Biochem. Cell. Arch. Vol. 19, No. 1, pp. 223-228, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

INVESTIGATION OF DNA DAMAGE AND SOME BIOCHEMICAL PARAMETERS ON ROSS BROILER FEEDING ON GUAR MEAL, SALINOMYCINE AND MYCOFIXE

Bushra M. W. AL-Oubaidi[1] and Mashaan A. AL–Zuhairi[2]

[1]College of Veterinary Medicine, University of Fallujah, Iraq.
[2]College of Veterinary Medicine, University of Baghdad, Iraq
e-mail : bushra.mohammed@uofallujah.edu.iq, khalid.rafea@yahoo.com

(Accepted 14 February 2019)

ABSTRACT : Target of this study to explained the influence of adding gaur meal 10% as partial replacements of soybean meal, salinomycine and mycofix to the diet of Ross broiler chicken on cytogentic affect and some biochemical parameters by measures catalase and peroxinitrate at 42 days. The experiment was conducted on 150 birds in one day divided to five groups, each groups include 30 birds with two replicate (15 birds) in each replicate, fed on diet contain : Ross: T1 control (1-42 day), T2 Guar meal 10% (1-42 day), T3- Salinomycin 500g\ton (1-42 day), T4- Salinomycin 500g\ton & gaur meal 10% (1-42 day) and T5- Salinomysine 500g\ ton & mycofix 2kg\ton & gaur meal 10% (1-42). Results demonstrated that 2nd group recorded maximum significant ($p \leq 0.05$) differences compared to other nourished groups in all comet assay parameters (% DNA in head, Tail length (px), % DNA in tail, tail moment and olive moment) compared with other groups feeding on salinomycine, mycofix (T3, T4, T5) regressed lowest value of all comet assay parameters, also T1, T2 recorded a significant ($p \leq 0.05$) decrement in catalase value compared with other studied groups. T1, T2 showed highest significant ($p \leq 0.05$) differences in peroxnitrate compared to other groups, which recorded increments significant ($p \leq 0.05$) differences. The addition of salinomycine at 500gm / ton and maycofix® at 2 Kg /ton to Ross broiler diet has an adverse effect on blood lymphocyte, catalase and peroxinitrate while supplementing guar meal at level 10%, which protect lymphocyte from damage as shown in comet assay, catalase enzyme, peroxynitrate.

Key words : Guar meal, Mycofix, salinomycin, comet assay, catalase and peroxinitrate.

INTRODUCTION

As a primary source of animal protein, the poultry section offers a valuable repository to bridge the distance between demand and the availability of balanced nutrition (Ravindran, 2013). The production of low quality feed has make variety of problems for the broiler industry resulting in weak performance and little returns (Kleyn and Chrystal, 2008). To enquiry available balance among the cost of diet, the broiler specification and quality of product, important additives are can be obtained in the market to be included in broiler share like anticoccidial, antioxidants, anticoccidialantimicrobials, toxin binder like mycofixe, pH control agents, enzymes and Phytogenic (Hashemi and Davoodi, 2010). Some of these additives are recommended for chemotherapeutic and prophylactic goal while others are reputed for the growth promoting effect like coccidiostats, toxin binder like mycofixe (Angelakis *et al*, 2013). Salinomycin is polyether ionophoric coccidiostat, which is broad used as a supplement in poultry diet to control with coccidia infection (Bolder *et al*, 1999). Contamination of poultry nutrition with mycotoxins is one of the important problems closed with feeding of poultry. Mycotoxins are the toxic metabolites production by a certain naturally growing fungi on animal feed, feed ingredients and other agricultural crop (Manafi *et al*, 2011). Mycofix® was effective way used for poultry to neutralized of ochratoxicosis and aflatoxin due to its dual actions, that are mode of adsorption of mycotoxins with appropriate located polar functional groups like aflatoxins by selective blend of minerals (Zahir, 2005). Guar (*Gyamopsistetra gonoloba*) is a drought resistant annual legume prominently produced in India and Pakistanthe plant is planting for its galactomannan ((Mudgil *et al*, 2014). Guar meal is a comparatively inexpensive high protein content of approximately 380 g/kg (Mishra *et al*, 2013) the minimum crude protein percentage of guar meal is rated at 50% compared to 48% of soy bean meal. Its crude fiber at 6.8% maximum, while that of soybean meal is at 3%; it has a minimum crude fat content of 5% versus 1%

of soybean meal and has a higher protein solubility of 89% than soybean meal with 78% (Mathur, 1989). Guar meal (GM) have energy, protein, enzyme, methionine and phosphorus in huge levels than that in soybean meal as a fractional replacement (<10%) of soybean meal (SBM) in poultryor domestic animal improve the economic plane for minimizing feed costs without any bad effects on production (Karman *et al*, 2002). Commercial poultry production is associated with different fatigue and stresses condition that affect on output and reproductive charectrctes of rising chicks, breeders and commercial layers. Oxidative stress is caused by imbalanced free radicals (FR), ROS which injury DNA, bio membrane, proteins, lipids and other macromolecules (Lu *et al*, 2010). Dietary compound have been shown to affect DNA methylation or histone modification by numerous pathways (Stefanska *et al*, 2012). So this experiment carried out to study the influence of guar meal with or without salinomycine and mycofixe on cytogenetic by using comet assay technique and biochemical performance of Ross broiler.

MATERIALS AND METHODS

This study was conducted in the poultry farm in the Agricultural ministry\ circle Agricultural Research / Baghdad, from 27/3/2016 to11/5/2016 (1-42) days of ages. The experiment was conducted on 150 birds Ross of one day old chicks. On arrival, chicks were weighed and randomly distributed into wood shavings covered floor pen then divided into 5 experimental sub groups 30 chicks in each. Each subgroup composed of two replicate pens with 15 chicks.

Dietary treatment

T1 control, T2 Guar meal 10%, T3- Salinomycin 500g\ ton-(1-42 day), T4- Salinomycin 500g\ton & gaur meal 10% (1-42 day) and T5- Salinomysine 500g\ ton & mycofix 2kg\ton &gaur meal 10% (1-42). The formulas and calculated nutrient of the basal diet are presented in Table 2. The feeding and water provided in ad libitum for the study. The diets were composition to complete requirement by the National Research Council (NRC, 1994) for broiler. Vaccination program /all vaccines were placed in free chlorine water after boiling and then cooling it one day before vaccination are presented in Table 1.

Blood collection

At day 42th of age, blood samples were collected from 4 birds from each group from the wing vein in a test tube without anticoagulant for biochemical and with coagulant for cytogenetic parameters according to Parasuraman (2010). The serum was separated by centrifugation for 10 minutes at 3000 rpm and stored in a deep freeze (-20) until analysis.

Table 1 : Vaccination program.

Age of chicks (by days)	Types of the vaccine	Rout of administration
1	Newcastle (single oil)	Injections in back of neck
7	Newcastle (lasota)	Drinking water
14	Infectious bursar disease	Drinking water
17	Newcastle (lasota)	Drinking water
27	Newcastle (lasota)	Drinking water

Biochemical parameters

A. Determination of catalase activity

The examination is detected with the enzymatic hydrolysis of H_2O_2 by CAT. H_2O_2 can be size at a colorimetric readout at 240 nm. Kits from Cohesion companyfrom Chinese

B. Determination of peroxynitrate level

The method was described by Beckman *et al* (1992), cited by Van Uffelen *et al* (1998). Kit from Bio Assay (USA).

Cytogenetic parameters

Evalution of DNA damage using single – cell gel electrophoresis (comet assay)

Procedure for SCGE, involving sample collection, lymphocyte separation, slide preparation, cell lysis, electrophoresis and neutralization is performed on Day I followed by fixation, staining and microscopy on Day II. The comet assay was performed under alkaline condition. Essentially according to the procedure described by Singh *et al* (1988) with a slight modification (Avishai *et al*, 2003). The slides stained with ethidiumbromide are observed under a bright-field light microscope and captured using CCD camera. Thus, captured images can be analyzed using commercially available software. Images of 100 randomly selected cell (50 cell from each of two replicated slides) were analyzed. Measurements of DNA density were performed using image analysis (comet score™) for slide detection used virtual lens(x100) and (x40), zoom lens (x1.9) to detected comet length (px), % DNA in head, tail length (px), %DNA in tail, tail moment and olive moment.

Statistical analysis

Data obtained were subjected by using analysis of variation ANOVA. Least significant difference (LSD) among different groups at 5% level was applied (Snedecor and Coehran, 1980).

Table 2 : The ingredients and chemical composition of diet used in experiment (starter, grower, finisher).

Ingredients (%)	Starter (1-11 day)		Grower (12-24 day)		Finisher (23-42 day)	
	GM(0)	GM(10%)	GM(0)	GM(10%)	GM (0)	GM (10%)
Corn	49.09	49.07	47.29	51.5	46.22	55
Soybean meal	35	24	31	21	27	17
Wheat	10	10	15.5	11	20	11
Oil	2	2.8	2.6	3	3.2	3.5
Premixes	2.5	2.5	2.5	2.5	2.5	2.5
Di calicium	0.5	0.6	0.4	0.4	0.3	0.4
Limmestone	0.8	0.8	0.6	0.6	0.6	0.6
Methionine	0.11	0.11	0.11	-	0.18	-
Lysine	-	0.12	-	-	-	-
Salt	-	-	–	-	-	-
Guar meal	-	10	-	10	-	10
Total weight (kg)	100	100	100	100	100	100
Total crude protein (%)	22.1	22	20.7	20.9	19.3	19.4
Fiber (%)	2.74	3.52	2.71	3.48	2.67	3.4
Fat (%)	4.65	5.55	5.09	5.84	5.73	6.4
Methionine + cyctine	1.03	1.21	0.99	1.08	1.02	1.03
Caicium	1.03	1.13	0.89	0.98	0.85	0.16
Phosphors	0.48	0.48	0.49	0.47	0.49	0.46
Methionine	0.66	0.88	0.64	0.76	0.68	0.74
Cyctine	0.37	0.33	0.35	0.32	0.33	0.29
Lysine	1.40	1.34	1.29	1.26	1.19	1.14
Total metabolizable energy (kcal/kg)	3029	3035	30.96	30.92	31.57	3157

RESULTS

As shown in Table 3 catalase values recorded a significant ($p \leq 0.05$) decrement in the first, 2nd groups compared to other studied groups which recorded significant ($p \leq 0.05$) increment. Groups 1, 2 showed highest significant ($p \leq 0.05$) differences in peroxnitrate compared to other groups, which recorded increments significant ($p \leq 0.05$) differences.

The means of comet assay parameters were listed in Table 4. Results demonstrated that 2nd recorded maximum significant ($p \leq 0.05$) differences compared to other nourished groups. Also the results revealed decrements values of all comet assay parameters in the others. There were no significant ($p > 0.05$) differences between some groups such as (1, 2) in %DNA in head, Tail length, % DNA in Tail and Tailmoment. The values in the Table 5 recorded lowest charge in comet length, Tail length, % DNA in Tail and Tail moment in groups 1, 2. Also regressed superior values in % DNA in head (99.440± 0.1568, 99.780 ±0.0489, respectively), while other groups (3,4,5) regressed superior values in % DNA in tail (36.620±1.500, 7.3200±3.43, 19.6400±6648, respectively) with lowest value of %DNA in head. Figs. 1, 2(A,B) represented normal lymphocyte (undamaged). Figs. 3(A,B), 4(A,B), 5(A,B) represented comet image showing migration of DNA damaged from embedded nuclei with head (undamaged DNA) and tail (damaged DNA) [Fig. 6(A, B, C, D, E). Measurements DNA damage, where (Comet length, %DNA in Head, Tail length, %DNA in Tail, Tail moment) in lymphocyte of Ross broiler nourished on different types of diet for 42 days of age (Table 4).

DISCUSSION

Catalase and peroxynitrate values showed a significant ($p \leq 0.05$) decrement in 2nd group compared to other studied groups. While other treatments recorded significant ($p \leq 0.05$) increment value in the end of studied. Treating broiler chicks with Guar meal for 6 weeks may be prevented the birds from oxidative stress by remaining the activities of enzymes such as catalase within normal value and peroxynitrate at lowest value compared with other nourished groups in the present study. Guar meal have chemical compound like flavonoids and tannins(Mukhtar *et al*, 2006). Studies have shown that chemical compound have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Ruberto *et al*, 2007). Continuous use of salinomycin may deactivated the physiological process of kidneys and liver because it causes metabolic disorded of ions within the tissues of the host animals or to oxidative

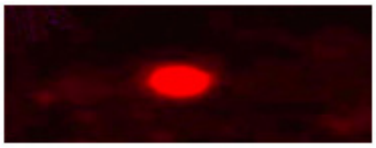
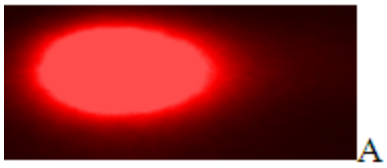


Fig. 1 : T1.



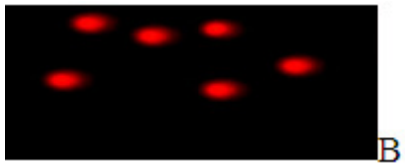
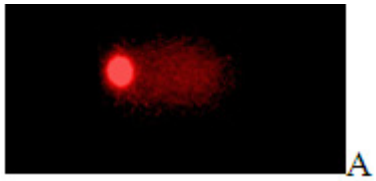


Fig. 2 : A(x190), B(x76).(T2).



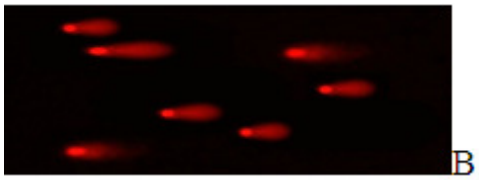
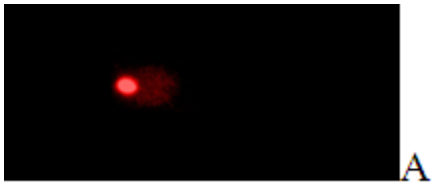


Fig. 3 : A(x190), B (x76). (T3).



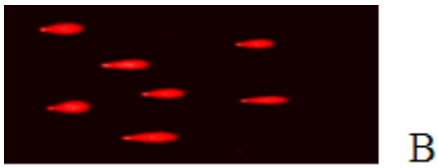
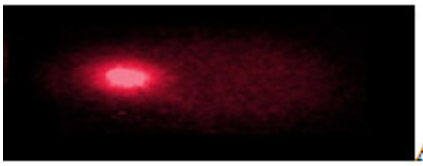


Fig. 4 : A (x190), B (x76). (T4).



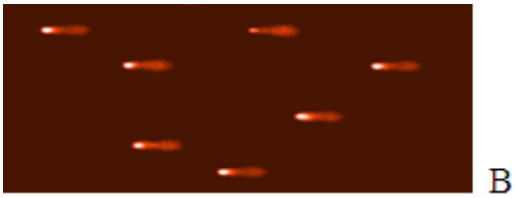


Fig. 5 : A (x190), B (x76). (T5).

damage. This oxidative damage generating free radicals and other cytotoxic chemical like Peroxynitrite (Kamashi *et al*, 2004). So this may be explained the reason of elevated value of Peroxynitrite in groups feed on salinomycin only and salinomycin&mycofix plus. Some workers (Van Vleet *et al*, 1983) proposed that toxicity of the drugs ionophores for myocardial tissue and probably also skeletal muscle, could be explained by excessive increases of intracellular Ca^{2+}, which exceeded the ability of cellular components such as mitochondria to sequester Ca^{2+} effectively. Subsequently, the Ca^{2+} overloaded cell would develop a series of degradative alterations with subsequent membrane damage followed by swelling of the whole cell. This reason may be explained the effect of adding salinomycine to the diet of broiler on DNA damage by causes degradative then damage to cell membrane, as well as structural tissue damage. They are genotoxic, not easily metabolised compounds, which are considered capable of irreversibly initiating the carcinogenic process even in small residue concentrations (Mei and Nan, 2010). Antioxidant enzyme like catalase was important for adaptation of cells to oxidative stress and preserved cells via degradation of the reactive hydrogen peroxide (Koinarski *et al*, 2005). The obtained result in present study recorded significant increments of peroxynitrite (ONOO-) in groups feed on salinomycin and mycofix®, and this peroxynitrite consider a strong oxidant, which leads to the formation of reactive intermediates due to spontaneous decomposition (Kontos, 2001). Really ONOO- reported damage or injured a wide diversity of bio molecules, including proteins (via nitration of tyrosine or tryptophan residues or oxidation of methionine or selenocysteine residues), DNA and lipids (Mruk *et al*, 2002). Chronic oxidative stress like continuous use of drug has long been associated with decreased longevity in animals (Salmon *et al*, 2010), then leads to formation of different oxidative DNA lesions, which can cause mutations (Young and Woodside, 2001). The result of

Table 3 : Effect of different types of diets on catalase (U/ ml) and peroxnitrate (µ mol/L) of Ross broiler (means ± SE) at 42 days of age, n = 4.

Catalase (U/ML)	T1	T2	T3	T4	T5	LSD
	1.863 ±0.015 D	1.636 ±0.07 E	2.518 ±0.06 C	2.827 ±0.014 B	3.25 ± 0.015 A	0.08
Peroxnitrate(µ mol/L)	1.287 ±0.0223 E	2.309 ±0.109 D	5.354 ±0.097 A	4.25 ±0.062 C	5.095 ±0.067 B	0.15

Different capital letters denoted significant (p≤ 0.05) differences among groups.

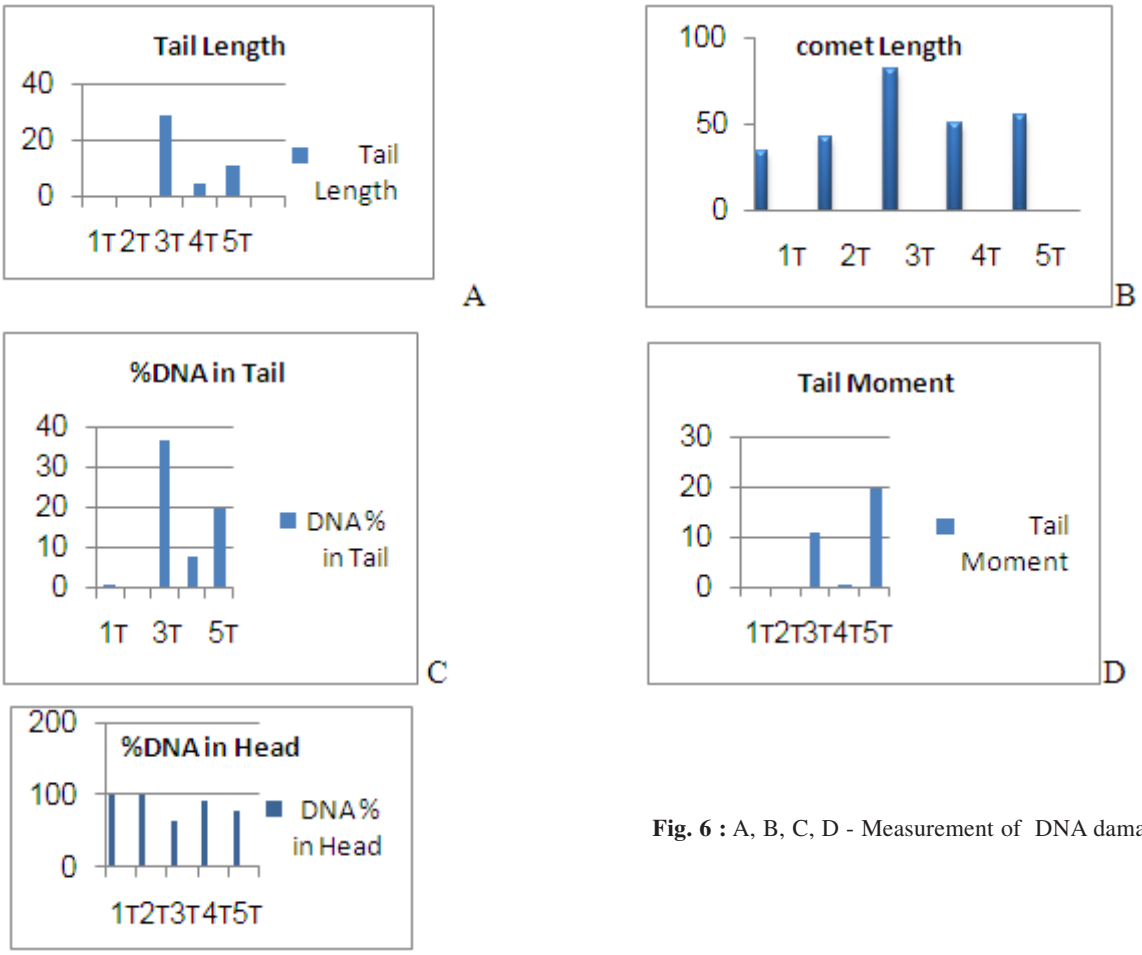


Fig. 6 : A, B, C, D - Measurement of DNA damage.

Table 4 : Effect of different types of diets on Comet assay (px) of Ross broiler sources (means ± SE), n = 4. Different capital letters denoted significant (p≤ 0.05) differences.

Parameters	Control T1	Gaur meal 10% T2	Salinomycine 500 gm/ton T3	Gaur meal 10% & Salinomycine 500 gm/ton T4	Gaur meal 10% & Salinomycine 500 gm/ton & mycofixe 2 kg /ton T5
%DNA in Head LSD=5.3	99.440 ± 0.1568A	99.780 ±0.0489A	63.2800 ± 1.500D	92.580 ± 3.4377B	78.2600 ± 5.4830 C
Tail length LSD=5.6	0.4600 ± 0.1568D	0.8000 ±0.583D	29.000 ± 1.923A	5.000 ± 1.8708C	11.4000 ± 2.8913Bb
% DNA in Tail LSD=4	0.6000 ± 0.6000D	0.0400 ±0.0400D	36.620 ± 1.500A	7.3200 ± 3.4377C	19.6400 ± 6.648B
Tail Moment LSD=0.5	0.00±0.0D	0.00±0.00D	10.680 ± 1.068A	0.5800 ± 0.3852C	2.9800 ± 1.3488B

present study is similar to the finding by Dar-ChihKuo *et al* (2007), which implicate that PHGG obtained from guar gum may have a direct antioxidant activity against oxidative stress and results found that PHGG is efficient to scavenge O_2^{-}•, H_2O_2 and HOCl. Plant derived antioxidants are gaining more demand in poultry nutrition because their meat has high content of polyunsaturated fatty acids and susceptible to lipid oxidation (Christaki, 2012). So, guar meal may be supported the machinery of repair or act as damage tolerance that counteracts DNA damage.

CONCLUSION

The addition of salinomycine at 500gm/ton and maycofix® at 2 Kg/ton to Ross broiler diet has genotoxic affect on blood lymphocyte and depressed health status as shown in comet assay, catalase enzyme, peroxynitrate. Supplementing guar meal in broiler diet at level 10% as a partial replacement of soybean, which improved broiler health status as shown in comet assay, catalase enzyme, peroxynitrate. Aadding guar meal at 10% with

salinomycine and with mycofix and salinomycin act as a meliorate or neutralizing a negative impact of these drugs on broiler performance and lead to decreased the geanotoxic effect on lymphocyte blood chicken by decrease level of Comet Assay parameters.

REFERENCES

Angelakis E, Merhej V and Raoult D (2013) Related actions of probiotics and antibiotics on gut microbiota and weight modification. *Lancet Infect. Dis*. **13**, 889-899.

Avishai N, Rabinowitz C and Rinkevich B (2003) Use of the comet assay for studying environmental genotoxiciyt : comparison between visual and image analysis. *Environ. Mol. Mutagen*. **42**, 155-165.

Bolder N M, Wagenaar J A, Putirulan F F, Veldman K T and Sommer M (1999) Environment and Health / The effect of flavophospholipol (flavocin) and salinomycin sodium (sacox) on the excretion of *Clostridium perfringens*, *Salmonella enteritidis* and *Campylobacter jejuni* in broilers after experimental infection. *Poult Sci.* **78**, 1681–1689.

Christaki E, Bonos E, Giannenas I and Florou-Paneri P (2012) Evaluation of oregano and α-tocopheryl acetate on laying Japanese quail diets. *J. Basic. Appl Sci*. **8**, 238-242.

Dar-Chih K, Shih-Ping H and Chiang-Ting C (2009) Partially hydrolyzed guar gum supplement reduces high-fat diet increased blood lipids and oxidative stress and ameliorates $FeCl_3$-induced acute arterial injury in hamsters. *J. Biomed Sci*. **16**(1), 15.

Hashemi S R and Davoodi H (2010) Phytogenics as New Class of Feed Additive in Poultry Industry. *Journal of Animal and Veterinary Advances* **9**, 2295-2304.

Kamran M, Pasha T N, Mahmud A and Ali Z (2002) Effect of commercial enzyme (Natugrain) supplementation on the nutritive value and inclusion rate of guar meal in broiler rations. *International Journal of Poultry Science* **1**(6), 167–173.

Kamashi K, Reddy A G, Reddy K S and Reddy V R (2004) Evaluation of zinc against salinomycin toxicity in broilers. *Indian J. Physiol. Pharmacol*. **48**, 89-95.

Kleyn R and Chrystal P (2008) Feeding the young broiler chicken in practice: a review. 23rd World's Poultry Congress, Brisbane, Australia.

Koinarski V, Georgieva N, Gadjeva V and Petkov P (2005) Antioxidant status of broiler chickens, infected with Eimeriaacervulina Revue. *Méd.Vét*. **156**(10), 498-450.

Kontos H A (2001) Oxygen radicals in cerebral ischemia: the 2001 Willis lecture. *Stroke* **32**, 2712-2716.

Lu T, Piao X L, Zhang Q, Wang D, Piao X S and Kim S W (2010) Protective effects of Forsythia suspensa extract against oxidative stress induced by diquat in rats. *Food Chem. Toxicol*. **48**, 764.

Manafi M, Mohan K and Noor Ali M (2011) Effect of Ochratoxin A on Coccidiosis-Challenged Broiler Chicks.

Mathur O P and Mathur C S (1989) Feeding of protected protein and urea supplementation for enhanced growth and feed utilization in Magra lambs. *Indian J. Anim. Nutr*. **6**(3), 274-278.

Mei N (2010) Metabolism, Genotoxicity, and Carcinogenicity of Comfrey. *Journal of Toxicology and Environmental.*

Mishra A, Sarkar S K, Ray S and Haldar S (2013) Effects of partial replacement of soybean meal with roasted guar korma and supplementation of mannanase on performance and carcass traits of commercial broiler chickens. *Veterinary World* **6**(9), 693-697.

Mudgil D, Barak S and Khatkar B S (2014) Guar gum: processing, properties and food .Applications - A Review. *Journal of Food Science and Technology* **51**(3), 409-418.

Mukhtar H M, Ansari S H, Bhat Z A and Naved T (2006) Antihyperglycemic Activity of *Cyamopsistetra gonoloba* Beans on Blood Glucose Levels in Alloxan-Induced Diabetic Rats. *Pharm Biol*. **44**(1), 10-13.

Mruk D D, Silvestrini B, Myand M O and Cheng C Y (2002) Antioxidant superoxide dismutase - a review: its function, regulation in the testis and role in male fertility. *Contraception* **65**, 305-311.

NRC (1994) *Nutrient Requirements of Poultry*. 9th Edn., National Academy Press, Washington, Dc., USA., ISBN-13: 9780309048927.

Parasuraman S, Raveendran R and Kesavan R (2010) Blood sample collection in small laboratory animals. *J. Pharmacol Pharmacother.*

Ruberto G A, Renda C, Daquino V, Amico C, Spatafora C, Tringali and Tommasi N (2007) Polyphenols constituents and antioxidant activity of grape pomace from five Sicilian red grape cultivars. *Food Chem.* 100, 203-210.

Salmon A B, Richardson A and Perez V I (2010) Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? *Free Radic. Biol. Med*. **48**, 642–655.

Singh N P, Mccoy M T, Tice R R and Schneider E L (1988) A simple technique for quantitation of low levels of Dnd damage in individual cells. *Experimental Cell Research* **175**, 184-191

Snedecor G W and Cochran W G (1980) *Statistical methods*. Iowa State University Press, Iowa.

Stefanska B, Karlic H, Varga F, Fabianowska-Majewska K and Haslberger A G (2012) Epigenetic mechanisms in anti-cancer actions of bioactive food components – the implications in cancer prevention. *Br J Pharmacol*. **167**, 279–297.

Van Vleet J F and Ferr Ansans V J (1983) Congestive cardiomyopathy induced in ducklings fed graded amounts of furazolidone. *American J. Vet. Res*. **44**, 76-85.

Van Uffelen Be, Van der Zee J, de Kostes B M, Van Stereninck J and Elferink J G (1998) Intracellular but not extracellular conversion of nitroxyl anion into nitric oxide leads to stimulation of human neutrophil migration. *Biochem J*. **330**, 719-722.

Young I and Woodside J (2001) Antioxidants in health and disease. *J. Clinpathol*. **54**, 176–186.

Zahir A J B (2005) The use of some adsorbents in decreasing T-2 toxin effect on broiler health and production. *M.Sc. Thesis*.