Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Molecular Sciences

Attitudes to feed fermentation for aflatoxin management in maize in Kenya

- Investigating aflatoxin reduction by yeast-fermentation

Inställningar till foderjäsning för hantering av aflatoxin i majs i Kenya

- Studie om reduktion av aflatoxin genom fermentation med jäst

Everlyne Hayo

Department of Molecular Sciences
Master's Thesis • 30 hec • Second cycle, A2E
Agriculture Programme – Food Science
Molecular Sciences, 2018:32
Uppsala, 2018

Attitudes to feed fermentation for aflatoxin management in maize in Kenya - Investigating aflatoxin reduction by yeast-fermentation

Inställningar till foderjäsning för hantering av aflatoxin i majs i Kenya - Studie om reduktion av aflatoxin genom fermentation med jäst

Everlyne Hayo

Supervisor: Su-lin Hedén, Swedish University of Agricultural Sciences, Department of Molecular Sciences

Field supervisor: Johanna Lindahl, International Livestock Research Institute, Department of Animal and Human Health, Nairobi, Kenya.

Assistant supervisor: Albina Bakeeva, Swedish University of Agricultural Sciences, Department of Molecular Sciences

Examiner: Volkmar Passoth, Swedish University of Agricultural Sciences, Department of Molecular Sciences

Credits: 30 hec
Level: Master, Second cycle, A2E
Course title: Independent project/degree project in Food Science – Master's Thesis
Course code: EX0425
Programme/education: Agriculture Programme - Food Science

Place of publication: Uppsala
Year of publication: 2018
Title of series: Molecular Sciences
Part Number: 2018:32
Online publication: https://stud.epsilon.slu.se

Keywords: Feed safety, food safety, dairy production, aflatoxin, contamination, maize, fermentation, yeast, Kasarani, Kisumu

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences

Abstract

Aflatoxins are toxins produced by certain strains of fungi belonging to the species *Aspergillus flavus* and *Aspergillus parasiticus* during infection in the field, post-harvest, storage and processing. Aflatoxins are found especially in maize and also carry over into milk when contaminated feeds are fed to dairy cows. Feed fermentation could possibly be used to reduce the level of aflatoxin in animal feed. The project investigates, with survey interviews, if Kenyan farmers are willing to use novel fermentation methods to reduce the level of aflatoxin. The project will give better understanding of the farmers' opinions and situation. The survey part involved 184 smallholder farmers in urban and peri-urban areas in Kasarani and Kisumu.

Feed fermentation for aflatoxin reduction is not well researched. To address this approach the second goal of this project was to investigate how different yeasts could be used to degrade aflatoxins in Kenyan maize. A laboratory pilot-trial was performed to find out if yeast-based feed fermentation of aflatoxin-contaminated maize might degrade or otherwise reduce toxin levels. Aflatoxin in the control treatment should have been constant during the incubation period, because the maize is dry and stable, and in the control would not be affected by addition of water or different yeasts. However, since the maize sample in the bottle was not homogeneous, each treatment, including the control, showed variation in aflatoxin content. The water added to the fermentation treatments was not sufficient to support good growth of inoculated yeasts or other microbes which could ferment the maize, so no clear effect of fermentation was observed.

The survey revealed that feed fermentation (or silage making) is not commonly practiced in Kenya. During the survey, it was found that only 22% of the farmers practice feed fermentation and that they desire more knowledge about it. According to the survey 95% of the Kenyan farmers were positive about using a specific yeast for feed fermentation.

Keywords: Feed safety, food safety, dairy production, aflatoxin, contamination, maize, fermentation, yeast, Kasarani, Kisumu

Sammanfattning

Aflatoxiner är toxiner som produceras av vissa svampstammar *Aspergillus flavus* och *Aspergillus parasiticus* vid infektion på fältet, efter skörd, förvaring och bearbetning. Aflatoxiner finns främst i majs men också i mjölk när förorenat djurfoder ges till mjölkkor. Fermentering kan eventuellt användas till att reducera nivån av aflatoxiner i djurföda. Projektet undersöker med hjälp av intervjuer om kenyanska bönder är villiga att använda nya fermentationsmetoder för att reducera nivån av aflatoxiner. Projektet ger en bättre uppfattning om jordbrukarnas åsikter och situation. Intervjudelen innefattar 184 småskaliga bönder i urbana och stadsnära områden i Kasarani och Kisumu.

Foderjäsning för reducering av aflatoxiner är inte väl utforskat. För att adressera detta var det andra målet med detta projekt att undersöka hur olika jästar kan användas för att bryta ned aflatoxiner i kenyansk majs. Ett laboratorieförsök gjordes för att ta reda på om jästbaserad foderjäsning av aflatoxinförorenad majs kan bryta ned eller annars minska toxinhalten. Aflatoxin i kontrollbehandlingen bör vara konstant under inkubation, eftersom majsen är torr, stabil och inte påverkas av vatten eller andra jästarter. Eftersom majsprovet i kolven inte var homogen visade varje behandling, däribland kontrollen, variation i aflatoxinhalten. Vattnet som tillsattes till fermentationsbehandlingarna var inte tillräckligt för att stödja god tillväxt av inokulerade jästar eller andra mikrober som skulle kunna jäsa majsen. Ingen klar verkan av jäsning observerades.

Intervjudelen uppdagade att foderjäsning inte brukar utföras i Kenya. Under undersökningen konstaterades att endast 22% av bönderna utför foderjäsning och de önskar mer kunskap om det. Enligt intervjudelen var 95% av bönderna positiva till att använda jäst i sin foderjäsning.

Nyckelord: Fodersäkerhet, livsmedelssäkerhet, mjölkproduktion, aflatoxin, förorening, majs, fermentering, jäst, Kasarani, Kisumu

Table of contents

List of tables

List of figures

Abbreviations

ACN	Acetonitrile
ANOVA	Analysis of variance
C	Control treatment
CGIAR	Consortium of International Agricultural Research Centres
DF	Degrees of freedom
F	Ferment treatment
FAO	Food and agriculture organization
IARC	International Agency for Research on Cancer
IITA	International Institute of Tropical Agriculture
ILRI	International livestock research institute
LAB	Lactic acid bacteria
LOD	Limit of detection
LOQ	Limit of quantitation
QPS	Qualified presumption of safety
SLU	Swedish University of Agricultural Sciences
Sq	Square
UPLC	Ultra-high performance liquid chromatography
WHO	World Health Organization

Y1	Yeast 1 treatment: *Wickerhamomyces anomalus*, strain J121
Y2	Yeast 2 treatment: *Meyerozyma guilliermondii*, strain J106
Y3	Yeast 3 treatment: *Kazachstania exigua*, strain J470
YPD	Yeast peptone dextrose

1 Introduction

1.1 Global food security

According the United Nations, the world needs to increase its agricultural food production by 70% by 2050 in order to prevent hunger. The agricultural lands of the world are not enough to support this increase in production (Dixelius, 2011). Since there is currently only 20% unused land that can be used for farming, the farmland we already have must be used in a more efficient way.

During the 1990s, food insecurity was an issue for developing countries in the southern hemisphere (Pain et al., 2015). However, during the economic crisis in 2008, food insecurity became more of a global issue, since more people could not afford food. More than 60 countries had food riots during 2008. The food crisis is no longer an issue only for developing countries but also a major threat globally.

Maize is consumed by more than 132 million people in East Africa as a staple food (ILRI, 2017). In the supply chain of cereal products at least 25% are contaminated by aflatoxin globally (Lee et al., 2017; ILRI, 2017; WHO, 2018). In Kenya maize is one of the staple foods in the area (EPZA, 2005). It is mostly used for both human consumption and for animal consumption and also as a source of income in Kenya (Oluoch, 2014; FAO, 2013; Groote et al., 2010).

1.2 Background and effects of aflatoxin in animal feed in Africa and the dangers to human health.

Aflatoxins are toxins produced by certain strains of fungi belonging to the species *Aspergillus flavus* and *Aspergillus parasiticus* during infection in the field, post-harvest, storage and processing. The fungi may contaminate food and feeds in many staple commodities, for example maize. About 4.5 billion people living in developing countries are chronically exposed to substantial uncontrolled amounts of the toxin (Williams et al., 2004; IITA, 2017). Figure 1 below shows the four main aflatoxins produced by moulds.

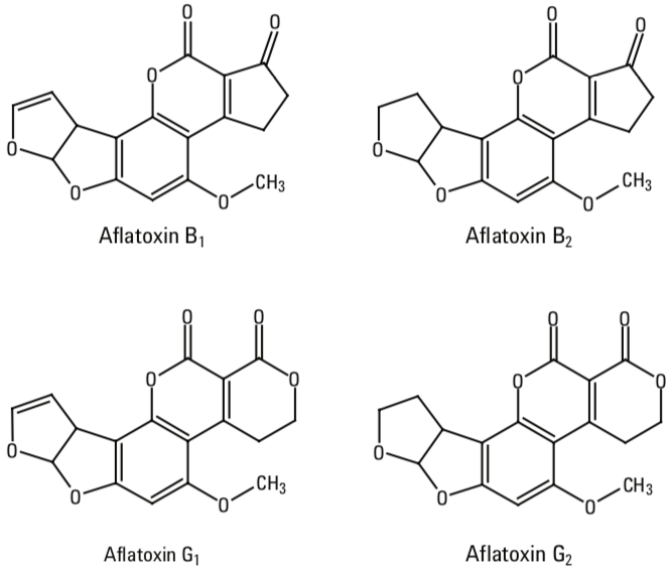


Figure 1. Chemical structure of the mycotoxin aflatoxin, hereafter abbreviated to AFB1, AFB2, AFG1 and AFG2. Source: Barbas et al. 2005

In developing countries, inadequate infrastructures, poor management of storage, high temperatures and unseasonal rain (causing heavy damage to crops), may contribute to aflatoxin contamination of crops. In developing countries such as Kenya, poor farmers who may earn only 200 KSH per day (Wageindicator, 2015) probably cannot afford to take their crops to an expensive laboratory for testing, which may cost more than 200 KSH (Ngotho, 2016). Much of the agricultural production in Kenya is done by smallholder farmers.

If an animal or a human consumes aflatoxin B1, the primary target of the toxin is the liver (Zain, 2011); at high concentrations, the toxin can cause liver damage and death, and at lower concentrations (chronic exposure), it greatly increases the risk for liver cancer. In Kenya 317 people became ill and 125 people died from consuming aflatoxin contaminated maize in 2004. During the aflatoxicosis outbreak in 2004, the level of aflatoxin in maize was more than 220 times greater than the 20 ppt recommended by the Kenyan authorities (Azziz-Baumgartner et al., 2005). If domestic animals are fed contaminated feed, they may pass it on to humans via milk or meat. Also, when humans breastfeed, they may pass it on to the baby. When dairy animals are fed with feed contaminated with AFB1, the AFB1 is hydroxylated by enzymes to AFM1 and AFM1 is excreted through milk within 12 hrs (Kang'ethe et al., 2009; Iqba et al., 2015; Ullah et al., 2016). Children between 4 – 6 months age are at risk to be exposed to AFM1 through breastfeeding, which has been demonstrated in Kisumu, Kenya (Obade et al., 2015b). A high number of pregnant women are exposed to aflatoxin in Kisumu (Obade et al., 2015a).

More than 160 million children in the world are stunted (IARC, 2016). Children from poor families, usually eat an undiversified diet and suffer from malnutrition-related diseases (Golden, 2010), but if they consume milk they can prevent those diseases. Cereals like maize, sorghum, millet and groundnuts are very important in Kenya. These cereals are those most commonly used for the weaning of the infants in Kenya, typically in a mixture containing approximately 29 percent flour (Okoth et al., 2004). Milk and cereals can be good sources of nutrition for children to prevent stunting, but on the other hand they can also lead to exposure to aflatoxins.

Much of the dairy milk produced in Kenya is sold by smallholder farmers selling at the local markets, and this is especially true in Kasarani and Kisumu. Producing safe and controlled milk is important for the health of infants. Unfortunately, discarding contaminated milk is not an option among impoverished smallholders in Kenya, and Kenya is already a country where most people live on less than one dollar per day (Wageindicator, 2015). Over 35% of the milk produced in Kenya contains AFM1 above the recommended 50 ppt limit and 55% of the feeds from manufacturers are above the limit which is recommended by the EU/FAO/WHO (WHO, 2018; Kang'ethe et al., 2009). Lindahl (2018), argued that we should address the issue of aflatoxin in production, rather than discouraging people not to consume the milk. Producing safe feed for animals will result in safe production of milk.

Around 27% of the children under the age of 5 in Kisumu are malnourished (Obade et al., 2015b) and there is a link between aflatoxin exposure and wasting in children under the age of 3 (Kang'ethe et al., 2009). Low growth rate and stunting has been observed among children living within low-income families in urban Kasarani, Nairobi, potentially related to aflatoxin exposure in the diet (Kiarie et al., 2016). Eating contaminated food with aflatoxin might also lead to other forms of malnutrition (Shetty et al., 2006), and it can also reduce the ability to cope with diseases, for example HIV/AIDS (IITA, 2017). Aflatoxicosis may lead to stunting (Sirma et al., 2018) which is 40% more frequent in areas with high aflatoxin exposure (IITA, 2017).

Aflatoxin in contaminated crops can be reduced or transformed. For example, a large part of the contamination may be reduced by cooking the crop in very hot temperatures, washing the crops and discarding mouldy food (Fandohan et al., 2005; Bullerman et al., 2007). Fermentation, certain fungi, enzymes and *Lactobacillus* may biologically transform the aflatoxin B_1, to other less toxic types of aflatoxin (B_2, B_3, M_1 etc.) (Wu et al., 2009). It has been shown that the fungus *Rhizopus oligosporus* could both inhibit production of AFB1 by more than 90% and degrade AFB1 in liquid culture medium (Kusumaningtyas et al., 2006). Also, *Saccharomyces cerevisiae* is able to reduce mycotoxins when added to ruminant feeds. Kusumaningtyas et al. (2006) used milled chicken feed artificially contaminated with AFB1, to test for reduction in aflatoxin when the feed was incubated with *R. oligosporus*, *S. cerevisiae* or the combination of the two fungi, at 28°C for 15 days in Erlenmeyer flasks. When compared with the two control treatments, one with aflatoxin and one without, the treatments *R. oligosporus*, *S. cerevisiae*

were most effective when used individually (34.9% and 29.8% reduction, respectively), instead of in combination.

Known mechanisms for the reduction of aflatoxin by fungi are varied. The yeast *S. cerevisiae* can bind AFB1 to its cell wall. A component on the cell wall of the yeast, called oligomannanes, is responsible for this binding. Moulds, for example *Aspergillus niger*, non-aflatoxin-producing *A. flavus*, *Eurotium herbariorum* and *R. oligosporus* are able to degrade AFB1 to aflatoxicol by reducing the cyclopentenone carbonyl of the aflatoxin (Wu et al., 2009). Possible interventions at an earlier stage of aflatoxin contamination include application of the yeast *Wickerhamomyces anomalus* (idem. *Pichia anomala*), which was shown to inhibit spore germination and aflatoxin production of the mould *A. flavus* (Hua et al., 2014).

Food fermentation is a relatively cheap technology. Lactic acid bacteria (LAB) are used for food preservation and sensory effects, but research has also found ways of using LAB fermentation to reduce AFB1 in foods and feeds (Dalié et al., 2010). LAB, similar to the yeast *S. cerevisiae*, may also be used in fermentation to bind aflatoxins to cell walls and thereby reducing uptake of aflatoxins by the consumer (Shetty et al., 2006; Mokoena et al., 2006)

Fermentation of feed, using specific fungi or bacteria, can degrade antinutritional compounds to improve the nutrient uptake in feed for domestic animals (Wang et al., 2018a). Feed fermentation can also provide probiotics and their metabolities, and can be a possible alternative to growth-promoting antibiotics (Wang et al., 2018a; Jazi et al., 2018). Apart from maize, feed fermentation has commonly been practiced on cereals such as soybean and other non-cereals such as hay. Feed fermentation can also be beneficial by influencing the function and the structure of pig gut microbiota (Wang et al., 2018b). Feed fermentation seems to be used for feeding cattle, pigs, poultry, sheep and goats (Jazi et al., 2018; Engberg et al., 2009).

1.3 Goals

One of the goals of this project was to investigate how different yeasts could be used to degrade aflatoxins in Kenyan maize. Feed fermentation may reduce aflatoxin levels in feed, and also have other nutritional benefits. The

project also investigated if Kenyan farmers are willing to use enhanced degrading methods and the project will give a better understanding of the farmers' opinions and situation. Smallholder farmers may have use for feed fermentation. The project also wanted to find out if farmers in Kenya were already practising feed fermentation in maize, which type of feed fermentations are used and how they are conducted. The project was also to investigate obstacles which prevent farmers in Kenya from practising feed fermentation.

In addition, the project wanted to find out how farmers in the study areas in Kenya monitor the general condition of feeds before and during storage. If feed fermentation was being practiced in Kasarani and in Kisumu, the project aimed to investigate which animals were fed with fermented feeds. It was also to understand how much knowledge and awareness farmers in Kasarani and Kisumu had about mould and aflatoxin.

1.4 Research Questions

- How does fermentation with the specific yeasts degrade aflatoxins in contaminated feed?
- Could feed fermented with the specific yeasts be fed to domestic animals?
- Is feed fermentation of maize for animal feed commonly used in Kenya? Why or why not? With which animals? How is it performed?
- How do farmers in Kenya monitor the feed quality, both before and during storage, and what is their awareness of aflatoxin?
- Is yeast fermentation of maize for animal feed commonly used in Kenya? Why or why not? For which animals?
- Would fermentation of maize with yeast be accepted by smallholder farmers in Kenya?

2 Methodology

The study involves both qualitative and quantitative studies to evaluate the research questions. The qualitative study was based on the questions asked to the interviewees. The quantitative study was mainly based on laboratory results, data collection and also descriptive statistics from the interviews.

2.1 Fieldwork in Kasarani and Kisumu

2.1.1 Background of the region and choice of areas for the survey

The survey part of this project involved smallholder farmers in urban and peri-urban areas in Kasarani and Kisumu. Kasarani is a subcounty located in Nairobi county. The surveys in Kasarani took place in the Mwiki, Kasarani, Claycity, Njiru and Ruai wards. Kisumu is a city and county located in western Kenya. The surveys in Kisumu took place in the four sub-counties Kisumu East, Kisumu West, Kisumu Central and Nyando. The major economic activities in these areas include livestock keeping, subsistence and cash crop farming, while others include fishing (Infotrak, 2018).

Kasarani and Kisumu were both selected for the survey because they represent urban and peri-urban areas with extensive dairy production. In these areas, the famers use more silage and concentrate feeds than farmers in rural areas. Kisumu has high temperatures and humidity which may have an effect on animal feeds during post-harvest and storage periods (Obade et al., 2015a).

Kisumu county is dominated by smallholder farmers mostly cultivating sorghum, millet, maize and groundnuts which are used both as food and feeds in this area. (Obade et al., 2015b). It was therefore important to know

what they do with their contaminated feeds since Kisumu has favourable temperatures for the mould growth.

Before this study, another study had been performed by staff at ILRI, where farmers were randomly sampled. Lists of dairy farmers in the selected sub-counties were obtained from the county veterinary and livestock production department. The lists were compiled, and 100 farms were randomly selected for the survey in each study area (Kisumu sub-counties and in Kasarani sub-counties).

During the previous study, the farmers were presented with some information about moulds and aflatoxin. A follow up study with the same farmers, was performed spring 2018 and this study joined the follow up study. Because the farmers had some previous knowledge, it could lead to some knowledge bias in this study. The average farmer in Kenya might have less knowledge than the farmers in this study.

2.1.2 Data Collection

Questionnaires were used to capture data on herd characteristics in terms of size, breed, previous training on dairy feeding including types, sources, storage conditions, animal feeding challenges, and local awareness of the link between aflatoxins in feed and human health.

The data were also collected in two different workshop seminars in Kasarani and in Kisumu. In attendance were International Livestock Research Institute (ILRI) representatives, the dairy stakeholders, members from Kenya government authorities and lectures from both Maseno and Nairobi universities.

The survey interviews were to find out if feed fermentations of maize are commonly used in Kenya and to find out the farmers' awareness of mould, aflatoxin and farming hygiene. An additional goal was to find out if feed fermentation with yeast would be accepted by smallholder farmers.

The survey had a mix of questions with quantitative answers, predefined answers and free text answers. The free text answers were normalised by grouping similar free text answers together.

2.2 Fermentation trials

A laboratory pilot-trial was performed to find out if yeast-based feed fermentation of aflatoxin-contaminated maize might reduce toxin levels. Unfortunately, an error was made in the experimental set-up which was not noticed until after data analysis, and there was then insufficient time to repeat the trial. But the methods are still described here, for the purpose of evaluating improvements if the trial can be repeated in the future.

Three yeast strains were selected that were previously used in feed fermentations at SLU, namely the biopreservative yeast *W. anomalus* strain J121, known to inhibit moulds and undesirable bacteria (Olstorpe et al., 2011), *Meyerozyma guilliermondii* strain J106, *Kazachstania exigua* strain J470.

Ultra-high performance liquid chromatography-fluorescence (UPLC) was used to detect the amount of aflatoxins in the samples taken at regular intervals during replicated fermentation batches (Leszcyñska et al., 2001), and compared with an uninoculated natural or wild control fermentation, and with unfermented maize as a control.

2.2.1 Sources of maize used

The contaminated dried maize kernels samples used were provided by ILRI. The maize kernels were collected from different parts of Kenya and in different seasons. The areas of interest were Eastern, Western and Nyanza regions. These areas have high temperatures and humidity that are conducive for aflatoxin accumulation. Due to these favourable conditions the crops are affected postharvest since they do not get conducive temperatures to dry completely before storage (Wangia, 2017).

The samples from Eastern region were collected in two counties Meru and Embu during the years of 2004 (Kang'ethe et al., 2009; Muthomi et al., 2009). These two counties were the most affected areas during an outbreak of aflatoxicosis during that time (Lewis et al., 2005).

The maize kernels from Western and Nyanza regions were collected from Busia, Kakamega Siaya and Kisumu counties in 2015.

2.2.2 Maize sample preparation

The dried maize kernels used for the experiment were kept at 4°C at ILRI laboratories before use. All provided dried maize kernels from different regions were thoroughly mixed in one container to form a composite sample before treatments. The maize kernels were then divided into five treatments. The total amount of maize used for experiment was 7500g. 3000g of maize was used to check the dry matter content. The remaining 4500g was used for five different treatments. The control treatment, natural fermented treatments, and fermented treatments with yeast 1, yeast 2 and yeast 3 were further divided into triplicate portions of 300g each in 15 Duran glass bottles labelled as control maize (C1, C2, C3), fermented maize with natural microbiota (F1, F2, F3), yeast inoculated maize (Y11, Y12, Y13), (Y21, Y22, Y23), (Y31, Y32, Y33). The control samples were packed first, followed by maize for fermentation with natural microbiota, and lastly, for fermentation with different yeasts. All samples were stored in separate Duran glass bottles at 4°C. The bottles used were 500 mL glass Duran laboratory bottles (218014459) with polypropylene screw-cap and pouring ring.

2.2.3 Yeast strains used

Three different strains of yeast were used for this experiment. The yeasts were provided by the Department of Molecular Sciences at Swedish University of Agricultural Sciences (SLU). The yeasts were prepared on YPD plates before traveling to ILRI Kenya and kept at 2°C until use. Below are the strains of yeast used.

- Yeast 1: *W. anomalus,* strain J121
- Yeast 2: *M. guilliermondii,* strain J106
- Yeast 3: *K. exigua,* strain J470.

2.2.4 Determination of Dry Matter Composition

The dry matter of the maize samples was determined to estimate the total amount of water to be added during fermentation. 200 g of the dry matter samples were collected in 15 (five treatments in triplicate) different brown paper bags. The samples were dried in a Gallenkamp BS oven for 24 hours

at a temperature of 105°C. The dry matter calculation is shown in the appendix 2.

2.2.5 Preparation of yeast inoculum (Media preparation and Counting the cells)

Yeast peptone dextrose broth (YPD) for yeast inoculation was prepared by adding 1L distilled water, 10 g yeast extract, 20 g peptone and 20 g dextrose (D-glucose). For the peptone water, 1L distilled water was added to 1 g of peptone. YPD and peptone water were autoclaved for 1 hour, at 121°C.

Yeasts 1, 2 and 3 were inoculated into 25ml YPD in sterile falcon tubes. The suspensions were thoroughly mixed by shaking vigorously and incubated at 25°C overnight.

The yeast cultures were then centrifuged for 10 minutes at 13000 rpm with a relative centrifugal force of 15900*g* (Eppendorf 5424 centrifuge with a 5424R FA-45-21-11 rotor, radius 84 mm). The pellets were resuspended in 0.1% peptone water. Yeast suspension was diluted 100 times and the number of cells estimated using a Bürker chamber.

The concentration of cells/ml calculated in the Bürker chamber is defined as

$$\frac{Number\ of\ cells}{Count\ area\ in\ a\ B\ddot{u}rker\ chamber \times Volume\ above\ squares \times Dilution}$$

The chamber depth had a constant of 0.1 mm and the smallest square length was 0.05 mm. which was converted into ml as shown below

$$= 10^{-2}cm \times (5 \times 10^{-3})cm \times (5 \times 10^{-3})cm$$
$$= 2.5 \times 10^{-7}cm^3\ or\ 2.5 \times 10^{-7}mL$$

The dilution in suspension in 100 times is equal to 0.01. Calculations for counting yeast 1, yeast 2 and yeast 3 cells are shown in appendix 3.

The final concentration of yeast inoculum should be $2 \times 10^5 cfu\ /\ g$ maize kernels, so the concentration of the yeast suspension needed to be adjusted.

The number of yeast cells needed is $900g\ maize\ kernels \times 2 \times 10^5 cfu\ /\ g = 180000000\ cfu = 1.8 \times 10^8$

The volume of yeast suspension to be used is

$$\frac{Number\ of\ cells\ needed}{\text{Concentration of cells/ml calculated in the Bürker chamber}}$$

$$= \frac{1.8 \times 10^8\ (cells)}{\text{Concentration calculated in Bürker chamber } (\frac{\text{cells}}{\text{ml}})}$$

$$= \text{volume of yeast suspension (ml)}$$

The calculation of yeast suspension to be used to inoculate 900 g maize, for each of the treatments yeast 1, yeast 2 and yeast 3 are shown in appendix 4.

2.2.6 Maize inoculation

Fermented triplicates by natural microbiota (F1, F2 and F3) were packed first to minimize the risk of cross-contamination between the treatments. The amount of water added was 22.68 ml water (Appendix 2). This was wrongly calculated as 25% of dry matter, and this mistake was repeated for the other treatments. The target was 25% dry matter in the final fermentation mixture, to simulate wet pig-feed in Sweden. But with the incorrect volume of water added, the dry matter was around 85%, which was too high to be a wet fermentation. For the control samples (C1, C2, C3) nothing was added.

- 0.024 ml of yeast 1 suspension J121 was added to 22.79 ml water. Each amount was used to inoculate 300 g of maize (Y11, Y12, Y13)

- 0.022 ml of yeast 2 suspension J106 was added to 22.60 ml water. Each amount was used to inoculate 300 g of maize (Y21, Y22, Y23).
- 0.023 ml of yeast 3 suspension J470 was added to 22.70 ml water. Each amount was used to inoculate each 300 g of maize (Y31, Y32, Y33).

2.2.7 Maize fermentation

After mixing the samples, the Duran glass bottles were tightly closed to avoid any air coming in during fermentation. The samples were taken before fermentation on day 0, and after incubation at 25°C for 2, 4, 6, 7 and 8 days. Thus, the total number of the samples or observations was 90 days.

The observations were grouped into a panel of six time periods and five treatments, whereof every combination of time period and treatment had three observations, so

$$the\ number\ of\ time\ periods\ *\ the\ number\ of\ treatments\ *\ 3 = 6\ *\ 5\ *\ 3\ =\ 90.$$

The six time periods were between 0 and 8 days. The treatments were control, natural fermented, fermented with yeast 1, fermented with yeast 2 and fermented with yeast 3.

2.2.8 Sampling for aflatoxin content in maize and milling process

At each sampling point, 50 g of each replicate (15 different samples in total) were weighed and placed in a brown paper bag. Contents of the Duran bottles were mixed well by shaking vigorously before and after sampling. The samples were collected using a laboratory stainless steel micro spatula spoon. The samples were then dried overnight before milling (homogenization) using two-speed Waring laboratory blenders with timer. The milling of the samples took place on different occasions due to different sampling points and the drying time.

The maize samples used for this experiment had five different types of treatments. Therefore, it was important to prevent cross contamination of aflatoxin between samples during the milling process, for example, if there are residues left in the blender between each milling of samples. The previously milled sample may contaminate the next sample or might affect the end result of all the experiments. Thus, decontamination of the blenders between samples was performed with 70% ethanol (v/v) disinfectant solution to rinse out residues. The ethanol helps to rinse out residues that might have been stuck between each milling process.

After milling, the samples were kept in a cold room before analysis. All the milling, clean-up and extraction procedures were done under the hood with the use of gloves and laboratory face mask.

2.2.9 Extraction of aflatoxin

Maize flour was weighed 5.00±0.05g into a 50ml polypropylene centrifuge tubes. 25ml of the extraction solvent (80% acetonitrile with 1% acetic acid) was added to 5.00±0.05g maize flour. The centrifuge tubes were tightly closed, placed in a mechanical shaker and shaken for 4 minutes. The extracted samples were allowed to settle for 15 minutes. The extracted samples were further diluted with 80% acetonitrile (ACN) in 2ml Eppendorf tubes (100 µl sample from extracted solution plus 900 µl ACN).

The samples were centrifuged for 3 minutes at 5000 rpm with a relative centrifugal force of 2350*g* (Eppendorf 5424 centrifuge with a 5424R FA-45-21-11 rotor, radius 84 mm). The samples were then diluted at a of ratio 1:1 (×2 dilution), 500 µl sample and 500 µl of 1% acetic acid and pipetted into UPLC sample vials. The vials were capped and loaded into the UPLC autosampler for analysis.

Stock solutions were commercially supplied and serially diluted to give concentrations of 1000 ng/ml, 500 ng/ml, 250 ng/ml, 125 ng/ml, 25 ng/ml, and 5 ng/ml total aflatoxin in acetonitrile solution according to ILRI standard operating procedure (ILRI, 2017).

2.2.10 UPLC conditions

The UPLC method is a sensitive, accurate and precise method for quantification of aflatoxins in maize (ILRI, 2017). It utilises reversed phase chromatography with fluorescence detection. The aflatoxins detected and quantified by the UPLC method were AFG1, AFG2, AFB1, AFB2.

The UPLC analyses were performed using a Nexera Liquid chromatograph LC-30AD with Nexera column oven CTO-30A. The Shimadzu prominence UPLC was equipped with Lab Solutions work station, an auto sampler (Nexera Auto sampler SIL-30AC), degasser, binary pump system, and a column and fluorescence detector.

The chromatographic separation was carried out with the C18 column (Phenomenex Synergi 2.5 μ Hydro - RP 100 mm $\times$ 3.00mm) at 50°C, and mobile phase of methanol and 1% acetic acid (40:60) with flow rate 0.4ml/min. The run time was 8 minutes. The column flushing solution was water/methanol (60:40). The prominence fluorescence detector RF-20AXS was set at excitation and emission wavelengths of 365nm and 455nm respectively (ILRI, 2017).

The limits of detection, limits of quantification and validated range for the aflatoxin analytes of maize for the method are listed in Table 1.

Table 1. *Limits of toxin residue analyses in maize using this UPLC method. The limits of detection, quantification and validated range for the aflatoxin analytes of maize. Source: ILRI, 2017*

Analyte	LOD (µg/kg)	LOQ (µg/kg)	Validated range (µg/kg)
AFG1	0.2	0.4	0.2-200
AFG2	0.05	0.1	0.05-50
AFB1	0.2	0.4	0.2-200
AFB2	0.05	0.1	0.05-50

3 Results

3.1 Fieldwork in Kasarani and Kisumu

The number of farmers that answered the survey were 184 and the number of targeted farmers were 200. All survey answers are found in appendix 5, referenced as Survey Question. During the survey, it was found that only 22% of the farmers practice feed fermentation. In Kasarani 33% of the farmers practice feed fermentation whereas in Kisumu 11% of the farmers practice feed fermentation. Only seven of the farmers mention that they routinely add yeast culture to their fermentation.

Farmers in both study areas Kasarani and Kisumu were asked about what they think about general feed fermentation (with or without added yeast) (Survey question 1.5.11). A total of 23% of the farmer mentioned that they do not have an opinion regarding general feed fermentation, whereof Kasarani 11% and Kisumu 36%. A total of 95% of the farmers were positive and willing to use specific yeast for feed fermentation, if available, in order to reduce the aflatoxin in feed (Survey question 1.6.9). Most farmers mentioned they would like to use feed fermentation.

Thirty-six farmers mentioned that when dairy cattle eat fermented feed the milk production increases. Forty-one farmers had no idea at all, while others thought it is an effective way of preserving food and very useful during dry seasons. Some farmers mentioned that it is good for animal digestion, increases animal appetite and is easy to feed (Survey question 1.5.11). In the interviews in Kasarani, most farmers were aware of feed fermentation even though most of them do not practise it because of economic difficulties. They said they have seen it from the neighbours that practise it and they produce a larger amount of milk. However, in the survey 35% in Kasarani

believed that fermentation increase milk production, compared to only 6% in the Kisumu survey. Some farmers in the survey mentioned that they have heard about feed fermentation, but they do not practice it since it is a source of diseases therefore not good for animal consumption. 9% of the farmers in Kisumu mentioned that feed fermentation is a source of disease, compared to 0% in Kasarani (Survey question 1.5.12). The difference can be due to local rumours about feed fermentation.

Lack of enough information, raw materials and high cost of production were the major challenges to why most farmers were not practicing feed fermentation. Some farmers mentioned that the challenges also can be labour intensiveness, lack of equipment and lack of experience on how to do feed fermentation. Some farmers also believed that feed fermentation only could be beneficial for dairy cattle, which can be seen in the survey where 80% of the farmers ferment feed only for cows (Survey question 1.5.9). Due to the belief that feed fermentation is primarily for dairy cattle, some mentioned that they have too few animals for it to be worth the effort (Survey question 1.5.11). However, other animals fed with fermented feeds in these areas include sheep, goats, pigs and poultry (Survey question 1.5.9).

Most of these farms were located in somewhat dense urban areas with limited space. Therefore, having adequate place to construct a vessel for silage fermentation can be a big challenge for some farmers. In the survey, 8% of Kasarani farmers mention lack of space as a challenge, compared to the less dense Kisumu, 2%.

A total of 40 farmers, 22%, were practicing feed fermentation, whereof Kisumu has 11% and Kasarani 33%. Maize products, such as maize stalks, were the most dominants feeds fermented compared to the other products (Survey question 1.5.5). Since there are some farmers using feed fermentation already, there is a possibility that the knowledge can spread between smallholder farmers. Some farmers said they mostly use brewery dairy meal (unga/mshisha) for fermentation. They buy it from the breweries, but they do not know what it contains. The brewery dairy meal, which is a left over from the breweries, is commonly used as a protein additive in animal feed. It contains for example the yeast *S. cerevisiae*, which is used for alcohol fermentation in the breweries, demonstrating that at least that this yeast is safe for feeding animals.

The farmers in Kasarani and Kisumu have two ways of preparing feed fermentation for their animals. Some make fresh fermentation every day,

while others keep a continuous fermentation by adding new feed and water. They said they keep adding water so that the feed they are fermenting would not get too dry and harden.

The number of incubation days varies between the farmers from a half day to 122 days (Survey question 1.5.8). The average number of days was 24 and the median number of days was 21 (Figure 2; Figure 3), and 50% of the farmers ferment between 7 and 30 days. The farmers in the survey who were practicing fermentation, were mainly fermenting low nutrient substrates like napier grass, maize stalks and hay, which take longer time to ferment compared to high nutrient substrates like maize or brewery dairy meal.

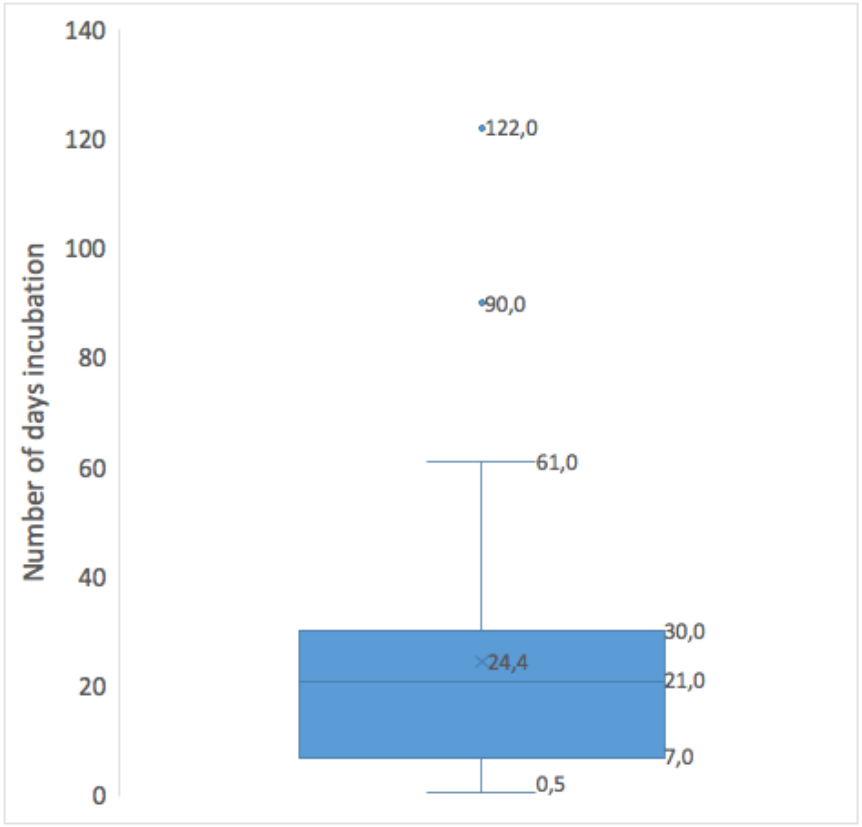


Figure 2. Box-Plot of number of incubation days of feed practiced by the farmers. The number of farmers answering the question was 39. The mean of number of incubation days was 24.4 and the median was 21.

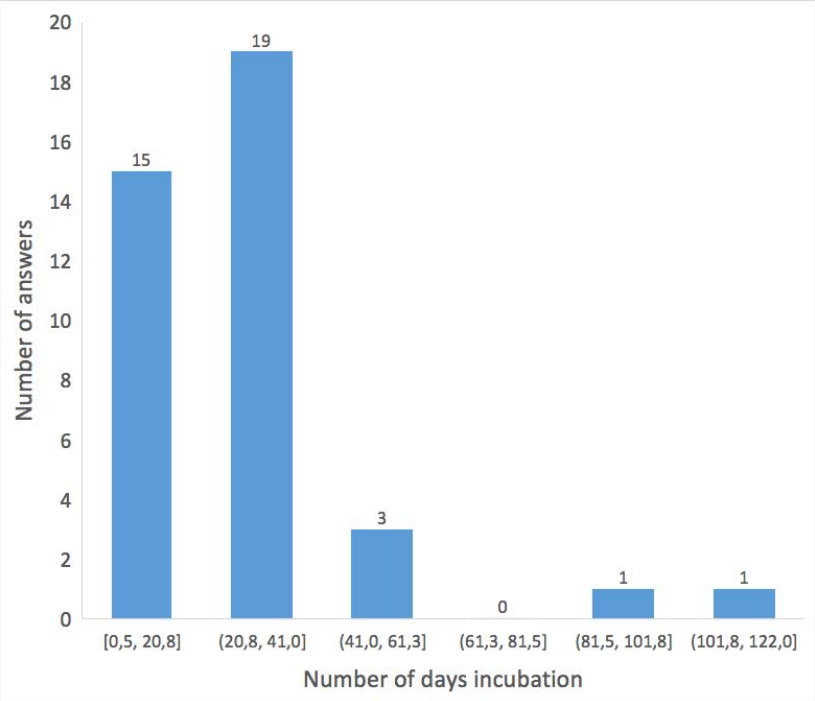


Figure 3. Histogram for number of incubation days of feed practiced by the farmers (the x-axis has five ranges of number of incubation days). The number of farmers answering the question was 39.

The majority of the farmers in both geographical areas of the study did not measure water during fermentation (Figure 4, Survey question 1.5.6). The process of feed fermentation varied within each area, both in number of incubation days and water to feed ratio used (Figure 4). They also added other supplements during fermentation, for example molasses, yeast of unknown type and salt. Since some farmers mentioned that they lack experience about how to do feed fermentation, they seemed to come up with their own ideas regarding number of days, water to feed ratio, what ingredients to add to the fermentation and how to properly mix the ingredients. This process should be researched further since the farmers are very interested in feed fermentation.

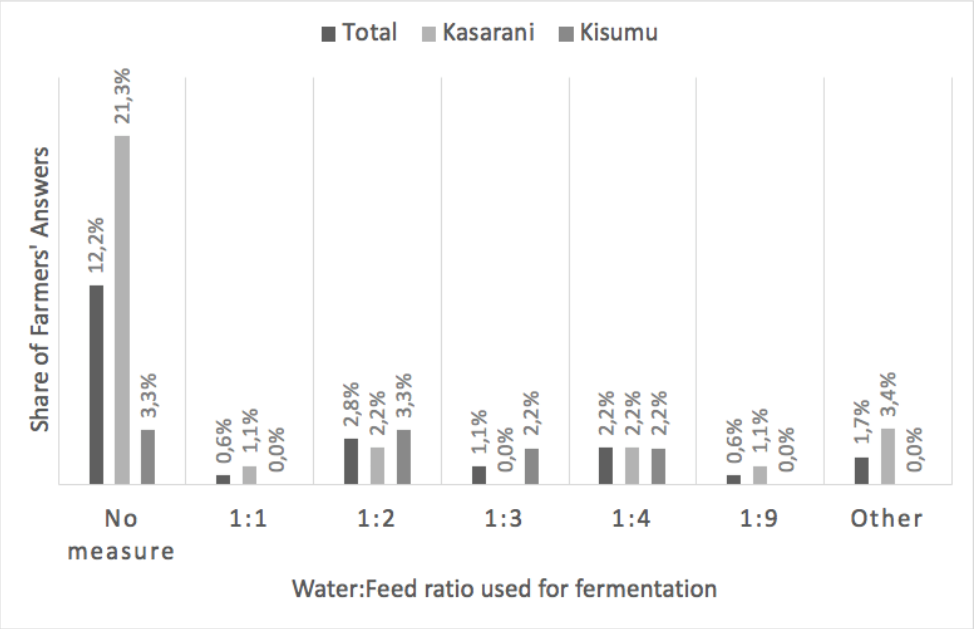


Figure 4. Ratio of water to feed, used by the farmers of the study, for fermentation of feed.

Most farmers were aware of mould and aflatoxin. In Kasarani and Kisumu, 44% said that they often see mould in their animal feed (Survey question 1.6.1). Farmers in these areas mentioned that the presence of mould in animal feeds can cause diseases and kill animals (Survey question 1.6.2). Another interesting result was that they mentioned that it can cause aflatoxin contamination and affect the milk quality. They also mentioned that mould is poisonous, kills animals, causes stomach upset, diarrhea and bloating. Most farmers in both areas also knew or had heard about aflatoxin. 82.9% of the farmers had heard about aflatoxin (Survey question 1.6.3) and they knew that aflatoxin is bad (Survey question 1.6.4), which is important, but they have different views of what it is. They mentioned that aflatoxin is a poison and is caused by mouldy feeds and grains. They also mentioned that aflatoxin is the rotting of feeds/grains and caused by improper storage.

In Kasarani most farmers reported some knowledge on how to reduce mould in the farm, but in Kisumu the knowledge was less. Mostly they said that feed should be stored in a dry place with a ventilation to avoid high moisture content (Survey question 1.6.7a), related to the fact that aflatoxin contamination can increase 10-fold in three days in high moisture (Mutegi et al., 2013). Some farmers mentioned it is important to maintain hygiene in the storage. Some farmers mentioned that feed should be stored for a

shorter period of time and that they should buy smaller amount for use in a shorter period of time (Survey question 1.6.7a). In Kisumu some farmers mentioned that it is important to monitor the presence of mould before and during the storage. The high temperature between 20°C and 35°C and high humidity between 40% to 89% in Kisumu is conducive beneficial for mould growth (Obade et al., 2015b).

In Kasarani, 89 % of the farmers discard feeds if they find it is contaminated by mould (Survey question 1.5.2), but in Kisumu only 8% discard contaminated feed. The knowledge of impacts of mould and actions that can be taken against mould, differed greatly between survey areas. The farmers in Kasarani had much more knowledge about how to reduce mould than the farmers in Kisumu. Some farmers said that contaminated feed can affect not only the animals, but also humans if the contaminated feed is consumed by animals. Some mentioned that it can cause cancer, which is true. Some farmers mentioned that they can dry already contaminated feeds in the sun and then use them to feed the animals instead of discarding it.

In Kasarani some farmers explained that animal feeds are very expensive to buy. Therefore, they find it difficult to throw away the contaminated feeds. Some farmers mentioned that they do not feed the animal with mouldy feeds, but they instead used it for mulching in their gardens.

One farmer in Kisumu explained that there is less land for them to cultivate crops, which means it is difficult to throw away food. The thought is that if humans cannot eat the rotten maize, they can still feed their livestock with it. Finding alternative options instead of discarding food is one of the main reasons for setting up this pilot study on yeast fermentation for feed.

Another way of reducing aflatoxin is making sure that the store where the feeds are kept are clean, with proper ventilations to prevent insects and rodents from entering the stores. Monitoring the feeds condition of the feeds to sort out and discard contaminated feeds minimises cross contamination (Mutegi et al., 2013). Farmers are aware that ventilating the storage can reduce both mould (Survey question 1.6.7a) and aflatoxins (Survey question 1.6.8a), but very few are practicing it in both Kasarani and Kisumu (Survey question 1.6.7b and Survey question 1.6.8b). Furthermore, the feeds should be well dried before storage to reduce the water activity and thereby inhibit mould growth.

Proper hygiene should be practiced by the farmers when handling the feeds to avoid cross contamination. During the survey in both study areas a

total of 2% of the farmers maintain hygiene while handling their storage facilities. 6% of the farmers practice general hygiene in their farms (Survey question 1.6.7b). This shows that more awareness is still needed for farmers in both Kasarani and Kisumu to produce safe milk and to reduce the spread of aflatoxin contamination in both feed and milk.

3.2 Fermentation trial

For each 50 g sample drawn from the same bottle, it was random which maize kernels were drawn.

Figure 5 below shows an average result of all the total aflatoxin levels over time, namely, the sum of AFB1, AFB2, AFG1 and AFG2. Each point in the figure represents sample from triplicate bottles.

Figure 6 below shows an average result of the aflatoxin AFB1 levels over time. Each point in the figure represents sample from triplicate bottles.

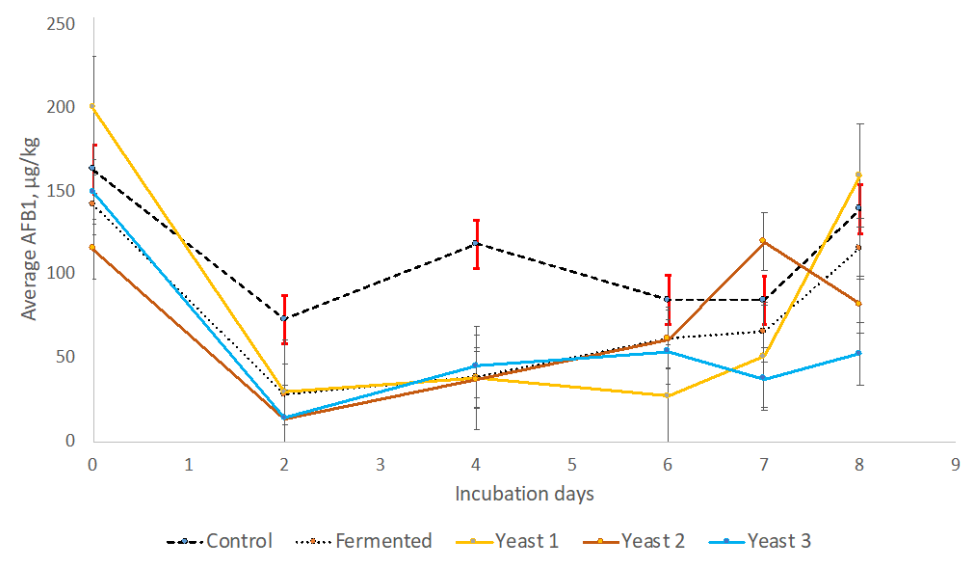


Figure 5. Average of total aflatoxin in different treatments during storage, when fermented maize had water added, and the yeast treatments had water added with yeast 1 *W. anomalus* strain J121, yeast 2 *M. guilliermondii* strain J106, yeast 3 *K. exigua* strain J470, respectively. The error bars show the standard error of mean for the treatment. The error bars are red for the control treatment.

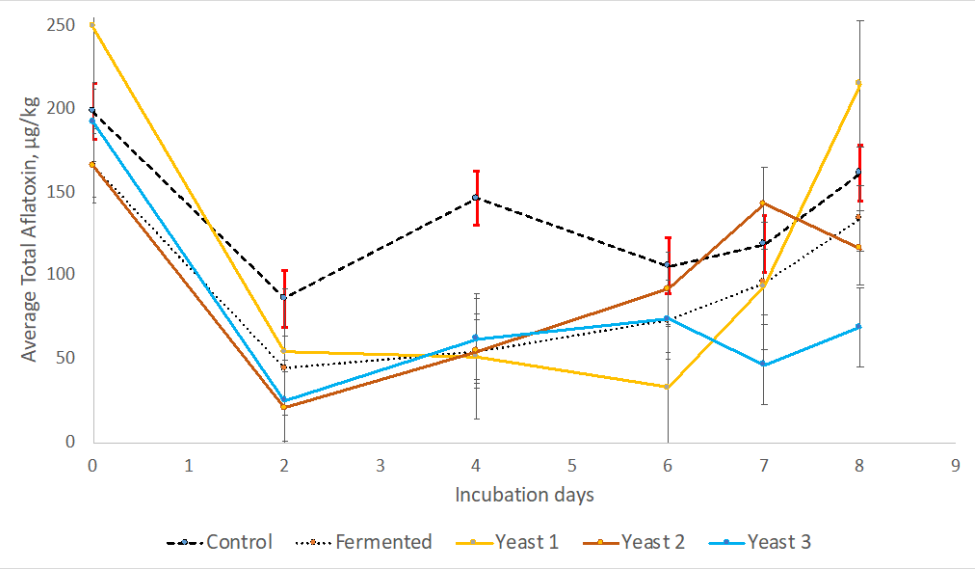


Figure 6. Average of aflatoxin B1 in different treatments during storage, when fermented maize had water added, and the yeast treatments had water added with yeast 1 *W. anomalus* strain J121, yeast 2 *M. guilliermondii* strain J106, yeast 3 *K. exigua* strain J470, respectively. The error bars show the standard error of mean for the treatment. The error bars are red for the control treatment.

Aflatoxin level in the maize population in the control group should have been constant over time during incubation. This is because the maize is dry and stable and would not be affected by addition of water or different yeasts. The maize kernel population in the bottle was however not homogeneous. Some kernels were more contaminated with aflatoxin than other kernels. When samples were randomly drawn from the non-homogeneous population, each treatment sample including the control sample, was non-homogeneous and therefore the replicates showed variation in aflatoxin content. There was a high variation in the control sample and also a high variation in the other treatment samples. This variation is natural because of the sampling of non-homogeneous population. This sampling variation is known as *within variation* and can be thought of as variation of aflatoxin levels within three bottles of the same treatment, within each time period (Olsson et al., 2005). An example of within variation is the control treatment in Figure 5 and Figure 6 which varied between the days. The non-homogeneity in the population caused two possible problems:

1. Control samples varied over time, within the three replicate bottles
2. The fermentation, yeast 1, yeast 2 and yeast 3 treatments, where we wished to observe if the aflatoxin content varied over time,

have large error bars due to variation within the three replicates (Figure 5 and Figure 6).

If there was high variation in the control sample, it is likely that there was a high variation in the other treatment samples as well. This may cause problem in trusting the difference between the control and the other treatments. However, the point of having a control group is to control for the normal variation which is present in the other treatment groups. If the variation is too high in all treatments, including the control, no statistical conclusion can be made. To make a conclusion if there is a difference between groups, the *between variation* is compared to the *within variation*. If the between variation is larger than the within variation, there is evidence for a statistical effect, which supports the theory of difference between treatments.

A Tukey's post hoc test was made to find evidence for a statistical effect of changes in control over time. When performing a pairwise comparison of control samples over time, all parameters of test were insignificant. This suggests that there is no evidence of a statistical effect of changes in control group over time (Table 11), despite the apparent fluctuations in aflatoxin content shown in Figure 5 and 6. Likewise, it is promising that the pairwise comparisons of the Day 0 samples for control and all fermented treatments did not differ significantly (Table 5), suggesting that the starting concentrations of aflatoxin in all the treatments was likely to be similar. However, at this detailed level, the degree of freedom is quite low. There are only three observations per combination of time period and treatment. The insignificant pairwise comparisons could have arisen because of too few observations to make a clear conclusion.

The between variation is a variation which is between the treatments or a variation which is between time periods. If the variation between the treatments is larger than the variation within the treatments, one can assume that there is a difference in AFB1 levels between the treatments. In order to check that, an ANOVA analysis was made, and it suggested that there was a difference between the treatments (Table 2).

Table 2. *Analysis of variance of aflatoxin B1 levels, per days, per treatments and per interaction between days and treatments.*

ANOVA	Df	Sum Sq	Mean Sq	F-value	p-value
DayID	5	148798	**29760**	16.301	p<0.001 ***
Treatment	4	26997	**6749**	3.697	p<0.001 ***
DayID:treatment	20	46749	2337	1.28	0.22769
Residuals	60	109540	**1826**		

Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

The ANOVA shows that there was a significant difference between days and significant difference between treatments, but the interaction between day and treatment was not significant (p-value larger than five percent). If the data were more robust with a control with constant aflatoxin, then we would have expected that the interaction days * treatments would be significant (control constant over time but fermented treatments differing over time). The non-significant interaction term weakens the reliability of the ANOVA for our experimental data set. Also, the ANOVA does not reveal how the treatments are different or how much they are different.

In order to understand how and how much the days and the treatments differed, a Tukey's post-hoc test was applied. The Tukey's test can be applied both on main effects, and also as a pairwise comparison of individual data points, as performed for the control above.

For comparing treatments, the Tukey's analysis suggested that there was a difference between control and yeast 2 and a difference between control and yeast 3 (Table 3). Yeast 2 and yeast 3 both appeared to reduce AFB1 compared to the control.

Table 3. *Tukey's comparison test. Comparing treatments by measuring the mean difference in aflatoxin B1 levels between different treatments.*

Compared treatments	diff AFB1	lwr	upr	p-value
F-C	-35.28	-75.34	4.78	0.11
Y1-C	-26.38	-66.44	13.67	0.354
Y2-C	-39.08	-79.14	0.97	0.059.
Y3-C	-51.86	-91.91	-11.80	0.005**
Y1-F	8.90	-31.16	48.95	0.971
Y2-F	-3.80	-43.86	36.25	0.999
Y3-F	-16.58	-56.63	23.48	0.772
Y2-Y1	-12.70	-52.76	27.36	0.899
Y3-Y1	-25.47	-65.53	14.58	0.39
Y3-Y2	-12.77	-52.83	27.28	0.897

Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Table 4 below shows the reduction of AFB1 from treatments and in relation to the initial value at day 0. The average levels of days 2 to 8 was compared to the initial average value at day 0. All treatments, including the control treatment had lower AFB1 levels for day 2 to day 8 than the initial average value at day 0. Control had the lowest reduction of 35% and yeast 3 had the highest reduction of 74%. Since this is an average over many days it may mean that yeast 3 reduced most in the same time period as the other yeasts or that yeast 3 had a more persistent effect than the other yeasts, i.e. an effect over a longer period than the other yeasts. The value of difference in % between the days for the control should have been 0% (and not -35%). However, the difference in % between days for control arises due to the sampling variance. For the other non-control treatments, it was expected that the values would decrease from day 0 if the yeasts were effective.

Table 4. *Relative differences of aflatoxin B1 levels per treatment. Initial average level of AFB1 at day 0 is the average of all the 15 sample flasks at day 0. Average level of AFB1 day 2 to 8 is the average of all day 2 to 8 per treatment. Average % diff between AFB1 level day 2 to 8 and initial average is the % difference between samples taken from day 2 until day 8 and the initial average aflatoxin level. Tukey's difference between AFB1 level of treatments and control are the estimated difference between the AFB1 levels of the treatments and control taken from the Tukey's test in Table 3. The Tukey's test contains all days. One treatment, namely the yeast 1 treatment, did not differ significantly from the control and is thereby not listed here.*

Treatment	**All**	**C**	**F**	**Y1**	**Y2**	**Y3**
Initial average level of AFB1 at day 0	154.3					
Average level of AFB1 day 2 to 8		100.2	62.0	61.1	62.8	40.6
Diff between average AFB1 level day 2 to 8 and initial average		**-35%**	**-60%**	**-60%**	**-59%**	**-74%**
Tukey's difference between AFB1 level of non-control and control treatments			-35.3	insign.	-39.1	-51.9

Yeast 1, did not have a total treatment effect in relation to control over the full trial period (Table 3), suggesting that over all there was no effect of the yeast. However, from the Tukey's test, yeast 1 had a significant interaction effect between days. This means that for yeast 1, the changes between days can be measured. For yeast 1, the change of aflatoxin level from day zero until day seven was significant. But the last day, the aflatoxin level was almost back to the aflatoxin value at day zero (Table 13), so that on average it would be back to the same level as the control. However, when putting yeast 1 in relation to control, there was no significant difference between the yeast 1 and control treatments on any day (Table 5 to 10).

According to Tukey's test, the fermentation group and the control group did not differ significantly by treatment (Table 3) nor the interaction (Table 12). The only treatment that had a significant interaction effect between days and treatment was yeast 1 (Table 11 to 15). There was a total treatment effect over 8 days in relation to control for yeast 2 and yeast 3 (Table 3). The total effect over 8 days for yeast 2 and yeast 3 (Table 3) might have left the interaction effect for those treatments insignificant (Table 14, Table 15), since they may be colinear. It might be a risk to compare the yeast treatments against the control as in Table 3, since the control varies (Figure 5); however, it can be argued that since the variation in control was not significant (Table 11) it may still be possible to guess at some trends of the yeast treatment effect against control (Table 3). Yeast 2 and yeast 3 displayed a significant treatment effect compared to the control over 8 days, but yeast 1 did not have a significant treatment effect.

On average the treatments went down in aflatoxin levels the first days and then went back up (Figure 5). This was because too little water was added to support the fermentation. During the first three incubation days, there were visible water droplets surrounding the maize in the bottles, meaning that the water was unevenly distributed amongst the maize kernels. There was also a smell from fermentation which indicated there was some fermentation active. At the same time the maize kernels started to swell and were much softer compared to before the water was added. The remainder of the days, the water droplets were invisible in the mixture as well as on the sides of the container, which suggests that they were absorbed by the maize kernels. There were no visible characteristics of mould growth.

The average sample dry matter was 90.77% or 90.77 g per 100 g wet sample. For 300 g maize on average 22.70 ml water was added, so per 100 g maize on average 7.56 g water was added. Then 100 g wet sample with

water weighs 107.56 g and dry sample weights 90.77. The average dry matter was then $\frac{90.77\ \mathrm{g}}{107.56\ \mathrm{g}} = 84.4\%$ and the average moisture level was $1 - 84.4\% = 15.6\%$.

4 Discussion

This paper reports on the knowledge and practice among peri-urban and urban dairy farmers in Kenya regarding fermentation of feed for their livestock.

Our research showed that feed fermentation is not commonly practiced in Kenya. However, farmers in Kasarani seemed more aware of feed fermentation, which maybe is due to lack of extra land space, which could be used to grow animal feeds such as maize. In contrast, farmers in Kisumu normally practise dairy farming on ancestral lands that are communally owned and therefore have more extra space to grow other feeds. Therefore, Kasarani farmers are more likely to buy processed feeds such as dairy meal than farmers in Kisumu.

Another plausible explanation could be due to historical farming tendencies, whereby farmers in Kasarani are located adjacent to the fertile highlands of central Kenya, and are thus more knowledgeable regarding value added feeds as compared to farmers in Kisumu who are in the plains and historically fishermen.

To the best of our knowledge we have found no article on feed fermentation practices in Kenya. However, evidence on food fermentation in Africa is abundant as was shown in a review by Franz et al. (2014). Therefore, further studies regarding feed fermentation are recommended.

The farmers mentioned that they lacked knowledge on how to practise feed fermentation. However, they desired to learn more about feed fermentation. A few farmers also mentioned that there was lack of space for practicing feed fermentation. This might reflect lack of knowledge of the various types of feed fermentation, since it is possible to do fermentation in small

containers. Some farmers were practicing feed fermentation in small containers using a very small space. The farmers also mentioned that another reason they did not practice feed fermentation was that it was costly to practice.

Our study showed that yeast fermentation is not commonly used in Kenya. In Kasarani, a few farmers use brewery dairy meal as a yeast fermented feed.

Few farmers were aware of how to prevent aflatoxin formation in their feed storage systems. It is important that farmers have knowledge of how to prevent aflatoxin contamination before it happens. Prevention of aflatoxin contamination that occurs in the field is outside the scope of this discussion. However, for farmers who buy or sell maize, aflatoxin contamination can occur during transportation of the maize after harvest (Mutegi et al., 2013), and it is therefore important for the farmers to consider that all the containers they use for transportation are clean. Contamination can also occur if the feeds are not stored properly, for example, in a dry and raised place with proper ventilation. It is also important to store for shorter periods of time and frequently monitor the feed for mould and other conditions.

The study also investigated how fermentation by yeasts could be used to reduce the level of aflatoxin. There was not enough evidence to demonstrate if the partial fermentation of feed with the specific yeasts reduce aflatoxins in the contaminated maize. However, some signs of a possible reduction of aflatoxin levels could be seen in the laboratory experiment.

An error in the experiment design of the yeast fermentation lab, caused the water mass to be less than required to support a full wet fermentation in all the non-control samples. In the fermentation Duran bottles, water droplets were visible on the glass up to around day 2. This coincided with the rapid decline of aflatoxin level in the fermented treatments (Figures 5), and could be because the amount of surface water was sufficient for the yeast to grow (as demonstrated by the fermentation smell) and reduce aflatoxin by some means, e.g. binding or degradation. Note that the changes in control between the days were statistically insignificant, so despite an apparent decrease in aflatoxin at day 2, the control samples may be considered to not vary. After day 2, it was visible that the fermented treatments had less water, which indicates that the water droplets outside the maize kernels were absorbed by the maize kernels. Also, after day 4, the fermentation smell was gone, which suggested that the yeast activity had slowed down or stopped,

probably due to lack of surface water. Shelled maize kernels contain so much moisture that, in order to prevent mould growth, they need to be dried to about 12% moisture level (if stored at 30°C) and 14 % for storage at 10°C (Herum, 1987). Since the average calculated moisture content in the fermented treatments was 16% and the water was unevenly distributed, some maize kernels must have had more water than 16%. It is likely that those maize kernels with much greater than 16% moisture and incubated at 25°C could support growth and aflatoxin production by *A. flavus* again, hence the slow increase in aflatoxin seen in Figure 5 from about day 4-6 onwards. In a proper wet fermentation, the lactic acid bacteria and yeast are able to grow fast and outcompete the *A. flavus*, because moulds, which are strict aerobes, need oxygen to grow and prefer to grow on surfaces (Olstorpe et al., 2011; Haugaard et al., 2001; Smith et al., 1986). There might have been some atmospheric changes in the fermentation bottles. When the bottles are tightly closed, the oxygen is used up by yeast and bacteria and the atmosphere will become anaerobic. But during sampling, the bottles were opened which means that oxygen was let into the bottles and the atmosphere became aerobic again. The oxygen might have allowed mould growth and mycotoxin production (Fredlund et al., 2002). In this way, the event of opening the bottles may itself have influenced the aflatoxin levels over the time course of the experiment.

In this experiment where the both yeast and moulds may have been growing and interacting, it is worth considering that *W. anomalus* and *M. guilliermondii* have been shown to inhibit mould growth (Petersson et al., 1995), and if mould growth is completely suppressed, then mycotoxins cannot be produced. A more recent study specifically examining aflatoxin production showed that *W. anomalus* produced a volatile compound, 2-phenylethanol, which inhibited the aflatoxin formation by down regulation of aflatoxin biosynthesis genes by 10,000-fold within 24 hours during incubation of 7 days (Hua et al. 2014). Other fungi, like *R. oligosporus*, are able to both degrade and inhibit the production of aflatoxin B1. The commonly used *S. cerevisiae* is also known to be able to reduce mycotoxin levels when added to ruminant feeds (Kusumaningtyas et al., 2006). It would be possible that the yeasts tested in this study, *W. anomalus*, *M. guilliermondii* and *K. exigua*, might do the same, that is, binding (Wu et al., 2009), degrading, and inhibiting production (Kusumaningtyas et al., 2006), and it may be the reason for a possible reduction in aflatoxin levels early in the time course of the laboratory experiment, like-wise, counteracting or slowing the new aflatoxin synthesis later in the time course.

Regarding our chosen time frame, it seems that Kenyan farmers practice longer duration of incubation period for fermentation, in both survey areas (only 9 answers in Kisumu), than this fermentation trial of 8 days. It could be that Kenyan farmers prefer to ferment for a longer period; the fermentation substrates also differed.

4.1 Limitations and challenges

Before this study, another study had been made (ILRI, 2018), where randomly sampled farmers answered an interview survey. Those farmers were introduced to the subjects of mould, aflatoxin, hygiene of milk handling and other relevant subjects. The farmers interviewed in this study were the same farmers as in the previous study. Because the farmers had some previous knowledge, it could lead to some knowledge bias in this study. The average farmer in Kenya might have less knowledge than the farmers in this study.

When farmers were interviewed they might have answered what they were thinking about at that present time. For example, they saw the problems that were affecting them at that particular time. The survey in Kasarani was done during dry season. When they were asked about mould they showed that the feed was dry. The interview in Kisumu was during the start of the rainy season and there had been some rainy days before the interview. When asked about if they have seen mould, they explained that it was raining and of course there was mould.

In both Kisumu and Kasarani, some of the farmers had other work to supplement their income, so they had labourers at the farm instead. These labourers were sometimes not willing to give all details about the farm, if the owner was not present. They also lacked information about some of the details about the farm. They wished to double check with their manager first. This might bias the answers, by not sharing important information.

If I would repeat the fermentation trial in the future I would use a more appropriate amount of water, more in line with what Kenyan farmers do, to simulate their style of fermentation and to support good growth of yeast and LAB. The previous target of 25% dry matter was based on Swedish wet pig

feed fermentation, because it was not possible to find out before the fieldwork how a normal feed fermentation in Kenya was conducted. But after the fieldwork, I now have a better idea of what Kenyan farmers do, for example, one farmer reported fermenting maize with 1:3 addition of water.

Instead of having three sample bottles for each treatment, a higher number of bottles could be used. Perhaps up to five bottles, which would imply five sample draws for each treatment for each period. Also, the sample size from each bottle could be increased from 50 g to 250 g, so that there is a higher probability of sampling contaminated maize kernels in each draw. That way the sample variation of the control and the other treatments become less problematic. Each bottle would need to contain 1500 g in order to satisfy six sampling points. The number of incubation days for the laboratory exercise might increase or decrease depending on the sample type, like for example high nutrient feed such as brewery dairy meal or maize kernels might take a shorter time compared to low nutrient feed such as to maize stalks. In the survey most farmers mentioned that they use low nutrient feed maize stalks or napier grass for feed fermentation.

The maize used for the laboratory experiment was extremely highly contaminated with aflatoxin B1. The maize was collected during 2004 in the regions in Kenya which had a major aflatoxin outbreak, which killed many people. This “extreme” maize may have given positively or negatively biased results in the laboratory experiments. For example, the treatment in the laboratory experiment might have a greater relative effect than a normally contaminated maize or *vice versa.*

4.2 Further research

It would be good to try other crops than maize. Apart from maize, the major crops for animal feed produced in Kenya are millet and sorghum (Sirma et al., 2016).

It could be possible to compare yeast fermentation with maize kernels and maize flour, and also try a mixed fermentation of maize with the brewery dairy meal which people were using in Kenya.

It would be good to try feed fermentation with the specific yeasts on some farms, to be able to understand the difficulties of using it. So many farmers

interviewed, 95.4%, were willing to use a specific yeast for their feed fermentation. The fermentation may show promising results during the lab, but it does not necessarily work in a on full-scale trial (Petersson et al., 1995). For example, the environment can be different. One of the yeasts, *W. anomalus*, originated from Sweden and the temperature and moisture might differ from the Kenyan environment. However, there are recent laboratory studies of bioethanol fermentation indicating that *W. anomalus* shows very high tolerance to diverse stress factors (Mukherjee et al., 2017).

It would be good to try feed fermentation in other neighbouring countries, like Uganda, Tanzania or Rwanda. Aflatoxin is a problem in those countries as well and it spreads between them through transportation.

Feed fermentation for animals is well researched. The *S. cerevisiae* yeast has been included in human food and animal feed for a long time. For example, in Kenya, the brewery dairy meal which is a leftover from fermenting different types of alcohol in breweries, contains the yeast *S. cerevisiae* and is used as animal feed. *S. cerevisiae* is one of the most widely used yeasts and considered to be generally safe to consume (de Hoog, 1996). However, safety assessments of non-*S. cerevisiae* yeast need to be made (Gil-Rodríguez et al., 2015). Feed safety regarding addition of *W. anomalus,* strain J121, has already been researched (Walker, 2011). For example, *W. anomalus* is considered to be safe and is classed at biosafety level 1 (de Hoog, 1996). It also has qualified presumption of safety (QPS) status from European Food Safety Authority (Sundh et al., 2010).

But feed fermentation with addition of the strains of the other two yeasts in this thesis, namely *M. guilliermondii*, strain J106, and *K. exigua*, strain J470, are not well researched. Therefore, it would be good to do more research on the effect of *M. guilliermondii* and *K. exigua* on animal health in a closed trial, before making a trial on farms. At this point, there is no proof that the two specific yeasts are safe to be included in animal feed.

5 Conclusion

Feed fermentation is not commonly practiced in Kenya. Very few farmers practiced it and they desire more knowledge about it. They believe that feed fermentation is good for the animals and they would like to use a specific yeast if it would reduce aflatoxin. The farmers that practice feed fermentation used it mainly for dairy cows. Most of the farmers did not know that they could use the feed fermentation for other animals than dairy cows. Only a few farmers monitor their storage regularly for moulds.

There is not enough evidence that fermentation of feed with the specific yeasts degrade aflatoxins in contaminated feed. In a following trial, more water is probably needed in the feed, because the fermentation in the laboratory ran out of water during day 3. Farmers in Kenya ferment with much more water than in the trial. Also, fermentation time could be optimized, to reflect the nutrient levels of the feed fermented. Further research is needed, with more water and incubation days, to see if feed fermented with the specific yeasts can degrade the aflatoxin so much that the feed can be used as animal feeds.

Few farmers use yeast for feed fermentation, but most farmers have a positive attitude to practice feed fermentation with yeast.

The institutions in Kenya should work together with farmers in order to give the farmers good advice regarding proper feed management and storage.

6 References

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Rogers, H., Kieszak, S., Njapau, H., Schleicher, R., McCoy, L., Misore, A., DeCock, K. (2005) Case–control study of an acute aflatoxicosis outbreak, Kenya, 2004. *Environ Health Perspective Vol 113*, pp.1779–1783

Barbas, C. Dams, A. and Majors, R. (2005) *Separation of Aflatoxins by HPLC.* Wilmington, USA: Agilent Technologies

Bullerman, L. and Bianchini, A. (2007) Stability of mycotoxins during food processing. *International Journal of Food Microbiology, 119.* pp.140-146

Dalié, D., Deschamps, A. and Richard-Forget, F. (2010) Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. *Food Control 21,* pp.370-380

Dixelius, C. (2011) ”Vårt vardagliga bröd – i morgon”. *Genetik som tar skruv,* Stockholm: Forskningsrådet Formas, pp. 61-72

Engberg, R., Hammershoj, M., Johansen, N., Abousekken, M., Steenfeldt, S. and Jensen, B. (2009) Fermented feed for laying hens: effects on egg production, egg quality, plumage condition and composition and activity of the intestinal microflora. *British Poultry Science, Vol 50(2).* pp.228-239

EPZA, (2005) *Grain Production in Kenya 2005.* Nairobi: Export Processing Zones Authority

Fandohan, P., Zoumenou, D., Hounhouigan, D., Marasas, W., Wingfield, M., Hell, K. (2005) Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *International Journal of Food Microbiology, 98.* pp.249-259

FAO. (2013) Analysis of Incentives and Disincentives for Maize in Kenya. Monitoring African Food and Agricultural Policies. Rome: FAO

Franz, C., Huch, M., Mathara, J., Abriouel, H., Benomar, N., Reid, G., Galvez, A. and Holzapfel, W. (2014) African fermented foods and probiotics. *International Journal of Food Microbiology, Vol 190,* pp.84–96

Fredlund, E., Druvefors, U., Boysen, M., Lingsten, K-J. and Schnürer, J. (2002) Physiological characteristics of the biocontrol yeast *Pichia anomala*, J121, *FEMS Yeast Research, Vol 2,* pp.395-402

Gil-Rodríguez, A., Carrascosa, A. and Requena, T. (2015) Yeasts in foods and beverages: In vitro characterisation of probiotic traits. *LWT - Food Science and Technology, Vol 64,* pp.1156-1162

Golden, M. (2010). Evolution of nutritional management of acute malnutrition. *Indian Pediatrics, 47*, pp.667-678

Groote, H., Nyanamba, T. and Wahome, R. (2010) Quality Protein Maize for the Feed Industry in Kenya. *Outlook on Agriculture, Vol 39(4),* pp.291-298

Haugaard, V., Udsen, A-M., Mortensen, G., Høegh, L., Petersen, K. and Monahan, F. (2001) Potential Food Applications of Biobased Materials. An EU-Concerted Action Project. *Starch/Stärke, Vol 53,* pp.189-200

Herum, F. (1987) Harvesting and post-harvest management, in *Corn: Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, pp 83-123

de Hoog, G. (1996) Risk assessment of fungi reported from humans and animals. *Mycoses, Vol 39,* pp.407-417

Hua, S., Beck, J., Sarreal, S. and Gee, W. (2014) The major volatile compound 2-phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression of aflatoxin biosynthetic genes of *Aspergillus flavus*. *Mycotoxin Research, Vol 30,* pp.71–78

Infotrak (2018) County Trak Kenya. Available online [2018-05-20]: http://countytrak.infotrakresearch.com/

IARC (2016) *New IARC report urges action against widespread mycotoxin contamination in developing countries.* Press Release: 17 February 2016. Lyon: IARC

IITA (2017) *Impact of Aflatoxin on Health and Trade in Africa.* Available online [2017-Jan-25]: http://ir4.rutgers.edu/GMUS/Nairobi%20Bio%20Eval-workshop/healthandtrade%20talkday1.pdf

ILRI (2017) *Standard Operating Procedure MYC002.00-Determination of Aflatoxins in Food using Ultra High Performance Liquid Chromatography-Fluorescence Detection.* Nairobi: ILRI

ILRI (2018) Personal communication with Kagera, I., Lindahl, J. and Owino, G. Nairobi: International Livestock Research Institute

Iqba, S., Jinap, A., Pirouz, A. and Ahmad Faiza, A. (2015) Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. *Trends in Food Science & Technology, Vol 46,* pp.110-119

Jazi, V., Ashayerizadeh, A., Toghyani, M., Shabani, A., Tellez, G. and Toghyani, M. (2018) Fermented soybean meal exhibits probiotic properties when included in Japanese quail diet in replacement of soybean meal. *Poultry Science, Vol 97(6),* pp.2113–2122

Kang'ethe, E. and Lang'a, K. (2009) Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centres in Kenya. *African Health Sciences Vol 9(4),* pp.218-226

Kiarie, G., Dominguez-Salas, P., Kang'ethe, S., Grace, D. and Lindahl1, J. (2016) Aflatoxin Exposure Among Young Children in Urban Low-Income Areas of Nairobi and association With Child Growth. *African Journal of Food, Agriculture, Nutrition and Development, Vol 16(3),* pp.10967-10990

Kusumaningtyas, E., Widiastuti, R. and Maryam, R. (2006) Reduction of aflatoxin B1 in chicken feed by using *Saccharomyces cerevisiae*, *Rhizopus oligosporus*, and their combination. *Mycopathologia Vol 162,* pp.307–311

Lindahl, J., Obade, M. and Andang'o, P. (2018) *Dairy stakeholders' workshop.* Kisumu: 2018-Mar-02

Lee, H., Nguyen-Viet, H., Lindahl, J., Thanh, H., Khanh, T., Hien, L. and Grace, D. (2017) A survey of aflatoxin B_1 in maize and awareness of aflatoxins in Vietnam. *World Mycotoxin Journal, Vol 10 (2),* pp.195-202

Leszcyñska, J., Maslowska, J., Owczarek, A. and Kucharska, U. (2001) Determination of aflatoxins in food products by the ELISA method. *Czech Journal of Food Science, 19(1).* pp. 8-12

Lewis, L., Onsongo, M., Njapau, H., Shurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A., Misore, A., DeCock, K. and Rubin, C. (2005) Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya, *Environmental Health Perspectives. Vol.113(12),* pp.1763-1767

Mokoena, M., Chelule, P. and Gqaleni, N. (2006) The toxicity and decreased concentration of aflatoxin B1 in natural lactic acid fermented maize meal. *Journal of Applied Microbiology, Vol 100.* pp.773-777

Mukherjee, V., Radecka, D., Aerts, G., Verstrepen, K., Lievens, B. and Thevelein, J. (2017) Phenotypic landscape of non-conventional yeast species for different stress tolerance traits desirable in bioethanol fermentation. Biotechnology for Biofuels, Vol 10:216, pp.1-19

Mutegi, C., Wagacha, M., Kimani, J., Otieno, G., Wanyama, R., Hell, K. And Christie, M. (2013) Incidence of aflatoxin in peanuts (*Arachis hypogaea Linnaeus*) from markets in Western, Nyanza and Nairobi Provinces of Kenya and related market traits. *Journal of Stored Products Research, Vol 52 (2013),* pp.118-127

Muthomi, J., Njenga, L., Gathumbi, J. and Chemining'wa, G. (2009) The Occurrence of Aflatoxins in Maize and Distribution of Mycotoxin-Producing Fungi in Eastern Kenya. *Plant Pathology Journal, Vol 8,* pp.113-119

Ngotho, A. (2016) *New aflatoxin test kit a boost for farmers.* Available online [2017-Feb-08]: http://www.the-star.co.ke/news/2016/07/29/new-aflatoxin-test-kit-a-boost-for-farmers_c1393463

Obade, M., Andang'o, P. Obonyo, C. and Lusweti, F., (2015a) Aflatoxin Exposure in Pregnant Women in Kisumu County, Kenya. *Current Research in Nutrition and Food Science, Vol.3(2).* pp.140-149

Obade, M., Andang'o, P. Obonyo, C. and Lusweti, F., (2015b) Exposure of Children 4 to 6 month of age to Aflatoxin in Kisumu County, Kenya. *African Journal of Food, Agriculture, Nutrition and Development, Vol.15(2).* pp.9949-9963

Okoth, S. and Ohingo M. (2004) Dietary aflatoxin exposure and impaired growth in young children from Kisumu District, Kenya. *African Journal of Health Sciences 2004, Vol 11.* pp.43-54

Olsson, U., Englund, J and Engstrand, U. (2005) *Biometri – Grundläggande biologisk statistik.* Lund: Studentlitteratur AB

Olstorpe, M and Passoth, V. (2011) *Pichia anomala* in grain biopreservation. *Antonie van Leeuwenhoek, 99*. pp.57-62

Oluoch, O. (2014) Report on Factors Limiting the Adoption of the Control Mechanisms of Striga Weed in Nyakach District-Kisumu County. *BSc. Thesis.* Nairobi: University of Nairobi

Pain, A. and Christopholos , I. (2015) *New challenges to food security. From climate change to fragile states.* Abingdon-on-Thames, UK: Routledge. pp.15-38

Petersson, S. and Schnürer, J. (1995) Biocontrol of Mold Growth in High-Moisture Wheat Stored under Airtight Conditions by *Pichia anomala*, *Pichia*

guilliermondii, and *Saccharomyces cerevisiae*, *Applied and Environmental Microbiology,* pp.1027–1032

Shetty, P. and Jespersen, L. (2006) *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. *Trends in Food Science & Technology, Vol 17,* pp.48-55

Sirma, A., Lindahl, J., Makita, K., Senerwa, D., Mtimet, N., Kang'ethe, E. and Grace, D. (2016) Aflatoxin B1 occurrence in millet, sorghum and maize from four agro-ecological zones in Kenya. African journal of food agriculture, nutrition and development, Vol 16(3), pp.10991-11003

Sirma, A., Lindahl, J., Makita, K., Senerwa, D., Mtimet, N., Kang'ethe, E. and Grace, D. (2018) The impacts of aflatoxin standards on health and nutrition in sub-Saharan T Africa: The case of Kenya. *Global Food Security, Vol 18,* pp.57–61

Smith, J., Ooraikul, B., Koersen, W., Jackson, E. and Lawrence, R. (1986) Novel approach to oxygen control in modified atmosphere packaging of bakery products. *Food Microbiology, Vol 3(4),* pp.315-320

Sundh, I. and Melin, P. (2011) Safety and regulation of yeasts used for biocontrol or biopreservation in the food or feed chain. *Antonie van Leeuwenhoek, Vol 99,* pp.113–119

Ullah, H., Durrani, A., Ijaz, M. and Javeed, A. (2016) Aflatoxin B1 Contamination Status of Concentrate Feeds of Dairy Goats in Lahore, Pakistan. *Sarhad Journal of Agriculture, Vol 32(2),* pp.57-61

Wageindicator (2015) *Minimum wages in Kenya with effect from 01-05-2015.* Available online: [2017-Feb-08]: http://www.wageindicator.org/main/salary/minimum-wage/kenya

Walker, G. (2011) *Pichia anomala*: cell physiology and biotechnology relative to other yeasts. *Antonie van Leeuwenhoek, Vol. 99,* pp.25–34

Wang, C., Lin, C., Su, W., Zhang, Y., Wang, F., Wang, Y., Shi, C. and Lu, Z. (2018a) Effects of supplementing sow diets with fermented corn and soybean meal mixed feed during lactation on the performance of sows and progeny. *Journal of Animal Science, Vol 96(1),* pp.206–214

Wang, C., Shi, C., Zhang, Y., Song, D., Lu, Z. and Wang, Y. (2018b) Microbiota in fermented feed and swine gut. *Applied Microbiology and Biotechnology, Vol 102,* pp.2941–2948

Wangia, R. (2017) Deaths from Consumption of Contaminated Maize in Easternprovince, Kenya. *KESSA Conference Proceedings 2017,* pp.39-42

WHO (2018) Aflatoxins, *Department of Food Safety and Zoonoses WHO/NHM/FOS/RAM/18.1*, pp.1-5

Williams, J., Phillips, T., Jolly, P., Stiles, J., Jolly, C. and Aggarwal, D. (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition, 80.* pp.1106-1122

Wu, Q., Jezkova, A., Yuan, Z., Pavlikova, L., Dohnal, V. and Kuca, K. (2009) Biological degradation of aflatoxins. *Drug Metabolism Reviews, 41(1).* pp.1-7.

Zain, M. (2011) Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society, 15(2).* pp.129-144

Acknowledgements

It is with immense gratitude that I acknowledge the support and help of my supervisors Su-Lin and Albina Bakeeva for assistance and support by giving me advice throughout the project.

I cannot find words to express my gratitude to thank my field supervisor Johanna Lindahl for proper arrangements of the fieldwork schedules and workshops in Kisumu and Kasarani and providing me with all the information I needed while in ILRI.

I also like to give thanks to all the IRLI personal at Animal and Human Health Department in Mara House, Nairobi.

I consider it an honour to work with Irene Kagera and Gladys Owino for massive help during my fieldwork in Kasarani and in Kisumu.

I would also give my special thanks to all 184 farmers which took part and participate to help me answer my questions. Special thanks to the veterinary officers in both Nairobi and in Kisumu for making it easy to identify all the farmers. Thanks also all the research assistance in both Kasarani and in Kisumu.

I will like to thank all the personals in Laboratory 4 and 7 in ILRI Nairobi, for allowing me to use their equipment. I would also like to give thanks to Fredrick Nganga, Maureen Kuboka, Nick Owiro, Richard Doose for the advice and help during the laboratory experiments.

My special thanks also go to SIDA, all the MFS committee at SLU and the NJ faculty for granting me with scholarship for my Master's thesis and for making it possible.

It is a great pleasure to acknowledge my family for always be there to support me. My husband Jonas Arvén and my daughter Sellah for letting me to travel miles away to achieve my dreams.

1 Appendix: Popular scientific summary

Maize and other crops can be contaminated by moulds. Moulds spread with the wind and rain between plants. Some types of mould produce very dangerous toxins, which can make animals and humans sick, and sometimes even kill them.

Year 2004 until 2010, a lot of people in Kenya lost their lives, after consuming contaminated maize. So many people are not aware of this toxin. It is called Aflatoxin and it affects the liver and is very carcinogenic. It can also reduce the growth of children. If animals consume aflatoxin, the toxin can spread to human by milk.

The moulds can spread by the wind, but it can also spread fast in poor storage, with insufficient ventilation. If there is moisture and high temperature, the mould can grow and spreads out over a large surface of the stored crop.

This thesis is making a trial on a new technique, to remove the toxin from contaminated maize, so that animals can consume it as feed. This way a lot of feed, which was to be thrown away, can be saved! The new technique implies that the contaminated maize should be fermented in water for some days and a specific yeast should be added, which inhibits the production of toxin and degrades the toxin to less severe forms. In that way, safe food will be produced.

The thesis also investigates what the farmers think about the new technique. A farmer must trust the technique in order to use it! The thesis finds that the farmers are aware of these problems of moulds and they like the new idea, so that they are willing to use the technique if it would be available to them.

2 Appendix: Determination of dry matter composition

2.1 Sample 1 (C1, C2, C3):

Empty brown natural paper bag weight = 6 g

Brown natural paper bag + maize = 200 g

Wet Sample = 200-6=194 g

Brown natural paper bag + Sample weight after 24 hrs in oven = 181.5 g

Dry sample weight 181.5-6=175.5 g

No water was added to the control sample.

2.2 Sample 2 (F1, F2, F3):

Empty brown natural paper bag weight $= 8\ g$

Brown natural paper bag + maize = $200\ g$

Wet Sample = $200 - 8 = 192\ g$

Brown natural paper bag + Sample weight after 24 hrs in oven = $182.2\ g$

Dry sample weight $182.2\ \mathrm{g} - 8\ \mathrm{g} = 174.2\ g$

An error in the experiment design caused the water mass to be 22.68.

2.3 Sample 3 (Y11, Y12, Y13):

Empty brown natural paper bag weight= $7\ g$

Brown natural paper bag + maize = $200\ g$

Wet Sample = $200 - 7 = 193\ g$

Brown natural paper bag + Sample weight after 24 hrs in oven = $182.9\ g$

Dry sample weight $182.9 - 7 = 175.9\ g$

An error in the experiment design caused the water mass to be 22.79.

2.4 Sample 4 (Y21, Y22, Y23):

Empty brown natural paper bag weight= $7\ g$

Brown natural paper bag + maize = $200\ g$

Wet Sample = $200 - 7 = 193\ g$

Brown natural paper bag + Sample weight after 24 hrs in oven = $181.5\ g$

Dry sample weight $181.5 - 7 = 174.5\ g$

An error in the experiment design caused the water mass to be 22.60 ml.

2.5 Sample 5 (Y31, Y32, Y33):

Empty brown natural paper bag weight= $7\ g$

Brown natural paper bag + maize = $200\ g$

Wet Sample = $200 - 7 = 193\ g$

Brown natural paper bag + Sample weight after 24 hrs in oven = $182.2\ g$

Dry sample weight $182.2 - 7 = 175.2\ g$

An error in the experiment design caused the water mass to be 22.70 ml.

3 Appendix: Calculation method for counting cells

3.1 Yeast 1: *Wickerhamomyces anomalus* strain J121

Number of cells counted = 370 Number of squares counted = 20

$$= \frac{370}{20 \times 2.5 \times 10^{-7} \times 0.01(dilution)} = 7.4 \times 10^{9} cells/mL$$

3.2 Yeast 2: *Meyerozyma guilliermondii* strain J106

Number of cells counted = 412 Number of squares counted = 20

$$= \frac{412}{20 \times 2.5 \times 10^{-7} \times 0.01(dilution)} = 8.2 \times 10^{9} cells/mL$$

3.3 Yeast 3: *Kazachstania exigua* strain J470

$\text{Number of cells counted} = 398$ $\text{Number of squares counted} = 20$

$$= \frac{398}{20 \times 2.5 \times 10^{-7} \times 0.01(dilution)} = 7.9 \times 10^{9} cells/mL$$

4 Appendix: Volume of yeast suspension to be used to inoculate maize kernels

4.1 Yeast 1: *W. anomalus* strain J121

$$= \frac{1.8 \times 10^8}{7.4 \times 10^9} = \mathbf{0.024}\ mL\ of\ yeast\ 1\ suspension\ \text{J121}$$

4.2 Yeast 2: *M. guilliermondii* strain J106

$$= \frac{1.8 \times 10^8}{8.2 \times 10^9} = \mathbf{0.022\ mL}\ of\ yeast\ 2\ suspension\ \text{J106}$$

4.3 Yeast 3: *K. exigua* strain J470

$$= \frac{1.8 \times 10^8}{7.9 \times 10^9} = \mathbf{0.023\ mL}\ of\ yeast\ 3\ suspension\ \text{J470}$$

5 Appendix: Statistical tables from the laboratory trial

Table 5. *Tukey's test, Compared interaction between Day 0:treatment. There was no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D0:F-D0:C	-20.94	-158.26	116.37	1.000
D0:Y1-D0:C	37.08	-100.23	174.40	1.000
D0:Y2-D0:C	-47.74	-185.05	89.58	1.000
D0:Y3-D0:C	-13.24	-150.56	124.07	1.000
D0:Y1-D0:F	58.03	-79.29	195.34	0.997
D0:Y2-D0:F	-26.79	-164.11	110.52	1.000
D0:Y3-D0:F	7.70	-129.61	145.02	1.000
D0:Y2-D0:Y1	-84.82	-222.14	52.49	0.807
D0:Y3-D0:Y1	-50.33	-187.64	86.99	1.000
D0:Y3-D0:Y2	34.49	-102.82	171.81	1.000

Table 6. *Tukey's test, Compared interaction between Day 2:treatment. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D2:F-D2:C	-45.13	-182.45	92.18	1.000
D2:Y1-D2:C	-43.54	-180.85	93.78	1.000
D2:Y2-D2:C	-59.77	-197.08	77.55	0.996
D2:Y3-D2:C	-59.07	-196.39	78.24	0.996
D2:Y1-D2:F	1.60	-135.72	138.91	1.000
D2:Y2-D2:F	-14.64	-151.95	122.68	1.000
D2:Y3-D2:F	-13.94	-151.25	123.38	1.000
D2:Y2-D2:Y1	-16.23	-153.55	121.08	1.000
D2:Y3-D2:Y1	-15.53	-152.85	121.78	1.000
D2:Y3-D2:Y2	0.70	-136.62	138.01	1.000

Table 7. *Tukey's test, Compared interaction between Day 4:treatment. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D4:F-D4:C	-79.88	-217.20	57.43	0.880
D4:Y1-D4:C	-80.66	-217.98	56.65	0.869
D4:Y2-D4:C	-81.52	-218.83	55.80	0.858
D4:Y3-D4:C	-73.40	-210.71	63.92	0.945
D4:Y1-D4:F	-0.78	-138.09	136.54	1.000
D4:Y2-D4:F	-1.64	-138.95	135.68	1.000
D4:Y3-D4:F	6.48	-130.83	143.80	1.000
D4:Y2-D4:Y1	-0.86	-138.17	136.46	1.000
D4:Y3-D4:Y1	7.26	-130.05	144.58	1.000
D4:Y3-D4:Y2	8.12	-129.19	145.44	1.000

Table 8. *Tukey's test, Compared interaction between Day 6:treatment. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D6:F-D6:C	-23.11	-160.42	114.21	1.000
D6:Y1-D6:C	-58.01	-195.33	79.30	0.997
D6:Y2-D6:C	-23.45	-160.76	113.87	1.000
D6:Y3-D6:C	-31.21	-168.52	106.11	1.000
D6:Y1-D6:F	-34.90	-172.22	102.41	1.000
D6:Y2-D6:F	-0.34	-137.65	136.98	1.000
D6:Y3-D6:F	-8.10	-145.41	129.22	1.000
D6:Y2-D6:Y1	34.57	-102.75	171.88	1.000
D6:Y3-D6:Y1	26.81	-110.51	164.12	1.000
D6:Y3-D6:Y2	-7.76	-145.07	129.56	1.000

Table 9. *Tukey's test, Compared interaction between Day 7:treatment. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D7:F-D7:C	-19.05	-156.37	118.26	1.000
D7:Y1-D7:C	-33.58	-170.90	103.73	1.000
D7:Y2-D7:C	35.12	-102.20	172.43	1.000
D7:Y3-D7:C	-47.26	-184.58	90.05	1.000
D7:Y1-D7:F	-14.53	-151.84	122.79	1.000
D7:Y2-D7:F	54.17	-83.14	191.49	0.999
D7:Y3-D7:F	-28.21	-165.53	109.11	1.000
D7:Y2-D7:Y1	68.70	-68.62	206.01	0.973
D7:Y3-D7:Y1	-13.68	-151.00	123.63	1.000
D7:Y3-D7:Y2	-82.38	-219.70	54.93	0.845

Table 10. *Tukey's test, Compared interaction between Day 7:treatment. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D8:F-D8:C	-23.56	-160.87	113.76	1.000
D8:Y1-D8:C	20.41	-116.91	157.72	1.000
D8:Y2-D8:C	-57.14	-194.46	80.18	0.998
D8:Y3-D8:C	-86.96	-224.28	50.35	0.770
D8:Y1-D8:F	43.97	-93.35	181.28	1.000
D8:Y2-D8:F	-33.58	-170.90	103.73	1.000
D8:Y3-D8:F	-63.40	-200.72	73.91	0.990
D8:Y2-D8:Y1	-77.55	-214.86	59.77	0.907
D8:Y3-D8:Y1	-107.37	-244.69	29.94	0.362
D8:Y3-D8:Y2	-29.82	-167.14	107.49	1.000

Table 11. *Tukey's test, Compared interaction between days:control. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D2:C-D0:C	-90.03	-227.35	47.29	0.712
D4:C-D0:C	-44.81	-182.12	92.51	1.000
D6:C-D0:C	-78.31	-215.62	59.01	0.899
D7:C-D0:C	-78.46	-215.77	58.86	0.897
D8:C-D0:C	-23.74	-161.06	113.57	1.000
D4:C-D2:C	45.22	-92.09	182.54	1.000
D6:C-D2:C	11.72	-125.59	149.04	1.000
D7:C-D2:C	11.57	-125.74	148.89	1.000
D8:C-D2:C	66.29	-71.03	203.60	0.983
D6:C-D4:C	-33.50	-170.82	103.81	1.000
D7:C-D4:C	-33.65	-170.97	103.66	1.000
D8:C-D4:C	21.06	-116.25	158.38	1.000
D7:C-D6:C	-0.15	-137.46	137.17	1.000
D8:C-D6:C	54.56	-82.75	191.88	0.999
D8:C-D7:C	54.71	-82.60	192.03	0.999

Table 12. *Tukey's test, Compared interaction between days:ferment. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D2:F-D0:F	-114.22	-251.54	23.10	0.249
D4:F-D0:F	-103.75	-241.06	33.57	0.431
D6:F-D0:F	-80.47	-217.79	56.84	0.872
D7:F-D0:F	-76.57	-213.88	60.75	0.918
D8:F-D0:F	-26.36	-163.67	110.96	1
D4:F-D2:F	10.47	-126.84	147.79	1
D6:F-D2:F	33.75	-103.57	171.06	1
D7:F-D2:F	37.65	-99.66	174.97	1
D8:F-D2:F	87.86	-49.45	225.18	0.753
D6:F-D4:F	23.27	-114.04	160.59	1
D7:F-D4:F	27.18	-110.14	164.49	1
D8:F-D4:F	77.39	-59.93	214.70	0.909
D7:F-D6:F	3.91	-133.41	141.22	1
D8:F-D6:F	54.11	-83.20	191.43	0.999
D8:F-D7:F	50.21	-87.11	187.52	1

Table 13. *Tukey's test, Compared interaction between days:yeast 1.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D2:Y1-D0:Y1	-170.65	-307.97	-33.34	0.003**
D4:Y1-D0:Y1	-162.55	-299.87	-25.24	0.006**
D6:Y1-D0:Y1	-173.41	-310.72	-36.09	0.002**
D7:Y1-D0:Y1	-149.12	-286.44	-11.81	0.019*
D8:Y1-D0:Y1	-40.42	-177.73	96.90	1
D4:Y1-D2:Y1	8.10	-129.21	145.42	1
D6:Y1-D2:Y1	-2.75	-140.07	134.56	1
D7:Y1-D2:Y1	21.53	-115.78	158.85	1
D8:Y1-D2:Y1	130.24	-7.08	267.55	0.086.
D6:Y1-D4:Y1	-10.85	-148.17	126.46	1
D7:Y1-D4:Y1	13.43	-123.88	150.75	1
D8:Y1-D4:Y1	122.13	-15.18	259.45	0.152
D7:Y1-D6:Y1	24.28	-113.03	161.60	1
D8:Y1-D6:Y1	132.99	-4.33	270.30	0.07.
D8:Y1-D7:Y1	108.70	-28.61	246.02	0.338

Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Table 14. *Tukey's test, Compared interaction between days:yeast 2. There were no significant values.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D2:Y2-D0:Y2	-102.06	-239.38	35.25	0.464
D4:Y2-D0:Y2	-78.59	-215.90	58.73	0.895
D6:Y2-D0:Y2	-54.02	-191.33	83.30	0.999
D7:Y2-D0:Y2	4.40	-132.92	141.71	1
D8:Y2-D0:Y2	-33.15	-170.46	104.17	1
D4:Y2-D2:Y2	23.47	-113.84	160.79	1
D6:Y2-D2:Y2	48.04	-89.27	185.36	1
D7:Y2-D2:Y2	106.46	-30.86	243.78	0.379
D8:Y2-D2:Y2	68.92	-68.40	206.23	0.973
D6:Y2-D4:Y2	24.57	-112.75	161.89	1
D7:Y2-D4:Y2	82.99	-54.33	220.30	0.836
D8:Y2-D4:Y2	45.44	-91.87	182.76	1
D7:Y2-D6:Y2	58.42	-78.90	195.73	0.997
D8:Y2-D6:Y2	20.87	-116.44	158.19	1
D8:Y2-D7:Y2	-37.54	-174.86	99.77	1

Table 15. *Tukey's test, Compared interaction between days:yeast 3.*

Compared Interaction days:treatments	diff AFB1	lwr	upr	p adj
D2:Y3-D0:Y3	-135.86	-273.17	1.46	0.056.
D4:Y3-D0:Y3	-104.96	-242.28	32.35	0.407
D6:Y3-D0:Y3	-96.27	-233.59	41.05	0.585
D7:Y3-D0:Y3	-112.48	-249.79	24.84	0.276
D8:Y3-D0:Y3	-97.46	-234.78	39.85	0.56
D4:Y3-D2:Y3	30.90	-106.42	168.21	1
D6:Y3-D2:Y3	39.59	-97.73	176.90	1
D7:Y3-D2:Y3	23.38	-113.93	160.70	1
D8:Y3-D2:Y3	38.40	-98.92	175.71	1
D6:Y3-D4:Y3	8.69	-128.62	146.01	1
D7:Y3-D4:Y3	-7.52	-144.83	129.80	1
D8:Y3-D4:Y3	7.50	-129.82	144.81	1
D7:Y3-D6:Y3	-16.21	-153.52	121.11	1
D8:Y3-D6:Y3	-1.19	-138.51	136.12	1
D8:Y3-D7:Y3	15.02	-122.30	152.33	1

Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

6 Appendix: Survey Questions and Answers

All answers from the survey questions, together with the survey questions, are listed below and numbered.

Appendix Table 6. *The number of individual/farmers who were questioned in the areas Kasarani and Kisumu.*

	Total	Kasarani	Kisumu
Number of individuals questioned	184	93	91

Analysis

1.5 Feeding

1.5.1 Do you routinely monitor the condition of your feed during storage, for any spoilage [yes] [no]

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Yes	155	93	62	85.6%	100.0%	70.5%	84.2%	100.0%	68.1%
No	26	0	26	14.4%	0.0%	29.5%	14.1%	0.0%	28.6%
Total	181	93	88						
Individuals answering	181	93	88						

1.5.2 If yes, what conditions do you routinely monitor for during storage
[1= moisture 2= warmth 3= ventilation 4= mold growth 5= dryness 6= pests / animal 7=other, please explain]

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
1	29	10	19	16.2%	11.4%	20.9%	15.8%	10.8%	20.9%
2	10	3	7	5.6%	3.4%	7.7%	5.4%	3.2%	7.7%
3	15	6	9	8.4%	6.8%	9.9%	8.2%	6.5%	9.9%
4	121	85	36	67.6%	96.6%	39.6%	65.8%	91.4%	39.6%
5	26	4	22	14.5%	4.5%	24.2%	14.1%	4.3%	24.2%
6	68	38	30	38.0%	43.2%	33.0%	37.0%	40.9%	33.0%
7	4	1	3	2.2%	1.1%	3.3%	2.2%	1.1%	3.3%
Total	273	147	126						
Individuals answering	179	88	91						

1.5.3 What actions would you take if you noticed your stored feed had molds *[1= dispose the feed 2= still give animals the feed 3= mix with good feed 4=other, please explain*

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
1	85	78	7	47.5%	88.6%	7.7%	46.2%	83.9%	7.7%
2	8	5	3	4.5%	5.7%	3.3%	4.3%	5.4%	3.3%
3	0	0	0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
4	17	8	9	9.5%	9.1%	9.9%	9.2%	8.6%	9.9%
Total	110	91	19						
Individuals answering	179	88	91						

1.5.4 Do you do any feed fermentation for any of your animals (feed fermentation = adding water and letting it stand for a period). [yes] [no]_____ If no go to 1.5.11

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Yes	40	30	10	22.0%	33.0%	11.0%	21.7%	32.3%	11.0%
No	142	61	81	78.0%	67.0%	89.0%	77.2%	65.6%	89.0%
Total	182	91	91				98.9%		
Individuals answering	182	91	91						

If above yes

-1.5.5 which kind of feed do you ferment?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Maize stalks	27	20	7	67.5%	66.7%	70.0%	14.7%	21.5%	7.7%
Napier grass	22	14	8	55.0%	46.7%	80.0%	12.0%	15.1%	8.8%
Molasses	5	0	5	12.5%	0.0%	50.0%	2.7%	0.0%	5.5%
Dairy meal	3	2	1	7.5%	6.7%	10.0%	1.6%	2.2%	1.1%
Hay	0	0	0	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Maize	3	2	1	7.5%	6.7%	10.0%	1.6%	2.2%	1.1%
Maize germ	2	2	0	5.0%	6.7%	0.0%	1.1%	2.2%	0.0%
Maize flour	1	1	0	2.5%	3.3%	0.0%	0.5%	1.1%	0.0%
Maize left overs from market	1	1	0	2.5%	3.3%	0.0%	0.5%	1.1%	0.0%
Phosphorus	1	0	1	2.5%	0.0%	10.0%	0.5%	0.0%	1.1%
Rice straws	1	0	1	2.5%	0.0%	10.0%	0.5%	0.0%	1.1%
Total	66	42	24						
Individuals answering	40	30	10						

-1.5.6 which ratio of water do you add to your feed? 0= No measure 1= 1:1 ratio 2= 1:2 ratio 3=other, please explain

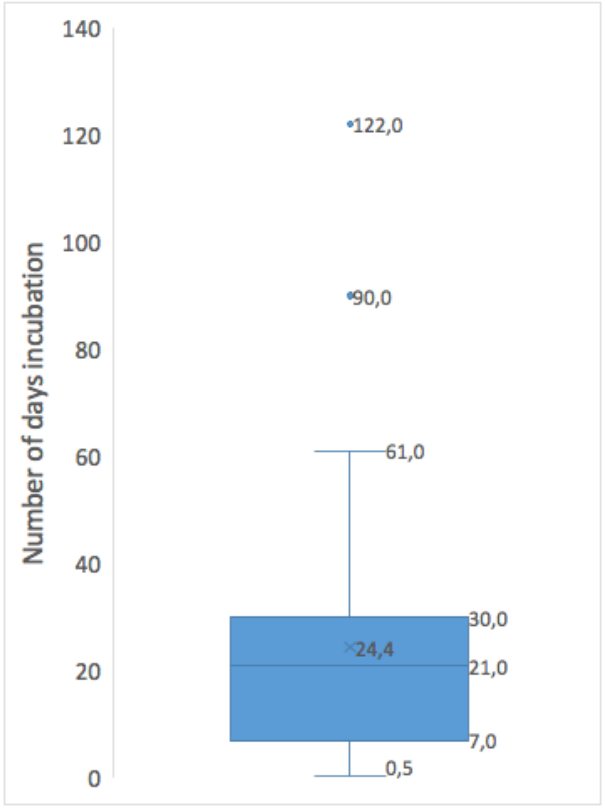
	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
No measure	22	19	3	12.2%	21.3%	3.3%	12.0%	20.4%	3.3%
1:1	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
1:2	5	2	3	2.8%	2.2%	3.3%	2.7%	2.2%	3.3%
1:3	2	0	2	1.1%	0.0%	2.2%	1.1%	0.0%	2.2%
1:4	4	2	2	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%
1:9	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Other	3	3	0	1.7%	3.4%	0.0%	1.6%	3.2%	0.0%
Total	38	28	10						
Individuals answering	180	89	91						

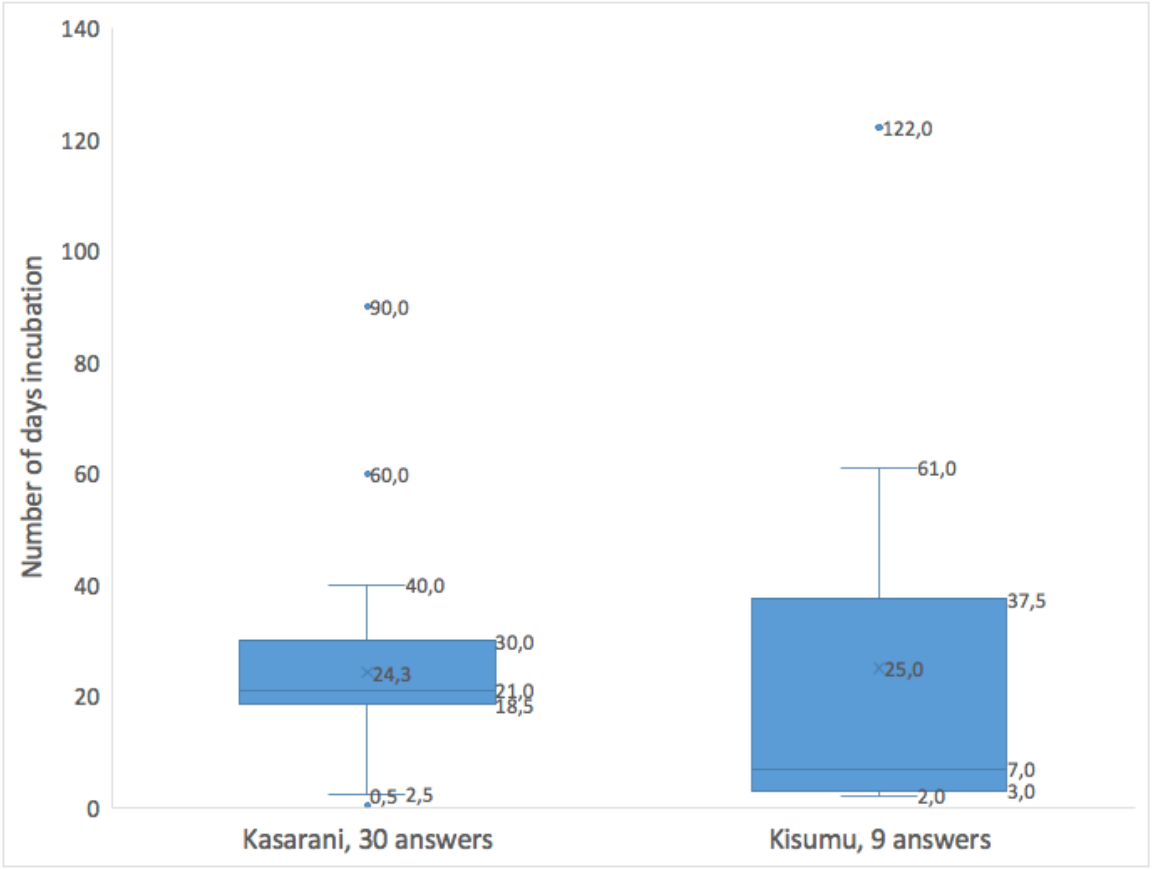
	Total							Kasarani						Kisumu			
Answer	No measure	1:1	1:2	1:3	1:4	1:9	Other	No measure	1:1	1:2	1:4	1:9	Other	No measure	1:2	1:3	1:4
Maize stalks	15	1	4	1	4	1	1	13	1	2	2	1	1	2	2	1	2
Napier grass	11	0	3	2	3	1	2	9	0	1	1	1	2	2	2	2	2
Molasses	1	0	1	2	1	0	0	0	0	0	0	0	0	1	1	2	1
Hay	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Maize	2	0	1	0	0	0	0	2	0	0	0	0	0	0	1	0	0
Maize germ	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
Dairy meal	2	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Phosphorus	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Maize flour	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rice straws	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Maize left overs from market	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Total	34	1	9	5	8	2	5										

-1.5.7 do you mix the feed and the water? 0 = No mixing 1= Mixing

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
0	14	13	1	45.2%	61.9%	10.0%	7.6%	14.0%	1.1%
1	17	8	9	54.8%	38.1%	90.0%	9.2%	8.6%	9.9%
Total	31	21	10						
Individuals answering	31	21	10						

-1.5.8 How many days do you let it to stand for incubation?





-1.5.9 Which animals do you feed with feed fermentation?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Cows	38	30	8	95.0%	100.0%	80.0%	20.7%	32.3%	8.8%
All animals	2	0	2	5.0%	0.0%	20.0%	1.1%	0.0%	2.2%
Sheep	2	1	1	5.0%	3.3%	10.0%	1.1%	1.1%	1.1%
Goats	2	1	1	5.0%	3.3%	10.0%	1.1%	1.1%	1.1%
Pigs	1	1	0	2.5%	3.3%	0.0%	0.5%	1.1%	0.0%
Poultery	1	1	0	2.5%	3.3%	0.0%	0.5%	1.1%	0.0%
Total	46	34	12						
Individuals answering	40	30	10						

-1.5.10 Do you make a fresh feed fermentation every day? Or do you keep a continuous fermentation by adding new feed and water, after you've taken out feed for the animals. 1= Fresh fermentation every day 2= Add water and feed to ongoing mixture

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
1	31	26	5	83.8%	92.9%	55.6%	16.8%	28.0%	5.5%
2	6	2	4	16.2%	7.1%	44.4%	3.3%	2.2%	4.4%
Total	37	28	9						
Individuals answering	37	28	9						

1.5.11 What do you think about feed fermentation?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Don't know	41	10	31	23.3%	11.2%	35.6%	22.3%	10.8%	34.1%
It increases milk production	36	31	5	20.5%	34.8%	5.7%	19.6%	33.3%	5.5%
Its good	32	14	18	18.2%	15.7%	20.7%	17.4%	15.1%	19.8%
Effective way of preserving feeds	17	13	4	9.7%	14.6%	4.6%	9.2%	14.0%	4.4%
Good for animals	14	3	11	8.0%	3.4%	12.6%	7.6%	3.2%	12.1%
Useful during dry seasons	13	10	3	7.4%	11.2%	3.4%	7.1%	10.8%	3.3%
Effective way of preserving feeds	10	6	4	5.7%	6.7%	4.6%	5.4%	6.5%	4.4%
Easy to feed	6	2	4	3.4%	2.2%	4.6%	3.3%	2.2%	4.4%
Desires to know and practice at th	5	2	3	2.8%	2.2%	3.4%	2.7%	2.2%	3.3%
Its not good	5	0	5	2.8%	0.0%	5.7%	2.7%	0.0%	5.5%
High cost of production	4	0	4	2.3%	0.0%	4.6%	2.2%	0.0%	4.4%
No wastage of feeds	4	4	0	2.3%	4.5%	0.0%	2.2%	4.3%	0.0%
Source of diseases	4	0	4	2.3%	0.0%	4.6%	2.2%	0.0%	4.4%
Laborious	3	2	1	1.7%	2.2%	1.1%	1.6%	2.2%	1.1%
Improve animals' appetite	3	3	0	1.7%	3.4%	0.0%	1.6%	3.2%	0.0%
Helps in digestion	2	2	0	1.1%	2.2%	0.0%	1.1%	2.2%	0.0%
Its good when all necessary resou	2	2	0	1.1%	2.2%	0.0%	1.1%	2.2%	0.0%
Its not good for animals	2	0	2	1.1%	0.0%	2.3%	1.1%	0.0%	2.2%
Supply of fresh feeds	2	2	0	1.1%	2.2%	0.0%	1.1%	2.2%	0.0%
Not easy to make	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Very important	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Doesn't help in production	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
High investment cost	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Cattle refuse to feed on it	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Its safe	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Low production cost	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Total	212	112	100						
Individuals answering	176	89	87						

1.5.12 What are some of the challenges you face when doing fermentation, or if you don't do it, why not?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Lack of information	49	25	24	27.8%	28.4%	27.3%	26.6%	26.9%	26.4%
Lack of raw materials	45	24	21	25.6%	27.3%	23.9%	24.5%	25.8%	23.1%
High cost of production	18	8	10	10.2%	9.1%	11.4%	9.8%	8.6%	11.0%
Labour intensive	17	5	12	9.7%	5.7%	13.6%	9.2%	5.4%	13.2%
Don't know	11	0	11	6.3%	0.0%	12.5%	6.0%	0.0%	12.1%
None	11	9	2	6.3%	10.2%	2.3%	6.0%	9.7%	2.2%
Feed quality risk	9	9	0	5.1%	10.2%	0.0%	4.9%	9.7%	0.0%
Lack of space	9	7	2	5.1%	8.0%	2.3%	4.9%	7.5%	2.2%
Source of diseases	8	0	8	4.5%	0.0%	9.1%	4.3%	0.0%	8.8%
Lack of equipment	6	6	0	3.4%	6.8%	0.0%	3.3%	6.5%	0.0%
High investment cost	5	4	1	2.8%	4.5%	1.1%	2.7%	4.3%	1.1%
Few animals	5	3	2	2.8%	3.4%	2.3%	2.7%	3.2%	2.2%
Time consuming	4	4	0	2.3%	4.5%	0.0%	2.2%	4.3%	0.0%
Lack of experience	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Rodents invasion	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Total	199	105	94						
Individuals answering	176	88	88						

1.5.13 Do you routinely add anything to your feeds today? Yes/No. If yes specify below

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Salt	70	36	34	40.9%	43.9%	38.2%	38.0%	38.7%	37.4%
Molasses	43	15	28	25.1%	18.3%	31.5%	23.4%	16.1%	30.8%
Dairy meal	23	4	19	13.5%	4.9%	21.3%	12.5%	4.3%	20.9%
Phosphorus	16	0	16	9.4%	0.0%	18.0%	8.7%	0.0%	17.6%
Minerals	7	3	4	4.1%	3.7%	4.5%	3.8%	3.2%	4.4%
Aflatoxin Binder	5	2	3	2.9%	2.4%	3.4%	2.7%	2.2%	3.3%
Cotton	5	5	0	2.9%	6.1%	0.0%	2.7%	5.4%	0.0%
Soya	5	5	0	2.9%	6.1%	0.0%	2.7%	5.4%	0.0%
Yeast	7	5	2	4.1%	6.1%	2.2%	3.8%	5.4%	2.2%
Sunflower	4	4	0	2.3%	4.9%	0.0%	2.2%	4.3%	0.0%
Desmodium	2	0	2	1.2%	0.0%	2.2%	1.1%	0.0%	2.2%
Lucina	2	0	2	1.2%	0.0%	2.2%	1.1%	0.0%	2.2%
Maize bran	2	0	2	1.2%	0.0%	2.2%	1.1%	0.0%	2.2%
Maize grain	2	0	2	1.2%	0.0%	2.2%	1.1%	0.0%	2.2%
Wheat bran	3	1	2	1.8%	1.2%	2.2%	1.6%	1.1%	2.2%
Cobler	1	1	0	0.6%	1.2%	0.0%	0.5%	1.1%	0.0%
Dicalcium phosphate	2	2	0	1.2%	2.4%	0.0%	1.1%	2.2%	0.0%
Fresh grass	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Omena	1	1	0	0.6%	1.2%	0.0%	0.5%	1.1%	0.0%
Total	201	84	117						
Individuals answering	171	82	89						

1.6 Awareness about molds and aflatoxins

1.6.1 Have you ever seen mold on cattle feed, in your farm_____ [yes] [no]

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Yes	81	40	41	44.0%	43.0%	45.1%	44.0%	43.0%	45.1%
No	103	53	50	56.0%	57.0%	54.9%	56.0%	57.0%	54.9%
Total	184	93	91						
Individuals answering	184	93	91						

1.6.2 1.6.2 If yes, do you think it has any impacts on cattle and if so what impact(s)

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Causes diseases	21	9	12	25.0%	22.0%	27.9%	11.4%	9.7%	13.2%
Causes diseases in animals	20	18	2	23.8%	43.9%	4.7%	10.9%	19.4%	2.2%
Causes diarrhea	13	4	9	15.5%	9.8%	20.9%	7.1%	4.3%	9.9%
Kills	11	3	8	13.1%	7.3%	18.6%	6.0%	3.2%	8.8%
Reduced milk production	9	5	4	10.7%	12.2%	9.3%	4.9%	5.4%	4.4%
NO	7	3	4	8.3%	7.3%	9.3%	3.8%	3.2%	4.4%
Bloating	6	2	4	7.1%	4.9%	9.3%	3.3%	2.2%	4.4%
Causes aflotoxin contamination	3	0	3	3.6%	0.0%	7.0%	1.6%	0.0%	3.3%
Stomach related problems	5	5	0	6.0%	12.2%	0.0%	2.7%	5.4%	0.0%
Affects the quality of milk	5	5	0	6.0%	12.2%	0.0%	2.7%	5.4%	0.0%
Kills animals	3	3	0	3.6%	7.3%	0.0%	1.6%	3.2%	0.0%
Poisonous	3	0	3	3.6%	0.0%	7.0%	1.6%	0.0%	3.3%
Causes aflotoxin contamination ir	2	2	0	2.4%	4.9%	0.0%	1.1%	2.2%	0.0%
Don't know	2	2	0	2.4%	4.9%	0.0%	1.1%	2.2%	0.0%
Spoilt milk	2	0	2	2.4%	0.0%	4.7%	1.1%	0.0%	2.2%
Stomach related problems in anin	2	2	0	2.4%	4.9%	0.0%	1.1%	2.2%	0.0%
Stomach upset	2	0	2	2.4%	0.0%	4.7%	1.1%	0.0%	2.2%
Loss of appetite	2	0	2	2.4%	0.0%	4.7%	1.1%	0.0%	2.2%
Causes aflotoxin contamination ir	1	0	1	1.2%	0.0%	2.3%	0.5%	0.0%	1.1%
Causes emaciation	1	0	1	1.2%	0.0%	2.3%	0.5%	0.0%	1.1%
Causes diarrhea in animals	1	1	0	1.2%	2.4%	0.0%	0.5%	1.1%	0.0%
Fungal infection	1	0	1	1.2%	0.0%	2.3%	0.5%	0.0%	1.1%
Total	122	64	58						
Individuals answering	84	41	43						

1.6.3 Have you heard of aflatoxins _____ [yes] [no]

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Yes	149	83	66	81.9%	89.2%	74.2%	81.0%	89.2%	72.5%
No	33	10	23	18.1%	10.8%	25.8%	17.9%	10.8%	25.3%
Total	182	93	89						
Individuals answering	182	93	89						

1.6.4 If yes, what are they?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Poison	32	18	14	20.9%	21.7%	20.0%	17.4%	19.4%	15.4%
Caused by mouldy feeds/grain	28	18	10	18.3%	21.7%	14.3%	15.2%	19.4%	11.0%
Caused by dampness of feeds/gr	26	23	3	17.0%	27.7%	4.3%	14.1%	24.7%	3.3%
Diseases	18	12	6	11.8%	14.5%	8.6%	9.8%	12.9%	6.6%
Poinson in feeds/grain	17	8	9	11.1%	9.6%	12.9%	9.2%	8.6%	9.9%
Don't know	16	6	10	10.5%	7.2%	14.3%	8.7%	6.5%	11.0%
Mould in feeds/grains	16	12	4	10.5%	14.5%	5.7%	8.7%	12.9%	4.4%
Causes diseases	11	6	5	7.2%	7.2%	7.1%	6.0%	6.5%	5.5%
Rottening of feeds/grains	9	6	3	5.9%	7.2%	4.3%	4.9%	6.5%	3.3%
Causes diseases in animals	7	4	3	4.6%	4.8%	4.3%	3.8%	4.3%	3.3%
Caused by improper storage of f	7	3	4	4.6%	3.6%	5.7%	3.8%	3.2%	4.4%
Poison in feeds/grain	7	4	3	4.6%	4.8%	4.3%	3.8%	4.3%	3.3%
Spoilage	6	1	5	3.9%	1.2%	7.1%	3.3%	1.1%	5.5%
Bacteria	5	2	3	3.3%	2.4%	4.3%	2.7%	2.2%	3.3%
Contmination in milk	5	0	5	3.3%	0.0%	7.1%	2.7%	0.0%	5.5%
Substance in mouldy feeds/grair	5	5	0	3.3%	6.0%	0.0%	2.7%	5.4%	0.0%
Caused by rotten/spoilt feeds/gr	4	4	0	2.6%	4.8%	0.0%	2.2%	4.3%	0.0%
Caused by poor hygene	3	0	3	2.0%	0.0%	4.3%	1.6%	0.0%	3.3%
From feed/grain	3	0	3	2.0%	0.0%	4.3%	1.6%	0.0%	3.3%
Substance in spoilt feeds/grains	3	3	0	2.0%	3.6%	0.0%	1.6%	3.2%	0.0%
Caused by mouldy of feeds/grair	2	0	2	1.3%	0.0%	2.9%	1.1%	0.0%	2.2%
Caused by bacteria	2	0	2	1.3%	0.0%	2.9%	1.1%	0.0%	2.2%
Kills animals	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
A disease that makes a cow not t	1	0	1	0.7%	0.0%	1.4%	0.5%	0.0%	1.1%
Bacteria in feeds/grains	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
Bacteria in milk	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
Cause cancer	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
Caused by poor hygene feeds/gr	1	0	1	0.7%	0.0%	1.4%	0.5%	0.0%	1.1%
Caused by poor hygene in milk	1	0	1	0.7%	0.0%	1.4%	0.5%	0.0%	1.1%
Color change of feeds/grain	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
Contmination in milk feeds/grair	1	0	1	0.7%	0.0%	1.4%	0.5%	0.0%	1.1%
Germs	1	0	1	0.7%	0.0%	1.4%	0.5%	0.0%	1.1%
Mouldy poison	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
Poisonous moulds	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
Milk quality	1	1	0	0.7%	1.2%	0.0%	0.5%	1.1%	0.0%
Caused by bad ventilation of fee	1	0	1	0.7%	0.0%	1.4%	0.5%	0.0%	1.1%
Total	246	143	103						
Individuals answering	153	83	70						

1.6.7a What **can** a farmer do to reduce **mould** in his or her farm?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Store feeds in a dry place	63	54	9	34.8%	59.3%	10.0%	34.2%	58.1%	9.9%
Store feeds that are dry	54	29	25	29.8%	31.9%	27.8%	29.3%	31.2%	27.5%
Store feeds in a ventilated place	26	15	11	14.4%	16.5%	12.2%	14.1%	16.1%	12.1%
Store feeds in a raised place	19	14	5	10.5%	15.4%	5.6%	10.3%	15.1%	5.5%
Store feeds in proper storage	19	6	13	10.5%	6.6%	14.4%	10.3%	6.5%	14.3%
Store feeds for shorter periods	12	3	9	6.6%	3.3%	10.0%	6.5%	3.2%	9.9%
Monitor feeds	6	0	6	3.3%	0.0%	6.7%	3.3%	0.0%	6.6%
Don't know	5	0	5	2.8%	0.0%	5.6%	2.7%	0.0%	5.5%
Maintain hygiene in storage facil	5	0	5	2.8%	0.0%	5.6%	2.7%	0.0%	5.5%
Add pesticides	4	1	3	2.2%	1.1%	3.3%	2.2%	1.1%	3.3%
Store feeds in a protected place	4	2	2	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%
Add aflatoxin binder to feeds	3	2	1	1.7%	2.2%	1.1%	1.6%	2.2%	1.1%
Use a veterinary	3	0	3	1.7%	0.0%	3.3%	1.6%	0.0%	3.3%
Avoid using grass	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Don't feed mouldy feeds	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Good maintnance	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Practice silage making	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Proper feed management	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Use nappier grass instead of gra:	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Use fermented feeds	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Put manure on farm	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Store feeds in a cool place	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Monitor the storage	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Sprinkle water on feeds	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Timely harvesting	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Store feeds not too close to a wa	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Total	236	132	104						
Individuals answering	181	91	90						

1.6.8a What **can** a farmer do to reduce **aflatoxins** in the milk produced by his or her cows?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Don't feed contaminated feeds	41	34	7	24.7%	44.2%	7.9%	22.3%	36.6%	7.7%
Don't know	38	4	34	22.9%	5.2%	38.2%	20.7%	4.3%	37.4%
Don't feed mouldy feeds	24	19	5	14.5%	24.7%	5.6%	13.0%	20.4%	5.5%
Add aflatoxin binder to feeds	10	9	1	6.0%	11.7%	1.1%	5.4%	9.7%	1.1%
Store feeds for shorter periods	9	0	9	5.4%	0.0%	10.1%	4.9%	0.0%	9.9%
Give feed that has been properly	7	7	0	4.2%	9.1%	0.0%	3.8%	7.5%	0.0%
Store feeds that are dry	6	0	6	3.6%	0.0%	6.7%	3.3%	0.0%	6.6%
Use a veterinary	6	0	6	3.6%	0.0%	6.7%	3.3%	0.0%	6.6%
Dry feed	6	6	0	3.6%	7.8%	0.0%	3.3%	6.5%	0.0%
Use Novasil binders in feed	6	0	6	3.6%	0.0%	6.7%	3.3%	0.0%	6.6%
Maintain hygiene	5	1	4	3.0%	1.3%	4.5%	2.7%	1.1%	4.4%
Maintain hygiene in storage facil	4	1	3	2.4%	1.3%	3.4%	2.2%	1.1%	3.3%
Monitor feeds	4	1	3	2.4%	1.3%	3.4%	2.2%	1.1%	3.3%
Store feeds in a dry place	4	0	4	2.4%	0.0%	4.5%	2.2%	0.0%	4.4%
Clean milk production	4	0	4	2.4%	0.0%	4.5%	2.2%	0.0%	4.4%
Store feeds in a ventilated place	3	0	3	1.8%	0.0%	3.4%	1.6%	0.0%	3.3%
Store feeds in proper storage	2	0	2	1.2%	0.0%	2.2%	1.1%	0.0%	2.2%
Call a veterinary for checks ofter	2	2	0	1.2%	2.6%	0.0%	1.1%	2.2%	0.0%
Proper feed management	1	1	0	0.6%	1.3%	0.0%	0.5%	1.1%	0.0%
Use nappier grass instead of gra:	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Give feed that had been stored i	1	1	0	0.6%	1.3%	0.0%	0.5%	1.1%	0.0%
I didn't know it can be found in r	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Keep feeds safe and fresh	1	1	0	0.6%	1.3%	0.0%	0.5%	1.1%	0.0%
Take care of feeds	1	1	0	0.6%	1.3%	0.0%	0.5%	1.1%	0.0%
Choose the proper feeds supplie	1	1	0	0.6%	1.3%	0.0%	0.5%	1.1%	0.0%
Total	188	89	99						
Individuals answering	166	77	89						

1.6.7.b Is there any particular thing you **do today** or you have been doing on your farm to reduce **mould** growth in feed ?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Store feeds in a dry place	50	44	6	28.6%	50.0%	6.9%	27.2%	47.3%	6.6%
Store feeds that are dry	33	22	11	18.9%	25.0%	12.6%	17.9%	23.7%	12.1%
Store feeds in a raised place	26	19	7	14.9%	21.6%	8.0%	14.1%	20.4%	7.7%
Store feeds for shorter periods	22	7	15	12.6%	8.0%	17.2%	12.0%	7.5%	16.5%
Store feeds in proper storage	20	16	4	11.4%	18.2%	4.6%	10.9%	17.2%	4.4%
Nothing	20	0	20	11.4%	0.0%	23.0%	10.9%	0.0%	22.0%
Store feeds in a ventilated place	12	6	6	6.9%	6.8%	6.9%	6.5%	6.5%	6.6%
Maintain hygiene	11	1	10	6.3%	1.1%	11.5%	6.0%	1.1%	11.0%
Store feeds in a protected place	8	8	0	4.6%	9.1%	0.0%	4.3%	8.6%	0.0%
Don't know	6	1	5	3.4%	1.1%	5.7%	3.3%	1.1%	5.5%
Add aflatoxin binder to feeds	3	2	1	1.7%	2.3%	1.1%	1.6%	2.2%	1.1%
Maintain hygiene in storage facil	3	1	2	1.7%	1.1%	2.3%	1.6%	1.1%	2.2%
Don't feed mouldy feeds	2	0	2	1.1%	0.0%	2.3%	1.1%	0.0%	2.2%
Monitor feeds	2	0	2	1.1%	0.0%	2.3%	1.1%	0.0%	2.2%
Monitor the storage	2	2	0	1.1%	2.3%	0.0%	1.1%	2.2%	0.0%
Use feeds directly without storin	2	0	2	1.1%	0.0%	2.3%	1.1%	0.0%	2.2%
Add fertilizer	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Give properly dried feeds to anir	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Store feeds for longer periods tc	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Fresh water for animals	1	0	1	0.6%	0.0%	1.1%	0.5%	0.0%	1.1%
Store feeds in a god temerature	1	1	0	0.6%	1.1%	0.0%	0.5%	1.1%	0.0%
Total	227	130	97						
Individuals answering	175	88	87						

1.6.8.b Is there any particular thing you **do today** you or have been doing on your farm to reduce **aflatoxins** contamination in feed?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Nothing	40	7	33	24.7%	9.2%	38.4%	21.7%	7.5%	36.3%
Store feeds in proper storage	19	12	7	11.7%	15.8%	8.1%	10.3%	12.9%	7.7%
Don't feed contaminated feeds	19	13	6	11.7%	17.1%	7.0%	10.3%	14.0%	6.6%
Don't know	16	1	15	9.9%	1.3%	17.4%	8.7%	1.1%	16.5%
Add aflatoxin binder to feeds	14	13	1	8.6%	17.1%	1.2%	7.6%	14.0%	1.1%
Don't feed mouldy feeds	11	11	0	6.8%	14.5%	0.0%	6.0%	11.8%	0.0%
Store feeds in a dry place	11	9	2	6.8%	11.8%	2.3%	6.0%	9.7%	2.2%
Store feeds for shorter periods	10	1	9	6.2%	1.3%	10.5%	5.4%	1.1%	9.9%
Store feeds that are dry	10	4	6	6.2%	5.3%	7.0%	5.4%	4.3%	6.6%
Monitor the storage	8	7	1	4.9%	9.2%	1.2%	4.3%	7.5%	1.1%
Maintain hygiene	5	0	5	3.1%	0.0%	5.8%	2.7%	0.0%	5.5%
Don't store contaminated feeds	5	5	0	3.1%	6.6%	0.0%	2.7%	5.4%	0.0%
Use Novasil binders in feed	3	0	3	1.9%	0.0%	3.5%	1.6%	0.0%	3.3%
Store feeds in a raised place	2	0	2	1.2%	0.0%	2.3%	1.1%	0.0%	2.2%
Maintain hygiene in storage facil	1	0	1	0.6%	0.0%	1.2%	0.5%	0.0%	1.1%
Monitor feeds	1	0	1	0.6%	0.0%	1.2%	0.5%	0.0%	1.1%
Store feeds in a protected place	1	1	0	0.6%	1.3%	0.0%	0.5%	1.1%	0.0%
Store feeds in a ventilated place	1	0	1	0.6%	0.0%	1.2%	0.5%	0.0%	1.1%
Total	177	84	93						
Individuals answering	162	76	86						

1.6.9 If there was a special way to ferment feed to prevent aflatoxins, by adding a yeast, would you do this and feed the cows?

	Number of answers			Share of individuals answered %			Share of individuals questioned %		
Answer	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu	Total	Kasarani	Kisumu
Yes	166	82	84	95.4%	97.6%	93.3%	90.2%	88.2%	92.3%
No	8	2	6	4.6%	2.4%	6.7%	4.3%	2.2%	6.6%
Total	174	84	90						
Individuals answering	174	84	90						