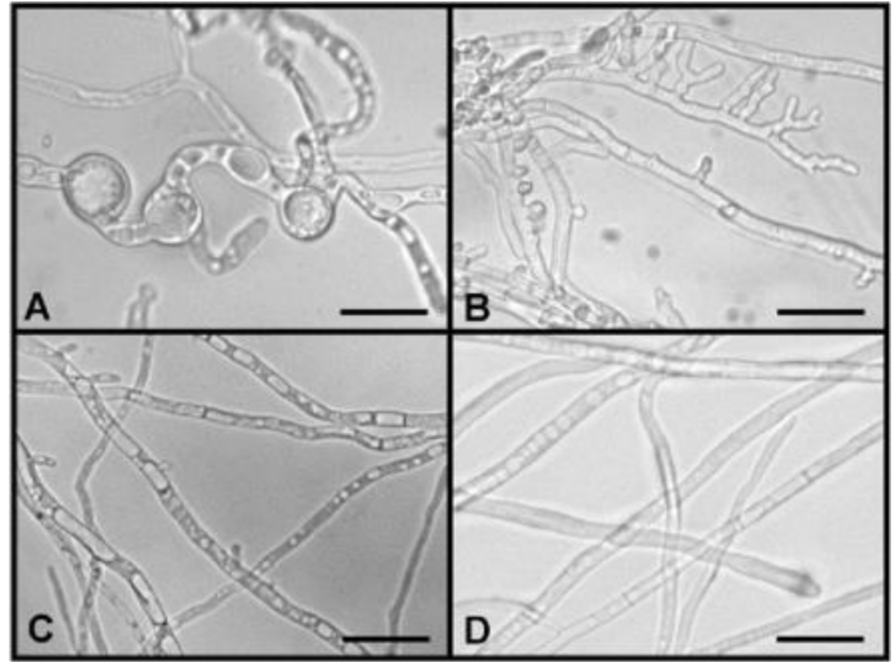
Description of some Claviceps species:

1. Claviceps purpurea (Fr.) Tul., Annales des Sciences Naturelles Botanique 20: 45 (1853)

≡Pseudocenangium purpureum (Fr.) A. Knapp
≡Sphaeria purpurea Fr., Systema Mycologicum 2: 325 (1823)
≡Cordyceps purpurea (Fr.) Fr., Summa vegetabilium Scandinaviae 2: 361 (1849)

Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales,Clavicipitaceae, Claviceps

Morphology of C. purpurea is variable. Sclerotial length ranges from 2 to 50 mm and the color of the stromata varies over a wide scale of red shades. Conidial size and shape also are polymorphic, ranging from oval spores 5 ìm in length to cylindric or elongated and up to 13 ìm in length (Loveless 1971, Sprague1950, Tanda 1979). The sclerotia contain peptide alkaloids that belong to three basic groups - ergotamines (with alanine as the first amino acid entering the cyclopeptide moiety), ergotoxines (with valine), and rarely found ergoxines (with 2-aminoisobutyric acid) (Walzel et al. 1997).
Three groups were identified: G1 from fields and open meadows G2 from shady or wet habitats G3 from Spartina salt marshes The sclerotia of G1 contained various ergotamines and ergotoxines, its conidia were 5-8 ìm long. G2 produced ergosine and ergocristine with small amounts of ergocryptine, conidia were 7-10 ìm long. G3 produced ergocristine and ergocryptine and conidial length was 10-12 ìm. Sclerotia of the G2 and G3 isolates floated on water.



Morphology of *C. purpurea* transformants overexpressing Ct*cdc42*(*G14V*) (A), Ct*cdc42*(*T19N*) (B), and unmodified Ct*cdc42* (C). *C. purpurea* wild-type strain 20.1 (D). Strains were grown for 5 days on Mantle medium. For details see text. Scale bars, 10 µm

2. **Claviceps africana Freder., Mantle & De Milliano, Mycological Research 95: 1106 (1991)**

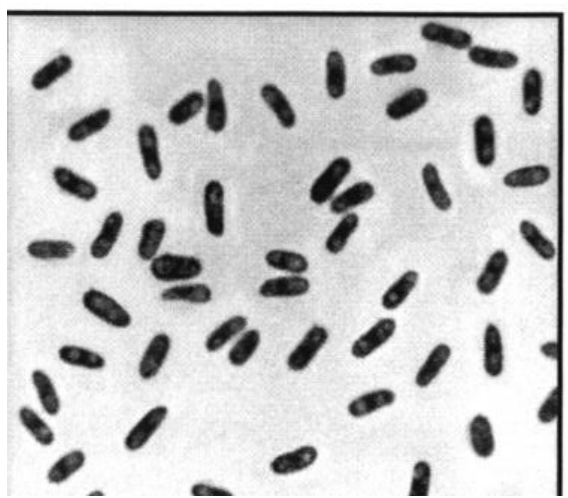
Morphology:

Teleomorph: Producing stromata with a purple stipe and dark purple capitula, 8-15 mm in height from reddish brown ergot (sclerotia). Ascocarps embedded on the surface of capitula, pyriform, producing cylindrical asci inside. Ascospores hyaline, filiform, -45 x 0.8-1.2 um. Anamorph: Macro conidia produced on the surface of sclerotia and in the honeydew, hyaline, oblong to oval, 9-17 x 5-8 um. Microconidia spherical, 2-3 um in diam. Secondary conidia rather constricted at the basal end, produced on the surface of the honeydew.

Secondary conidia on the surface of honeydew, Macroconidia and microconidia
Natural Resources Inventory Center, NIAES. http://www.niaes.affrc.go.jp/

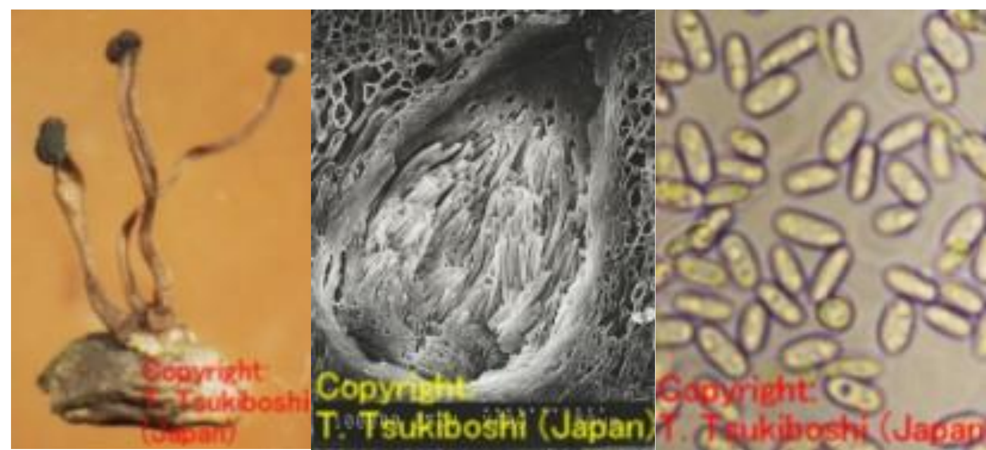
3. Claviceps sorghi B.G.P. Kulk., Seshadri & Hegde, Mysore Journal of Agricultural Science 10 (2): 288 (1976)

In culture on a defined medium, *C. sorghi* isolates (from young sphacelia) grew as white, cottony to velvety, even colonies with diffuse margins and no puckering or sulcation. Macro- and microconidia were produced in pale-brown honeydew-like droplets at the centre of the colony Sphacelia cream-white to grey; elongate, straight to curved, 3-14 x 1.0-2.5 mm, forming two types of spore: oblong macroconidia, with polar vacuoles and slight central constriction, 8-19 x 4-6 mm and spherical microconidia, 2.5 mm diameter. The proximal tissues form the sclerotium largely within the glumes, with distal sclerotial tissue constituting the thin, red core of the protruding sphacelium. Germination of the sclerotium gives rise to two or three stromata, stipes bronze to terracotta-coloured, 6-8 x 0.5 mm, capitulum buff 0.7 mm diameter, perithecial ostioles dark, papillate. Stipe insertion point surrounded by a white frill. Ascomata (perithecia) 130-250 x 60-125 mm diameter. Asci cylindrical 56-114 x 2.5-3.0 mm ends tapering, apical caps hyaline. Ascospores eight, 40-97 x 0.4-0.8 mm


Macroconidia of Claviceps sorghi

4. Claviceps sorghicola Tsukib., Shiman. & T. Uematsu 1999

Sphacelia elongated, producing conidia in brownish honeydew, ellipsoid to ovoid, hyaline, aseptate, 5–11.3 x 2.5–3.6 µm; no microconidia observed in nature. Sclerotia cylindrical to conical, straight to curved, grooved longitudinally, purple black to black, 2.5–20 mm x 1.9–3.5 mm, with covering and small cap of white sphacelium. Stromata 1-4, with stipes brown to bronze-colored, 3.5–17 mm, capitula globose-subglobose, dark brown, papillate, 0.5–1.6 mm diam. Perithecia in capitula ovoid to pyriform, 21–300 x 105–140 µm, ostioles erumpent. Asci cylindrical, 122–315 x 2.5–3.8 µm, with thickened apex. Ascospores hyaline, filiform, eight per ascus, 92–205 x 0.5–1 µm (Tsukiboshi et al., 1999).



Stromata on a sclerotium | Ascocarp in stromata and asci (longitudinal section) | Conidia

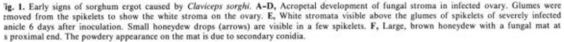
Reports:

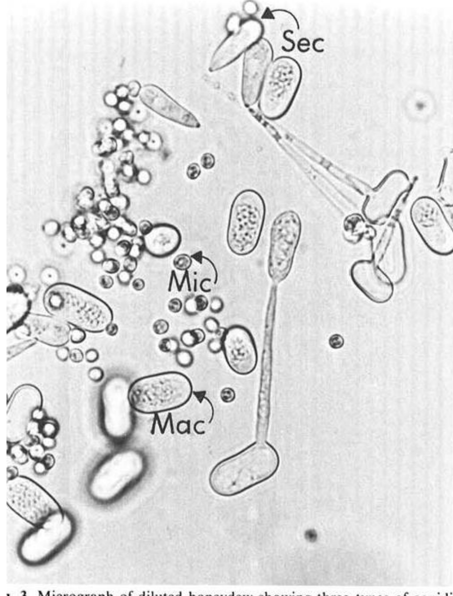
Young and. Marquardt (1982) evaluated the nutritional and toxicological effects of feeding ergotamine tartrate over the range of 0 to ca. 800 ppm in the diet to chickens.

In 7- to 10-day feeding trials with broiler and Leghorn chicks, 30–40 ppm of ergotamine tartrate in the diet did not alter feed consumption or weight gains. Pure alkaloid (at ca. 800 ppm) had only a slight effect on the feed:gain ratio, whereas 4% wheat ergot decreased the feed utilization efficiency twofold. Gross pathological effects in brain, liver, and muscle tissues were not observed, even at the highest (ca. 800 ppm) levels, although toe necrosis occurred at about 250 ppm. Hearts were enlarged in birds at or above 250 ppm, likely due to back pressure arising from vasoconstriction. In a 51-day trial with broilers, similar performance and pathological effects similar to those noted in the short-term studies were observed. Reduced weight gains were apparent only for the first 2–3 wk; thereafter, chicks maintained nearly constant average weights relative to control. Ergotamine tartrate did not accumulate in tissues and only when the highest levels were fed could trace amounts (< 10 ppb) be detected. About 5% of the alkaloid fed was excreted unchanged with an additional 15–20% detected as a complex mixture of 16 possible metabolites. Key words: Chickens, mycotoxins, ergot, ergotamine tartrate

Rorrnn *et al.* (1985) studied the effects of increasing concentrations of dietary wheat ergot (0.308Vo total alkaloids) on the performance of growing male Single Comb White Leghorn and commercial broiler chicks in two experiments. As the concentration of ergot increased from I to 8Va \n the diet, there was a progressive decrease in the performance of both strains of chicks relative to birds given the control diets that contained no ergot. The broiler chicks were slightly more sensitive than the Leghorn chicksto the effects of ergot. In general, however, after 3 and 4 wk of exposure, birds which consumed lTc dietary ergot had an approximately ll%o lower relative weight gain than the control birds, whereas those exposed to 8Vo ergot had an 80Vo lower relative weight gain. During the first 2 wk.of both experiments, there was a progressive decrease in relative weight gain in all dietary ergot concentrations, with the exception of the lVa ergot diet. After 2 wk, feed consumption and weight gain of birds consuming the intermediate concentrations of ergot (2-5%) stabilized or tended to increase slightly relative to the controls. Mortality was low on diets containing up to 3Va dietary ergot but above this concentration there was a dramatic and progressive increase in deaths with increasing ergot concentrations.

Bandyopadhyay *et al.* (1990) mentioned that The first sign of ergot (*Claviceps sorghi*) disease in sorghum was the appearance of superficial mycelial growth on the proximal end of the ovary 3 days after inoculation with conidial suspension. The ovary was converted into a fungal stroma 2 days later, followed by honeydew exudation from the stroma. Honeydew contained three types of conidia?macroconidia, secondary conidia, and microconidia. Macroconidia were elliptical in shape and were the first to be released in the honeydew. Under humid conditions some macroconidia on the surface of the honeydew germinated by germ tubes that enmeshed to form a hyphal mat; others germinated by erect conidiophores on which apical, pyriform secondary conidia were formed outside the honeydew surface. Small, obovate microconidia were later found in the honeydew. All three conidial forms germinated on and penetrated the stigma. Stromata developed at 14?35 C. Honeydew and conidial production occurred at 14?28 C and RH above 90% for 12?16 hr day$^{?1}$. Sclerotia developed at 28?35 C and RH below 90% for 2 hr day$^{?1}$. Above 90%, RH, stromata, and honeydew were colonized by saprophytic fungi and sclerotia were not formed.


'ig. 1. Early signs of sorghum ergot caused by *Claviceps sorghi*. **A–D,** Acropetal development of fungal stroma in infected ovary. Glumes were emoved from the spikelets to show the white stroma on the ovary. **E,** White stromata visible above the glumes of spikelets of severely infected anicle 6 days after inoculation. Small honeydew drops (arrows) are visible in a few spikelets. **F,** Large, brown honeydew with a fungal mat at s proximal end. The powdery appearance on the mat is due to secondary conidia.



;. 3. Micrograph of diluted honeydew showing three types of conidi acroconidia, Mac; secondary conidia, Sec; and microconidia, Mic oduced by *Claviceps sorghi* in infected sorghum spikelets (×2,640) te the hilum in secondary conidia and difference in sizes and shape the three conidial types.

Bandyopadhyay *et al.* (1990)

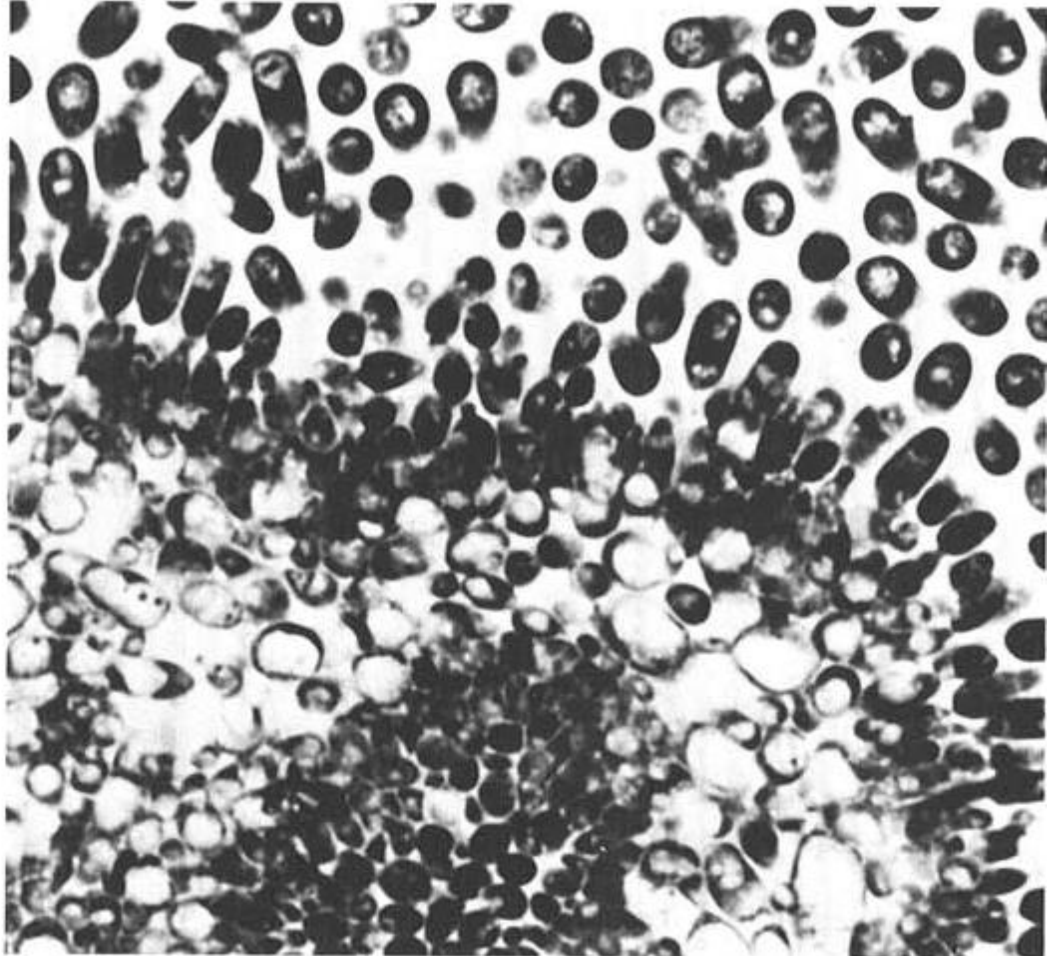


Fig. 4. Longitudinal section of a stroma of *Claviceps sorghi* in a sorghum ovary showing macroconidia originating from conidiophores on the surface of the stroma and in locules inside the stroma (×2,490).

Bandyopadhyay *et al.* (1990)

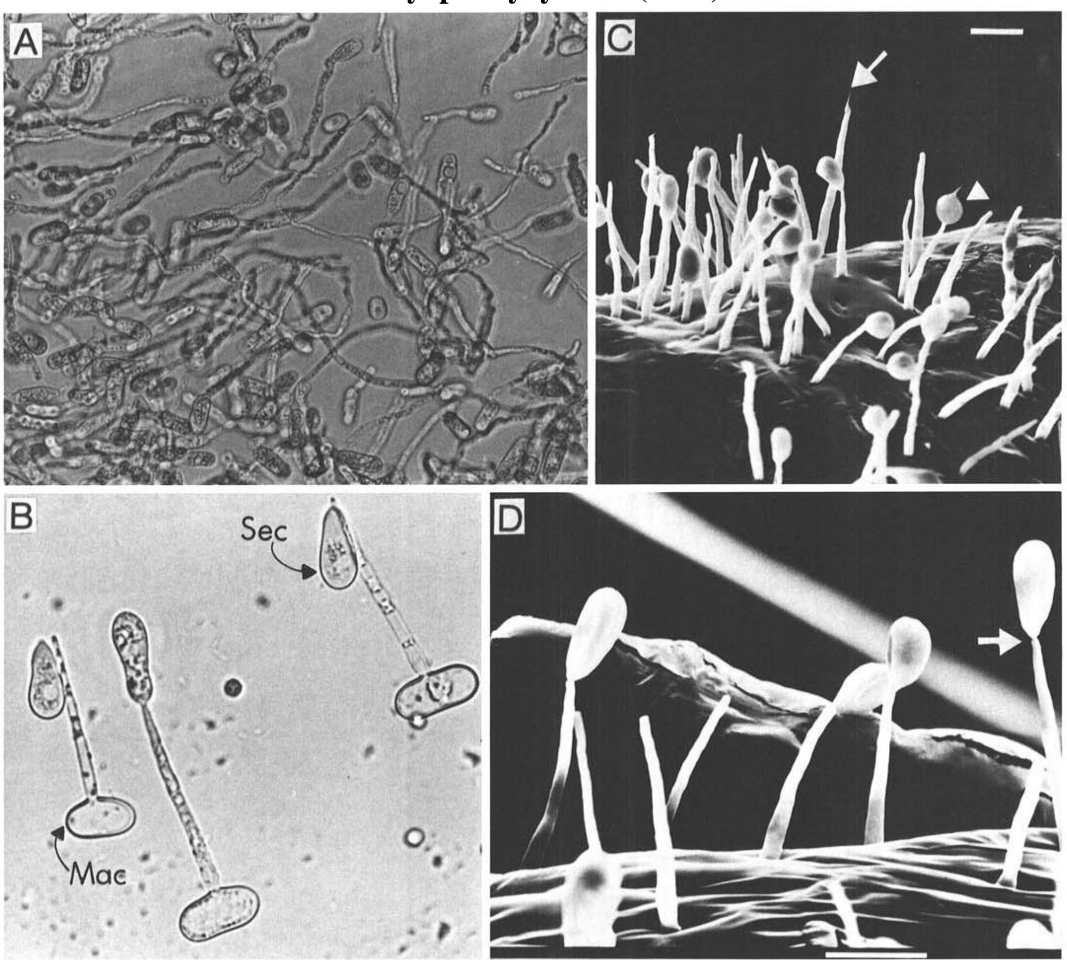


Fig. 6. Events related to formation of secondary conidia of *Claviceps sorghi* on the surface of honeydew in infected sorghum spikelets. **A,** Macroconidia lining the honeydew surface germinated from apical ends and produced germ tubes that enmeshed to form a fungal mat. The mat was crushed on a slide before it was photographed (×1,210). **B,** Several macroconidia in the mat produced aerial conidiophores on which secondary conidia were borne apically. Also note the difference in shapes of macroconidia (Mac) and secondary conidia (Sec) (×2,500). **C,** Scanning electron micrograph of the honeydew surface showing conidiophores with a pointed structure at the apical end (arrow) that formed a bulb (arrowhead) and finally differentiated into secondary conidia. Scale bar = 10 μm. **D,** Close-up scanning electron micrograph of mature secondary conidia on the honeydew surface. Note the delicate hilar attachment (arrow) between the secondary conidium and conidiophore. Scale bar = 10 μm.

Bandyopadhyay *et al.* (1990)

BAILEY *et al.* (1999) conducted 3 experiments to evaluate the performance of broilers fed sorghum ergot consisting of sphacelia/sclerotia of Claviceps africana present in tailings removed by conditioning of seed from grain sorghum hybrid seed production fields near Uvalde (Experiments 1 and 2) and Dumas (Experiment 3), Texas. Percentage sphacelia/sclerotia and total alkaloid content, respectively, in sorghum ergot tailings were 8% and 11.3 ppm for Uvalde and 75% and 235 ppm for Dumas. Sorghum ergot and control sorghum diets were based on the NRC (1994) requirements for starting broilers. In Experiment 1, neither growth nor feed efficiency were significantly reduced in male broilers fed sorghum ergot from hatch to 3 wk of age, but liver weights were significantly greater than those in the control.

Fazzino (1999) conducted four experiments to evaluate the performance of broiler chickens fed sorghum contaminated with ergot sphacelia/sclerotia of Claviceps africana present in tailings removed by conditioning of seed from grain sorghum hybrid seed production gelds near Uvalde (Experiments 1 and 2) and Dumas

(Experiments 3 and 4). Percentage of sphacelia/sclerotia and total alkaloid content, respectively, in the sorghum contaminated with ergot tailings were 8% and 11.3 ppm for Uvalde, and 75% and 235 ppm for Dumas in Experiment 3. Total alkaloid content in the extracted Dumas sample in Experiment 4 was 266.9 ppm. All diets were based on the NRC (1994) requirements for broilers. Hatch to 3-week-old male broilers in Experiment 1 fed sorghum contaminated with ergot showed significant reduction in growth at week three. Relative liver weights in ergot fed birds were significantly greater than control. Hatch to 6-week-old straight-run broilers in Experiment 2 were raised on a three-phase feeding program. Sorghum contaminated with ergot significantly reduced growth in broilers at Weeks 4, 5, and 6. Feed conversion was significantly reduced during all three phases of feeding. In Experiment 3, control sorghum and the 75% ergot tailings were added to corn-soy basal diets at 2.5, 5, and 10% by weight. These male chicks were fed from hatch to 3-weeks of age. Sorghum contaminated with ergot did not significantly reduce growth, but, during Weeks 2 and 3, feed conversions were significantly higher. Neither type nor concentration of sorghum contaminated with ergot significantly affected relative liver weights. In Experiment 4, alkaloids were extracted from ergot sphacelia/sclerotia, added to a corn-soy basal diet, and fed from hatch to 4-week-old male broilers. Sorghum contaminated with ergot significantly increased feed conversion in Week 2. Significantly higher levels of glucose and triglycerides were found in broilers fed sorghum contaminated with ergot. We did not observe significant mortality or obvious signs of ergot toxicity, such as necrotic lesions of the feet or comb, in any of the four experiments. We can conclude that the effects of sorghum contaminated with ergot on broilers will be negligible to broiler production operations.

Dingle and Blaney (2003) conducted 3 experiments.The first experiment was conducted to determine the effect of sorghum ergot (Claviceps africana) alkaloid (dihydroergosine, DHES) and Mycosorb® binding agent on the production of laying hens. A total of 96 commercial ISA Brown laying hens were paired caged in a semi-controlled environment room, and fed different levels of ergot contaminated sorghum. The basal diets were :(1) 24 mgkg-1 DHES, (2) 12 mgkg-1 DHES, (3) 6 mgkg-1 DHES and (4) zero DHES (normal sorghum). Mycosorb was added to half of each basal diet, making 8 diets in total. The diets were fed and egg production, feed intake and egg weight were measured, and feed conversion ratio (FCR) was calculated for a period of 6 weeks. Egg production from the diet containing 24 mgkg-1 DHES was significantly less than that produced from the other diets. The addition of Mycosorb was beneficial. Egg weight at week four was significantly decreased by 24 mgkg-1 DHES. Egg mass decreased as the level of DHES in the diets increased but Mycosorb addition increased egg mass. Neither DHES nor Mycosorb had any significant effect on feed intake or feed conversion ratio overall. This work shows that if the maximum limit of sorghum ergot was increased from the present level of 0.3% to 1% (i.e. from approximately 1 mgkg-1 to 5 mgkg-1 DHES) it would not significantly affect the production or efficiency of laying hens.

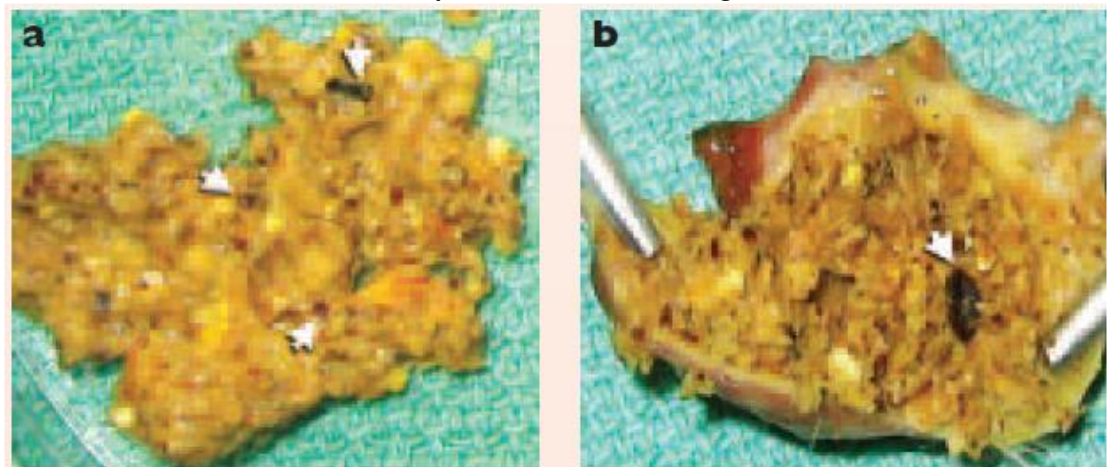
The second experiment was conducted to determine the effect of DHES on dry matter digestibility in laying hens. A total of 32 commercial Brown laying hens 66 weeks old were pair caged in a semicontrolled environment room, and fed different levels of ergot contaminated sorghum. The diets were formulated to contain :(1) 24 mgkg-1 DHES, (2) 12 mgkg-1 DHES, (3) 6 mgkg-1 DHES and (4) zero DHES or normal sorghum. The calculated nutrient content of the diets was estimated to be adequate for good production for ISA

Brown hens. Mycosorb® was added to half of each diet, making 8 diets in total. The diets were fed and total faeces were collected from two replicate 2-bird cages per treatment for a period of 24 hours. Representative samples of all diets and faecal collections were analyzed for dry matter content and the apparent dry matter digestibility of each of the eight diets was calculated. There was no significant (P>0.05) effect of DHES or Mycosorb addition on dry matter digestibility in laying hens. However, there was a trend of decreasing digestibility with increasing level of DHES in the diet. However it was concluded that increasing the maximum allowable concentration of ergot in layer diets from 0.3% to 1% (from 1 to 5 mg DHES/kg diet) would not significantly affect the digestibility of the diet.

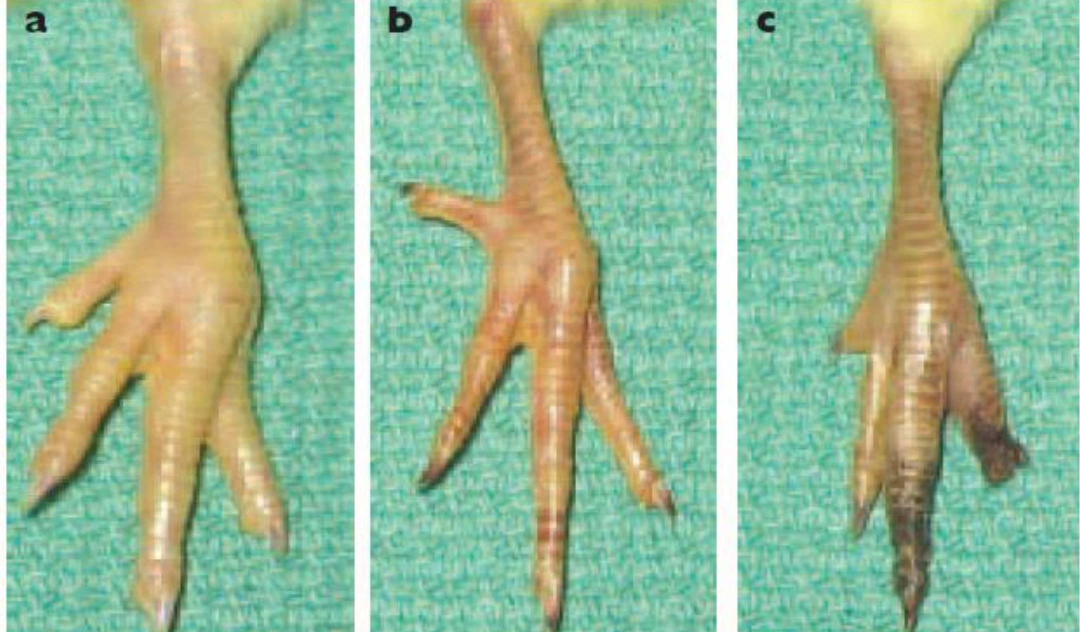
The third experiment was conducted to investigate whether feeding sorghum ergot to hens produces alkaloid residues in eggs. Hens were fed diets containing up to 24 mg DHES/kg for several weeks and eggs were collected daily. Over 80 eggs from ergot-fed birds and 80 from control birds were blended and assayed by an ELISA that is very specific for DHES. The ELISA had a detection limit of 0.005 mg/kg DHES, but DHES was not detected in any egg. Over 40 eggs from the ergot-fed birds were also assayed by HPLC with fluorescence detection, also with negative results. On average, only 29% of ingested DHES was recovered in the excreta, suggesting that DHES was rapidly degraded in the intestine. The regulatory limit for ergot in feed for laying hens might be raised from 0.3% to 1% (about 1 mg DHES/kg to about 5 mg DHES/kg) without significantly increasing the risk of adverse production effects or residues in eggs.

Wojnarowicz *et al.* (2004) reported an experience where the cause of mortality was eventually associated with ergot poisoning, even though initially ergot toxicity was not considered. In case one, In mid July of 2004, the owner of a freshly placed flock of broiler breeders noticed that the feet of several five day old chicks turned purple to black. Some birds died within the next 14 hours. He submitted four dead and three live birds for examination. All live birds were reluctant to move. Their feet were uniformly dark reddish purple and slightly dehydrated. A few black grains, considered to be ergot's sclerotia, were found in the crops and gizzards of some birds. No other lesions were found in the affected birds. A random feed sample, obtained a few days later, showed numerous sclerotia throughout the sample. The sample was analysed by the reference laboratory (University of Missouri, Columbia, USA). The levels of ergopeptaine alkaloids were 8.08ppm, which was approximately 40 to 80 times higher than considered acceptable. Case two, in mid November of 2004, the owner of a freshly placed commercial broiler flock noticed that the claws, toes, shanks and beaks of several three day old chicks were purplish-black. The feed contained many black kernels characteristic of ergotinfested wheat. The ergot sclerotia were also found in the crops and gizzards of the birds investigated in the present case study. It is noteworthy that the feet of the chickens that died of causes not related to ergot appeared normal. Those that ingested ergot infested grains had dark purple toenails and in more advance cases necrotic toes. Similar differences were apparent in the beaks of the birds that died of ergot unrelated causes (Fig. 4a) versus those from ergot exposed birds

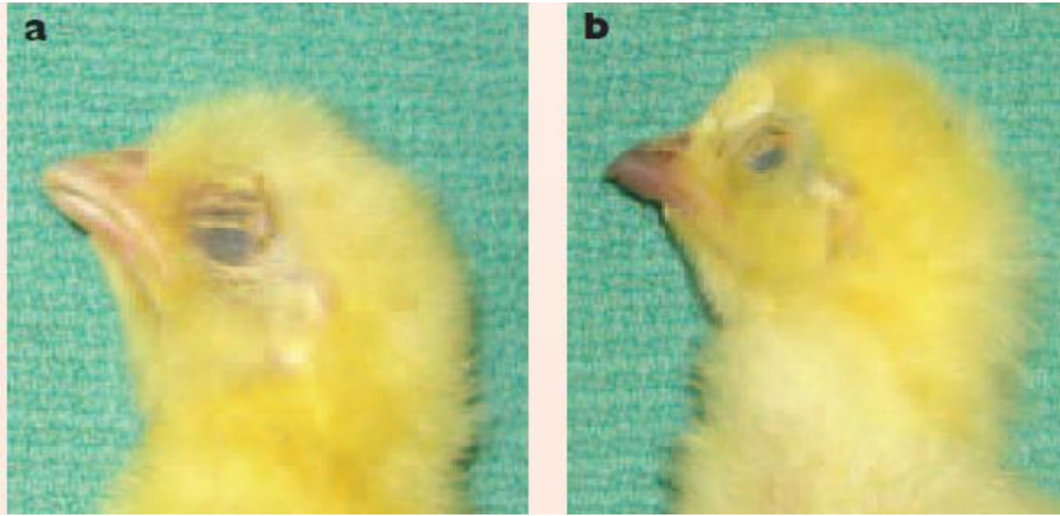
Poultry feeds infected with ergot



Ergot infested kernels (white arrows) were found in the crop (a) and gizzard (b). a b



A foot from a normal chick (a) is contrasted with the feet of ergot exposed birds. Moderate exposure (b) is marked by multifocal browning of the digit and toenails. Extensive and severe necrosis of the digits (c) indicates heavy exposure to ergot alkaloids.



The creamy pink beak of a normal bird (a) provides a stark contrast to the purple beak of an ergot exposed bird (b)

Mainka *et al.* (2005a) conducted 2 dose response trials with piglets and chickens to study the effects of increasing amounts of ergot (Claviceps purpurea) with a defined alkaloid content and pattern on performance, biochemical serum characteristics and organ weights of chickens). The ergot was mixed into the cereal-soybean meal based diets at levels of 0, 0.5, 1, 2 and 4 g/kg. The total alkaloid content of the ergot was analysed to be 2775 mg/kg and showed the following composition: ergometrine 8.1%, ergotamine 5.4%, ergocomine 3.2%, alpha-ergocryptine 1.9%, ergocristine 14.9% and residue 66.5%. Each treatment was tested with 28 male chickens for 21 days (43 g initial live weight). The experiment with chickens demonstrated no significant effects on performance due to dietary ergot exposure. The serum activities of glutamate dehydrogenase and alanine aminotransferase were not significantly influenced by dietary treatment while serum activities of gamma-glutamyltransferase and aspartate aminotransferase and the concentrations of albumin and total bilirubin were significantly affected. Heart weights showed a significant linear decrease due to ergot feeding. Ergot effects on signs of inflammation in the proximal duodenum occurred in chickens fed diets containing 2.8 mg and 11.1 mg total ergot alkaloids/kg although live performance remained unaffected. Further studies are necessary to define the critical level of ergot alkaloids in dependence on alkaloid

Mainka *et al.* (2005b) compared the effect of ergot contaminated feed on performance and health of chickens. Five groups of 28-day old male chickens ("Lohmann Meat") were formed, the average initial weight of the chickens within each group being 43.2 ± 3.0 g. Feed and water was available ad libitum, the different groups being offered feed with a content of 0, 0.5, 1, 2 and 4 g of ergot/kg diet. The ergot was analysed to contain 2 775 mg of total alkaloids per kg, the EAs being ergocristine (14.9 %), ergometrine (8.1 %), ergotamine (5.4 %), ergocornine (3.2 %) and α–ergocryptine (1.9 %) (expressed as percentage of the total dry weight of the ergot, which in addition contained 66.5 % of unknown alkaloid residue). The total alkaloid contents of each of the diets were 0, 1.4, 2.8, 5.6 and 11.1 mg/kg. Serum activities of glutamate dehydrogenase (GLDH), γ-glutamyltransferase (γ-GT), alanine aminotransferase (ALT) were determined together with albumin and total bilirubin. After slaughter, weights of liver, heart, spleen and bursa fabricii were recorded. Inner

organs were examined and the proximal duodenum was scored for inflammation. No mortality was observed in the groups fed 0, 0.5, 1 and 2 g of ergot per kg feed; however, three chickens were taken out in the highest dosed group apparently due to difficulties not related to the experiment. Feed intake was not affected by the dietary composition, neither was the cumulative daily weight gain. The serum activities of GLDH and ALT were not affected. However, the γ-GT as well as bilirubin showed a significant linear increase, while albumin decreased also in a linear manner. The weight of hearts decreased in a linear manner while moderate inflammations were found in the proximal duodenum of the ergot fed groups from 2.8 mg/kg of alkaloids and upwards. Severe inflammation was only seen for two animals in the highest dosed group. The authors concluded that the highest dose did not reach a critical level for performance depression, but that the obvious adverse effect on the integrity of the mucosa needs to be further studied The authors did not identify a NOAEL but it seems that this could be identified at 1.4 mg of EAs/kg feed. During the study the animals gained weight to reach a final weight of around 700 g.

Dänicke (2015) carried out a growth experiment (Day 0-49, n = 54/group) with the aim to titrate the lowest observed adverse effect level (LOAEL) for total ergot alkaloids (TEA). A control diet was prepared without ergots, and the diets designated Ergot 1 to 4 contained 1, 10, 15 and 20 g ergot per kg diet, respectively, corresponding to TEA contents of 0.0, 0.6, 7.0, 11.4 and 16.4 mg/kg. Sensitivity of ducks to EA was most pronounced at the beginning of the experiment when feed intake decreased significantly by 9%, 28%, 41% and 47% in groups Ergot 1 to 4, respectively, compared to the control group. The experiment was terminated after two weeks for ducks exposed to Ergot 3 and 4 due to significant growth retardation. Ergot alkaloid residues in edible tissues were lower than 5 ng/g. Bile was tested positive for ergonovine (=ergometrine = ergobasine) with a mean concentration of 40 ng/g. Overall, the LOAEL amounted to 0.6 mg TA/kg diet suggesting that ducks are not protected by current European Union legislation (1 g ergot/kg unground cereal grains).

Coufal-Majewski (2016) mentioned that ergot is found worldwide, with even low concentrations of alkaloids in the diet (<100 ppb total), reducing the growth efficiency of livestock. Extended periods of increased moisture and cold during flowering promote the development of ergot in cereal crops. Furthermore, the unpredictability of climate change may have detrimental impacts to important cereal crops, such as wheat, barley, and rye, favoring ergot production. Allowable limits for ergot in livestock feed are confusing as they may be determined by proportions of ergot bodies or by total levels of alkaloids, measurements that may differ widely in their estimation of toxicity. The proportion of individual alkaloids, including ergotamine, ergocristine, ergosine, ergocornine, and ergocryptine is extremely variable within ergot bodies and the relative toxicity of these alkaloids has yet to be determined. This raises concerns that current recommendations on safe levels of ergot in feeds may be unreliable. Furthermore, the total ergot alkaloid content is greatly dependent on the geographic region, harvest year, cereal species, variety, and genotype. Considerable animal-to-animal variation in the ability of the liver to detoxify ergot alkaloids also exists and the impacts of factors, such as pelleting of feeds or use of binders to reduce bioavailability of alkaloids require study. Accordingly, unknowns greatly outnumber the knowns for cereal ergot and further study to help better define allowable limits for livestock would be welcome.

References:

4. BAILEY, C. A., J. J. FAZZINO, JR., M. S. ZIEHR, M. SATTAR, A. U. HAQ, G. ODVODY, and J. K. PORTER. Evaluation of Sorghum Ergot Toxicity in Broilers. Poult Sci. 1999 Oct;78(10):1391-7
5. Bandyopadhyay,R., L. K. Mughogho, S. K. Manohar, and M. V. **Stroma Development, Honeydew Formation, and Conidial Production in *Claviceps sorghi*.**
6. Satyanarayana(Phytopathology 80:812-818.1990. 1990
7. Belser-Ehrlich S, Harper A, Hussey J, Hallock R. Human and cattle ergotism since 1900: symptoms, outbreaks, and regulations. Toxicol Ind Health (2012) 29:307–16
8. Coufal-Majewski, Stephanie , Kim Stanford, Tim McAllister, Barry Blakley, John McKinnon, Alexandre Vieira Chaves and Yuxi Wang. Impacts of Cereal Ergot in Food Animal Production. Front. Vet. Sci., 25 February 2016
9. Dänicke S. Ergot alkaloids in feed for Pekin ducks: toxic effects, metabolism and carry over into edible tissues. Toxins (Basel). 2015 Jun 2;7(6):2006-23.
10. Dingle, John and Barry Blaney Impact of sorghum ergot in layer hens A report for the Australian Egg Corporation Limited and the Grains Research and Development Council. Hurstville, N.S.W. : Australian Egg Corporation Limited, c2003.
11. Duckett SK, Agrae JG, Pratt SL.. Exposure to ergot alkaloids during gestation reduces fetal growth in sheep. Front Chem (2014) 2:1–7.
12. Edwards NM, Hatcher DW, Fu BX. Quality of western Canadian wheat. Canadian Grain Commission. Winnipeg, MB: (2011). p. 1–40.
13. Fazzino, Johnny Joseph (1999). The evaluation of sorghum contaminated with ergot on broiler chicken performance. Master's thesis, Texas A&M University. Available electronically from http://hdl.handle.net/1969.1/ETD-TAMU-1999-THESIS-F366
14. Fink-Gremmels J. The impact of mycotoxins in animal feeds. In: Leslie JF, et al., editors. , editors.Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. Cambridge, MA: CAB Intentional; (2008). p. 155–67.
15. Government of Canada, Agriculture and Agri-Food Canada. Section 1: Mycotoxins in Livestock
16. Government of Saskatchewan. Ergot of Cereals and Grasses. (2015). Available from:http://tinyurl.com/z76zh4f
17. Kirchhoff H. Beitrage zur Biologie und Physiologie des Mutterkornpilzes. Zentbl Bakt Parasitkde (Abt 11) (1929) 77:310–69.
18. Klotz JL, Bush LP, Smith DL, Shafer WD, Smith LL, Vevoda AC, et al. Assessment of vasoconstrictive potential of d-lysergic acid using an isolated bovine lateral saphenous vein bioassay. J Anim Sci (2006)84:3167–75.10.2527/jas.2006-038
19. Krebs J. Untersuchungen über der Pilz den Mutterkorns *Claviceps purpurea* Tul. Ber Schweizen Bot Geselschaft (1936) 4:71–165.
20. Krska R, Crews C.. Significance, chemistry and determination of ergot alkaloids: a review. Food Addit Contam (2008) 25:722–31.
21. Leroux E. Management of Mycotoxin Contamination in Raw Materials and Feeds. (2012). Available from: https://en.engormix.com/MA-mycotoxins/articles/management-mycotoxin-contamination-raw-t2275/p0.htm
22. Lovell A. Ergot on the Rise in Western Canada. Grainews; (2013). Available from: www.grainews.ca
23. Mainka S., Dänicke S., Böhme H., Wolff J., Matthes S., Flachowsky G. Comparative studies on the effect of ergot contaminated feed on performance and health of piglets and chickens. Arch. Anim. Nutr.2005;59:81–98.
24. Miller JD, Richardson SN. Mycotoxins in Canada: A Perspective for 2013. Ottawa, ON: Carleton University; (2013).

25. Mitchell DT, Cooke RC. Some effects of temperature on germination and longevity of sclerotia in*Claviceps purpurea*. Trans Br Mycol Soc (1968) 51:721–9.10.1016/S0007-1536(68)80092
26. Nicholson SS. Ergot. In: Gupta RC, editor. , editor. Veterinary Toxicology. Oxford: Academic Press; (2007). p. 1015–8.10.1016/B978-012370467-2/50179-6
27. Pearse P. Ergot of cereals and grasses. Canadian Grain Commission (2006). Available from:www.grainscanada.gc.ca
28. Perumbakkam S, Rattray RM, Delorme MJM, Duringer JM, Craig AM. Discovery of Novel Microorganisms Involved in Ergot Alkaloid Detoxification: An Approach. New Zealand Grassland Association, Endophyte Symposium; (2007).
29. Rorrnn, R. G., Meneu,qnor, R. R. ,qNo Cnow, G. H. 1985. A comparison of the effect of increasing dietary concentrations of wheat ergot on the performance of Leghorn and broiler chicks. Can. J. Anim. Sci. 65:963-974.
30. Schumann GL. 'Ergot', The Plant Health Instructor. (2000). Available from:http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Ergot.aspShelby RA. Toxicology of ergot alkaloids in agriculture. In: Kren V, Cvak L, editors. , editors. Ergot Toxicology. Amsterdam: Overseas Publishers Association; (1999). p. 469–77.
31. **Wojnarowicz, Chris Andrew Olkowski and Susantha Gomis (2013). Prevalence of ergot toxicity.** International Poultry Production — Volume 14 Number 1**.14-15. 2004**
32. Young J. C. and R. R. Marquardt, "Effects of Ergotamine Tartrate on Growing Chickens," Canadian Journal of Animal Science, Vol. 62, 1982, pp. 1181-1191.