Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 55 (1):180-197. Oct. 2017
DOI: 10.5455/ajvs.269041

Protective Effect of Chemical and Biological Mycotoxin Binder on Growth Performance, Serum Biochemistry and Carcass Traits in Broiler Chicks Fed on Aflatoxin Contaminated Diet

Mohamed I. El-Katcha[1], Mosad A. Soltan[1], Set A. El-Shobokshy[1], Ahmed Shokry[2]
[1]Nutrition and veterinary clinical nutrition Department, Faculty of Veterinary Medicine, Alexandria University, Egypt
[2]Veteriaian at General Authority of Veterinary Medicine

Key words:
Broiler chickens – Growth performance – Immune response – Aflatoxin B_1 – Chemical and Biological Mycotoxin Binder.

Correspondence to: *
seet1989@yahoo.com

ABSTRACT

A total of 210 one-day-old dual purpose chicks of mixed sex were used to determine the activity of the chemical (based on organic acids salt) or biological (containing live yeast, yeast cell wall and some enzymes) commercial mycotoxin binder and minerals and to evaluate the protective effects of the binder on performance, carcass quality, some blood biochemical changes and immune status of broilers fed with Aflatoxin B_1. The chicks were randomly allotted into 6 equal groups, the first group fed on the basal diet without any supplementation and considered as control, while groups 2–3 fed on the same basal diet with chemical and biological mycotoxin binder supplementation through drinking water respectively, while group 4–6 fed as mentioned for the first three groups with aflatoxin B_1 (AFB_1) contamination from 22th days of broiler age at a rate of 1mg of AFB_1/Kg diet. It was found that aflatoxin feed contamination without mycotoxin binder supplementation significantly ($P \leq 0.05$) reduced the growth performance and deteriorated the feed efficiency parameters compared with broiler chicken group fed on the same diet without aflatoxin B1 contamination. On the other hand, chemical or biological mycotoxin binder supplementation with aflatoxin B1 contamination counteract the deleterious effect of aflatoxin B1 and significantly ($P \leq 0.05$) improved the growth performance compared with broiler chicken fed on the contaminated diet without mycotoxin binder supplementation. Our data indicated that mycotoxin binder supplementation significantly ($P \leq 0.05$) improved the immune response via increase the serum proteins level and improvement the WBCs level especially lymphocytes and neutrophils, phagocytic activity and index, antibodies production and immune organs weight of broiler chicken compared with control group fed on the basal diet without mycotoxin binder supplementation. It can be concluded that biological binder more immune stimulant in broiler chicken compared with chemical mycotoxin binder source.

1. INTRODUCTION

Mycotoxins are the toxic metabolites produced by certain fungi. They are always a hazard to man and domestic animals and had come to public interest since the past 30 years. Among them, Aflatoxins are a class of potent mycotoxins produced mainly by Aspergillus flavus, Aspergillus parasiticus, and occasionally by other Aspergillus species (Smith et al., 1995). Aflatoxins constitute a great threat to the health of animals and humans due to their teratogenic, carcinogenic, mutagenic, and immunosuppressive effects (Guan et al., 2008 and Yunus et al., 2011). Additionally, in terms of the livestock industry, aflatoxins cause huge economic loss by retarding animal growth, increasing feed consumption, and reducing meat production (Fan et al., 2013 & Do and Choi, 2007). Among the various types of aflatoxins, aflatoxin B_1 (AFB_1) is known to be the most biologically active component.

The immune system in poultry is the first target to be influenced by mycotoxins. Immunosuppression can be observed in poultry ingesting aflatoxins at levels below those that cause over symptomatology, and explained, in part, by atrophy of the bursa of Fabricius, thymus, and spleen (Peir et al., 1972). The control of mycotoxicosis is based on preventing fungal development in the feedstuffs, and on detoxifying toxin contaminated feed. At present, unfortunately, aflatoxins are considered unavoidable contaminants of feed and foods. The Food and Agriculture Organization (FAO) estimates that at least 25% of world cereal production is contaminated with mycotoxins (Dowling, 1997). For this reason, developments of detoxification procedures are needed. Such detoxification procedures should not only reduce the concentration of toxins to "safe" levels (below regulatory limits), but also to prevent production of new toxic products derived from the aflatoxin degradation, and of course non-reduction of

the nutritional value of the treated commodities. A good toxin binder should restore the nutritional values of aflatoxin contaminated feeds. The quality of a toxin binder is expressed in four different parameters: binding capacity, absorption efficacy, activation time and inclusion rate (Van Kessel and Hiang Chek, 2004). A number of methods have been investigated in connection with their effectiveness to inactivate aflatoxins in contaminated feedstuffs; the aims of these methods are either to remove or to destroy the toxin, and can be classified into physical, biological and chemical methods.

Organic acid is considering one of the most widely-used food additives, which is commonly used as a preservative, acidulant, pH control agent, flavor enhancer, and antioxidant in many foods. Organic acid is one of the important organic acids, which is responsible for the characteristic odor and sour taste of vinegar with antibacterial and antifungal properties (Shakhashiri, 2008). Organic acids have been used to decrease the growth of harmful fungi and reduce secretion of aflatoxins. In addition, they have many properties on poultry health including good growth performance, decrease feed conversion ratios, and enhance hematological and pathological parameters through the detoxification of aflatoxin (Hassan et al., 2012 and Hassan et al., 2015).

Among mycotoxin binder, the use of biological methods, using microorganisms and their metabolites to eliminate aflatoxins, can be a highly promising approach owing to its specific, efficient, and environmentally sound detoxification (FAO, 2001). Some microbes, including fungal and bacterial isolates, such as Live yeast, Sacchromyces cerevisiae (Aravind et al., 2003), Flavobacterium aurantiacum (Line et al., 1994), Stenotrophomonas Maltophilia (Guan et al., 2008) Myxococcus fulvus (Zhao et al., 2011), and Aspergillus niger (Zhang et al., 2014) were reported to effectively biodegrade aflatoxins in vitro. However, little is known about their efficiency in the biodegradation of aflatoxins and effect on aflatoxicosis in vivo. As a biological product, several studies have revealed that esterified glucomannan derived from cell wall of Saccharomyces cerevisiae (Girish and *Smith*, 2008) have shown considerable promise in countering aflatoxins. Studies of using biological mycotoxin based on S. cerevisiae, showed that using that binder in contaminated feeds was responsible for reducing liver residual aflatoxin levels (Gargees and Shareef, 2009), and in ameliorating the negative effect of aflatoxins on Newcastle antibody production (Gargees and Shareef, 2008).

The aims of the current study were to examin the toxic effect of aflatoxins and protective efficacy of chemical (based on organic acids salt) or biological (containing live yeast, yeast cell wall and some enzymes) commercial mycotoxin binder on performance, carcass quality, some blood biochemical changes and immune status of broilers exposed to feed contaminated by Aflatoxin B_1.

2. MATERIALS AND METHODS

2.1. Birds used: A total of 210 one-day-old dual purpose chicks of mixed sex were used in this experiment. They were obtained from a local Egyptian private hatchery. The chicks were randomly allotted into 6 equal groups (35 chicks/group).

2.2. Accommodation and management: The broiler chicks were housed in a clean well-ventilated room previously disinfected by fumigation using formaldehyde gas produced by mixing formalin 40 % with potassium permanganate powder. The room was provided by gas heater in addition to electric lamps of 200 watt over each partition to obtain the suitable temperature needed for broiler chicks. The room floor was partitioned into 6 partitions. Each compartment was bedded by fresh clean wheat straw forming a deep litter of four centimeters depth. Each compartment was provided by suitable feeder and waterer. Prophylactic measures against the most common infectious diseases were carried out. The chicks were vaccinated against Newcastle disease using different types of Newcastle disease vaccines (Hitchner B1, Gumboro and lasota). After vaccination the broiler chicks received AD_3E vitamins (1 ml/L of drinking water) to improve vitality of chicks.

2.3. Experimental design and feeding program: Broiler chicken were fed on commercial starter, grower and finisher diets and considered as basal diet which composed from corn, soybean, corn gluten, vegetable oil, mineral and vitamin mixture at different percentage. Chemical analyses of the basal diets used in the experiment are presented in tables 1. Experimental period lasted for five continuous weeks. First group fed on the basal diet without any supplementation and considered as control, while groups 2 – 3 fed on the same basal diet with chemical and biological mycotoxin binder supplementation through drinking water respectively, while group 4 – 6 fed as mentioned for the first three groups with aflatoxin B1 (AFB1) contamination from 22^{th} days of broiler age at a rate of 2mg of AFB1/Kg diet. The applied experimental design is shown in table 2.

Table (1): Chemical analyses of the basal diets.

Items	Feed Type		
	Starter diet	**Grower diet**	Finisher diet
Moisture%	11.05	12.38	11.22
Crude protein%	22.56	20.37	19.08
Ether extract%	4.6	4.49	5.89
Crude fibre%	2.99	3.07	3.13
Ash%	6.45	5.98	6.03
NFE%*	52.35	53.71	54.65
Calcium%	1.11	1.07	0.86
Phosphorus%	0.71	0.69	0.78
ME Kcal/kg diet**	3069.6	3084.6	3178.9
Calorie/protein ratio***	136.1	151.4	166.6

* NFE= Nitrogen free extract (calculated by difference "100-(moisture% + CP% + EE% + CF% + ash%)". **Calculated according to Lodhi et al. (1976) as follows: Metabolizable energy MJ/Kg = 1.549+ (CP%*0.102) + (EE %*0.275) + (NFE%*0.148) + (CF%*0.034). ***Calorie/protein ratio = ME kcal/CP%

Table (2): The applied experimental design.

Group No.	Diet	Mycotoxin binder supplementation		AFB1 feed contamination
		Chemical[1] (1ml/L)	Biological[2] (0.5ml/L)	
1	Basal diet	--	--	--
2	""""	+	--	--
3	""""	--	+	--
4	""""	--	--	+
5	""""	+	--	+
6	""""	--	+	+

[1]Chemical mycotoxin binder (Detox) 2Biological mycotoxin binder (Ochra-mat)

2.4. Growth performance: Individual bird body weight at the beginning of the experiment was recorded. Body weight, weight gain and feed intake for each pen were recorded weekly; feed conversion ratio (FCR), protein efficiency ratio (PER), efficiency of energy utilization (EEU) and performance index (PI) were calculated.

2.5.Chemical composition:

Dry matter and crude nutrients: Analytical DM contents of feed samples were determined by oven-drying at 105ºC for 48 h **(**AOAC, 2000; method 930.15). Ash contents of feed and liver samples were determined by incineration at 550ºC overnight. Crude protein in feed samples was determined by using Kjeldahl method according to Randhir and Pradhan (1981) and ether extract was determined according to Bligh and Dyer (1959) technique as modified by Hanson and Olly (1963).

2.5.Analysis of AFB1 from ileum, cecal contents and liver tissue samples: Extraction of fecal samples was modified from a method developed by **(Mykkänen et al., 2005).** After collection, ingesta and liver samples were weighed and stored in plastic bags at -20ºC until analysis. Samples were mixed for 180 seconds (Stomacher 400 Laboratory Blender, GW Berg & Co, Vantaa, Finland) with 2.5 x volume of 0.2 M sodium acetate in 10% NaCl. Aliquots (2 ml) of the mixture were spiked with AFG2 (18.6 pmol/sample) as internal standard and centrifuged (3000×g, 15 min, 4ºC). Pellets were suspended in 4 ml 80% methanol (in 10% NaCl, v/v), vortexed and homogenized thoroughly (MICCRA D-8, ART Labortechnik, Mühlheim, Germany). Following a second centrifugation, the supernatant was reduced to a volume of 1 ml (under N2-stream, 50°C), diluted with 9 ml of Milli-Q water and aflatoxin residues were isolated using solid phase extraction columns (Strata C18-E 55um, 70A, Phenomenex, Fenno Medical, Vantaa, Finland). Columns were pre-activated with 10 ml methanol followed by 10 ml Milli-Q water. Samples were loaded at a flow rate of 1 ml/min and columns were washed with 5 ml of 5% methanol. Aflatoxin residues were eluted from the columns with 3 ml of acidified methanol (0.5% acetic acid in 50/50 methanol/water) followed by 5 ml of methanol, evaporated to approximately 100 µl volume and diluted with 2 ml of Mili-Q water for Immuno affinity column (IAC) cleanup. IACs (AflaTest, Vicam, Fleurs, Belgium) were washed with 10 ml PBS and 10 ml MQ-water. Samples were loaded, washed with 5 ml PBS and 10 ml MQ-water and eluted with 4 ml of 95% methanol. Cleaned samples were evaporated to dryness under vacuum (SPD1010 SpeedVac® System, Thermo Savant, Waltham, MA, USA), reconstituted in 500 µl methanol and stored at -20°C prior to HPLC analysis.

2.6.Immune response measurements:

2.6.1.Hemagglutination Inhibition test for detection of Newcastle antibodies: Blood samples were collected at days 21, 35 and 42th of age from four chickens of each group. Blood samples were left

without anticoagulant to clot. The serum was separated by centrifugation at 3000 rpm for 10 minutes. Microtechnique of haemagglutination inhibition test was done according to Takatasy (1955). Geometric mean titer (GMT) was calculated according to Brugh (1978).

2.6.2.Phagocytosis and differential leukocytic counts: Four blood samples were collected from each group of the experimental birds at 42th days of age in a clean dry vials containing anticoagulant (0.1ml sodium citrate 3.8%) for determination of phagocytic activity, phagocytic index, some blood pictures (total leukocytic count (WBCs), red blood cells (RBCs) counts, hemoglobin and differential leukocytes count).

2.7.Determination of phagocytic activity and phagocytic index: Phagocytic activity was determined according to Kawahara et al. (1991). Fifty micrograms of *Candida Albicans* culture were added to 1 ml of citrated blood, collected at the end of experiment slaughtering four birds from each group. Treated blood samples were put in shaker water bath at 23 – 25C for 3 – 5 hrs. Smears of blood were made and then stained with *Geimsa* stain. Phagocytosis was estimated by determining the proportion of macrophages which contain intracellular yeast cells in a random sample of 300 macrophages and expressed as percentage of phagocytic activity (PA). The number of phagocytized candida cells was counted in the phagocytic cells to calculate the phagocytic index.

Determination of total leukocytic count (WBCs) and other blood pictures (RBCs count, and Hb%): They were determined after previous methods according to Maxine and Benjamine (1985).

2.8. Determination of differential leukocytic count: Blood film was prepared according to the method of Lucky (1977). Ten drops from *May-Grunwald* stain stock solution on a dry, unfixed smear were added to equal amount of blood, then mixed and left for 1 minute for staining. The dye was decanted without rinsing. Diluted Giemsa´s solution (10 drops of the dye were added to 10 ml of distilled water) was poured over the film as counter stain and left for 20 minutes, then rinsed in water current and examined under an oil immersion lens. The percentage and absolute value for each type of cells were calculated according to Schalm (1986).

2.9. Lymphoid organs weight and some carcass traits: At the end of experimental period, four birds from each dietary treatment were randomly taken, fasted for 6 hours then weighed and slaughtered to complete bleeding and weighed to determine relative weight of immune organs (spleen, bursa and thymus gland) and some carcass traits (liver, gizzard, proventriclus and abdominal fat).

2.10. Assessment of some blood parameters: At the 42th day, of age blood samples were collected from four birds of each group, and the blood were left to drop on the side of the tube to prevent destruction of RBCs. Each blood sample was left to coagulate at room temperature. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 10 minutes. The clear serum was kept in a freezer (-20 C) until analysis for determination of serum total protein, globulin, albumin, GOT, GPT, urea, uric acid, creatinine, calcium, phosphorus, serum lipids concentrations (cholesterol, triglyceride, HDL, LDL and VLDL) and glucose were estimated using specific commercial kits (Roche Diagnostica, Basel, Switzerland).

2.11. Mortality rate and European production efficiency factor (EPEF): Mortalities were recorded throughout the growing period by dividing the number of dead birds in a certain group by the total number of birds in this group. European production efficiency factor was calculated according to Sainsbury (1984).

Statistical analysis: The analysis of variance for the obtained data was performed using Statistical Analysis System (SAS, 2004) to assess significant differences among the different examined groups.

3. RESULTS AND DISCUSSION

Growth performance and feed efficiency parameters:

Broiler fed chemical or biological mycotoxins binder supplementation significantly ($P \leq 0.05$) improved final body weight, total gain, feed intake, FCR, PER, EEU and PI of broiler chicken when compared with broiler chicken fed on the basal diet without supplement. These data are in harmony with those obtained by Salgado-Tránsito et al. (2011) reported that LBW was significantly improved in birds fed diet supplemented with 12.5, 25 and 50 g citric acid/kg compared with control. Moreover, Yildirim et al. (2011) concluded that final body weights were statistically higher in yeast glucomannans supplemented birds than control group. However, These data are in harmony with Che et al. (2011) Supplementation with 0.1% CMA (mixture from esterified glucomannan and hydrated sodium calcium aluminosilicate) in the contaminated diet significantly improved ADG and ADFI during 10-42 d ($p<0.05$).

Regarding feed contamination by aflatoxin B1 at three weeks of broiler age, it was found that aflatoxin feed contaimantion without mycotoxin binder supplementation significantly ($P \leq 0.05$)

reduced the growth performance and deteriorated the feed efficiency parameters compared with broiler chicken group fed on the same diet without aflatoxin B1 contamination. On the other hand, chemical or biological mycotoxin binder supplementation with aflatoxin B1 contamination counteract the deleterious effect of aflatoxin B1 and significantly (P≤0.05) improved the growth performance compared with broiler chicken fed on the contaminated diet without mycotoxin binder supplementation. Previous study (Hedayati et al., 2014) showed that the addition of binder (composed from minerals (extra purified clay containing diatomaceous earth mineral), antioxidants (Curcuminoids extracted from Turmeric) and enzymes (Epoxidase and Esterase)}, significantly alter the adverse effects of aflatoxin (AF) and in absence of AF, when binder alone was fed to chicks; the better performance was recorded, when compared with control group.

The most economically significant effect of aflatoxicosis in poultry is reduced growth rate. The results of our experiment indicated that dietary AFB1 severely affected the performance. The negative of AF in broiler chickens demonstrated in this study have been reported previously (Santin et al., 2003, Giacomini et al., 2006). The primary effects that have been reported include decreased BWG and FC, and increased FGR (Ortatatli et al., 2005 and Zhao et al., 2010). Our results agree with those presented by Fernandez et al. (1994) and Denli et al. (2004) with broiler chickens. The adverse effects of AFB1 on growth performance have been related with a decrease in the protein and energy utilization (Dalvi and Ademoyero, 1984 and Verma et al., 2002), probably as a consequence of a deterioration of the digestive and metabolic efficiency of the birds.

3.1. Blood picture:

Effect of chemical or biological mycotoxin binder supplementation without or with aflatoxin B1 feed contamination on blood picture of broiler chicken is presented in table (4). Statistical analysis of the obtained data indicated that mycotoxin binder supplementation significantly (P≤0.05) increased Hb % of broiler chicken compared with control group fed on the basal diet without mycotoxin binder supplementation. Feed contamination by AFB1 reduced WBCs, RBCs and Hb % compared with broiler chicken group fed on the basal diet without AFB1 contamination, moreover, it was observed that chemical or biological mycotoxin binder supplementation with AFB1 feed contamination significantly (P≤0.05) increased and improved WBCs, RBCs and Hb % compared with broiler chick group fed on the contaminated diet without mycotoxin binder supplementation.

The adverse effects of mycotoxins on animal health are expressed in a diverse range of symptoms including haemostasis blood system damage (Abbès et al., 2006). Hematological parameters of broiler chicks are determined as an index of their health status. In our study, the AFB1 contaminated diet decreased the levels of WBCs, Hgb and RBCs. Similarly, Abbès et al. (2006) reported that mice treated with 500 mg/kg ZEN caused a significant decrease in RBC level. Our results indicate that haemostasis blood system damage was induced by mycotoxins.

Regarding types of mycotoxin binder (chemical or biological) supplementation without or with AFB1 contamination, it can be concluded that biological binder more immune stimulant in broiler chicken compared with chemical mycotoxin binder source in improvement the level of WBCs. The present data are in harmony with those obtained by Ali (2014) revealed that yeast extract supplementation more effective against mycotoxin toxicity of broiler chicken than acidic acid supplementation. Enhancement of humoral immune response after mycotoxin binder supplementation is in line with Ibrahim et al. (2000) who detected that addition of binder was significantly effective in ameliorating the negative effect of mycotoxin due to improvement the level of WBCs, RBCs and Hb % of broiler chicken.

3.2. Blood serum units

Effect of chemical or biological mycotoxin binder supplementation without or with aflatoxin B1 feed contamination on serum proteins and glucose levels of broiler chicken is presented in table (5). Statistical analysis of the obtained data indicated that mycotoxin binder supplementation significantly (P≤0.05) increased the serum total proteins, albumin, globulin and glucose levels of broiler chicken compared with control group fed on the basal diet without mycotoxin binder supplementation. Feed contamination by AFB1 reduced serum proteins compared with broiler chicken group fed on the basal diet without AFB1 contamination, moreover, it was observed that chemical or biological mycotoxin binder supplementation with AFB1 feed contamination significantly (P≤0.05) increased serum proteins and glucose, compared with broiler chick group fed on the contaminated diet without mycotoxin binder supplementation.

Regarding types of mycotoxin binder (chemical or biological) supplementation with AFB1 contamination, it can be concluded that biological binder more immune stimulant in broiler chicken compared with chemical mycotoxin binder source in

improvement the level of serum total proteins and globulin. The present data are in harmony with those obtained by Ali (2014) revealed that yeast extract supplementation more effective against mycotoxin toxicity of broiler chicken than acidic acid supplementation. Enhancement and improved the liver function after mycotoxin binder supplementation is in line with Ibrahim et al. (2000) who detected that addition of binder was significantly effective in improving the level of serum proteins. Our results agreed with those of (Shareef and Aziz 2012) who make an experiment to investigate the effect of mycotoxin adsorbant supplementation on some blood parameters alterations of T2 toxin (a trichothecene mycotoxin) challenge broilers and they observed that serum protein was significantly ($p<0.05$) affected by feeding T-2 toxin to birds. Intermediate restoration of serum protein level was approached by addition of the adsorbents to the diet.

3.3.Some blood serum minerals levels:

Effect of mycotoxin adsorbant supplementation on calcium and phosphorus of broiler chicken is presented in table (6). Statistical analysis of the obtained data indicated that there is a significant difference between different groups in its content in calcium and phosphorus serum levels. The amount of calcium and phosphorus increased in the group treated with chemical (based on organic acids) or biological (based on yeast cell wall, β-glucan and some enzymes) when compared with broiler chicken control fed on the basal diet without supplement.

Regarding types of mycotoxin binder (chemical or biological) supplementation without or with AFB1 contamination, it can be concluded that biological increased the serum calcium and phosphorus content in broiler chicken compared with chemical mycotoxin binder. These data are in harmony with those obtained by Yildirim et al. (2011) concluded that final calcium and phosphorus content were statistically higher in yeast glucomannans supplemented birds than control group. Previous study (Hedayati et al., 2014) showed that the addition of binder (composed from minerals (extra purified clay containing diatomaceous earth mineral), antioxidants (Curcuminoids extracted from Turmeric) and enzymes (Epoxidase and Esterase)}, significantly alter the adverse effects of aflatoxin (AF) and in absence of AF, when binder alone was fed to chicks; the better performance and minerals content was recorded, when compared with control group. These results agree with Abdalla et al. (2012) who reported a significant improvement in calcium and phosphorus levels after addition of Nutritox to contaminated ration. The supplementation of 0.1% Yeast Glucomannan alone to the aflatoxin-contaminated diet significantly prevented the adverse effects of aflatoxin on serum biochemical parameters (Azizpour and Moghadam, 2015).

3.4. Liver and kidney blood serum related parameters:

Effect of chemical or biological mycotoxin binder supplementation without or with aflatoxin B1 feed contamination on liver and kidney related enzymes levels of broiler chicken is presented in table (7). Statistical analysis of the obtained data indicated that mycotoxin binder supplementation significantly ($P\leq0.05$) decreased the level of serum urea, uric acid but increased the liver related enzymes including the GOT and GPT levels of broiler chicken compared with control group fed on the basal diet without mycotoxin binder supplementation. Feed contamination by AFB1 increased serum urea, uric acid and serum enzymes levels that include GOT and GPT levels compared with broiler chicken group fed on the basal diet without AFB1 contamination which indicate kidney and liver deterioration, moreover, it was observed that chemical or biological mycotoxin binder supplementation with AFB1 feed contamination ($P\leq0.05$) improved the kidney function (significantly reduced serum urea, creatinine and uric acid concentration) compared with broiler chick group fed on the contaminated diet without mycotoxin binder supplementation.

The serum activities of AST and alanine transaminase (ALT) have been recognized as sensitive serological indicators in the impairment of the hepatic tissues and biliary system, and the serum level of total protein (TP) is the indicator of protein synthesis (Abdel-Wahhab and Aly, 2005). Therefore, in our study, the increased serum AST and ALT activities observed in the broiler chicks group fed diets contaminated by AFB_1 indicates that at least certain damage occurred in the liver. This is because AST and ALT originally located in the cytoplasm, is released into the blood system only when hepatic structural integrity is affected. This was consistent with a previous report in which broilers were exposed to diets containing AFB_1 at a level of 82.4 µg/kg of diet (Yang et al., 2012). However, some reports demonstrated that a high level of AFB_1 (2500 µg/kg) significantly decreased serum TP content and/or increased serum ALT and ALP activities in animals (Bagherzadeh Kasmani et al., 2012). Regarding types of mycotoxin binder (chemical or biological) supplementation with AFB1 contamination, it can be concluded that biological binder was more effective in improvement the kidney and liver function (significantly decreased serum urea, uric acid, GOT and GPT levels) compared with chemical mycotoxin binder source.

Table (3): Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 contamination on some growth performance and feed efficiency parameters of broiler chicken.

Parameter	Types of mycotocin binder supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Initial Wt (g/chick)	33.35±0.68^{A}	32.65±0.43^{A}	36.00±0.65^{A}	35.41±0.48^{A}	36.03±0.59^{A}	35.35±0.48^{A}
Final body Wt (g/chick)	2060.55±55.12^{F}	2385.31±41.13^{C}	2577.67±42.95^{A}	1948.96±58.81^{E}	2403.17±53.39^{B}	2332.23±61.19^{D}
Total gain (g/chick)	2028.31±54.57^{e}	2353.03±40.79^{b}	2541.94±42.36^{a}	1914.65±58.43^{d}	2367.90±52.94^{b}	2297.32±60.75^{c}
Total Feed intake (g/chick)	3966.80	4055.60	4268.00	4150.20	4044.50	4096.30
Average FCR value	1.99±0.05^{B}	1.74±0.03^{D}	1.70±0.03^{D}	2.23±0.09^{A}	1.74±0.04^{D}	1.82±0.05^{C}
Average PER value	2.59±0.07^{C}	2.94±0.05^{B}	3.02±0.05^{A}	2.34±0.07^{C}	2.97±0.07^{B}	2.84±0.07^{B}
Average EEU value	6.19±0.16^{A}	5.41±0.10^{B}	5.27±0.10^{B}	6.94±0.27^{A}	5.39±0.14^{B}	5.66±0.16^{B}
Average performance index	107.48±5.94^{D}	139.68±4.73^{C}	154.88±4.99^{A}	91.98±4.98^{E}	142.72±6.05^{B}	133.52±6.84^{C}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (4): Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 contamination on blood picture of broiler chicken.

Parameter	Types of mycotocin adsorbant supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
WBCS	6.20±0.37^{B}	2.73±0.17^{D}	3.45±0.96^{C}	2.20±0.15^{D}	1.65±0.18^{E}	7.28±0.33^{A}
RBCs	2.17±0.05^{B}	2.06±0.05^{C}	1.98±0.09^{D}	1.91±0.06^{D}	1.67±0.12^{E}	2.52±0.10^{A}
Hb %	11.75±0.32^{AB}	11.88±0.43^{AB}	12.75±0.32^{A}	11.75±0.32^{AB}	11.13±0.66^{B}	11.50±0.54^{AB}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (5): Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 contamination on serum total proteins and glucose levels of broiler chicken.

Item	Types of mycotocin adsorbant supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Total protein (g/dl)	5.60±0.09^{B}	5.78±0.08^{AB}	5.65±0.05^{AB}	5.65±0.06^{AB}	5.75±0.09^{AB}	5.85±0.03^{A}
Albumin (g/dl)	3.85±0.21^{B}	3.80±0.14^{B}	3.90±0.04^{AB}	3.85±0.13^{AB}	3.98±0.09^{A}	3.93±0.09^{A}
Globulin (g/dl)	1.75±0.29^{B}	1.98±0.09^{A}	1.75±0.09^{B}	1.80±0.18^{B}	1.78±0.15^{B}	1.93±0.10^{A}
Glucose (mg/dl)	199.95±1.21^{A}	200.90±0.41^{A}	201.25±0.36^{A}	201.10±0.15^{A}	200.53±0.59^{A}	200.30±0.20^{A}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

The present data are in harmony with those obtained by Suksombat et al. (2011) and Ali (2014) where they revealed that live yeast, yeast and algae extract supplementation more effective against mycotoxin toxicity of broiler chicken than acidic acid supplementation. Enhancement and improved the liver and kidney function after mycotoxin binder supplementation is in line with Ibrahim et al. (2000) and Suksombat et al. (2011) where they detected that addition of binder was significantly effective in decreasing serum urea, uric acid and serum liver related enzymes levels that include GOT and GPT. Also, Shareef and Aziz (2012) observed that, the liver function indicator enzymes, ALT and AST, were statistically affected by T-2 toxin inclusion to the bird's diet, these enzymes were increased with T-2 toxin inclusion compared with the negative control group.

3.5. Blood serum lipid profile:

Effect of chemical or biological mycotoxin binder on serum lipids levels of broiler chicken is presented in table 8. The obtained data indicated that mycotoxin binder supplementation significantly ($P \leq 0.05$) decreased the level of triglycerides, total cholesterol and LDL levels, and increased the level of HDL and VLDL of broiler chicken compared with control group fed on the basal diet without mycotoxin binder supplementation. Feed contamination by AFB1 increased triglycerides, total cholesterol and LDL with reduction of HDL and VLDL serum lipids compared with broiler chicken group fed on the basal diet without AFB1 contamination.

Regarding types of mycotoxin binder (chemical or biological) supplementation without or with AFB1 contamination, no significant differences were observed in the serum lipids levels between the two mycotoxin binder types. The present data are in harmony with those obtained by Suksombat et al. (2011) and Ali (2014) revealed that yeast extract supplementation more effective against mycotoxin toxicity of broiler chicken than acidic acid supplementation. Improved the serum lipids after mycotoxin binder supplementation is in line with Ibrahim et al. (2000) and Yildirim et al. (2011) who detected that addition of binder was significantly effective in improving and increasing the level of serum lipids.

3.6. Immune response:

3.6.1. Differential leukocyte:

Effect of chemical or biological mycotoxin binder supplementation without or with aflatoxin B1 feed contamination on differential leucocytic counts of broiler chicken is presented in table (9). Statistical analysis of the obtained data indicated that mycotoxin binder supplementation without or with AFB1 contamination significantly ($P \leq 0.05$) increased the WBCs level especially lymphocytes and neutrophils of broiler chicken compared with control group fed on the basal diet without mycotoxin binder supplementation. Feed contamination by AFB1 reduced WBCs and its differentials especially neutrophils, basophils and monocytes compared with broiler chicken group fed on the basal diet without supplement.

Regarding types of mycotoxin binder (chemical or biological) supplementation without or with AFB1 contamination, it was observed that biological binder non-significantly reduced the neutrophils but significantly increased the lymphocytes compared with chemical mycotoxin binder source.

The present data are in harmony with those obtained by Ali (2014) revealed that yeast extract supplementation more effective against mycotoxin toxicity of broiler chicken than acidic acid supplementation. Enhancement of humoral immune response after mycotoxin binder supplementation is in line with Ibrahim et al. (2000) who detected that addition of binder was significantly effective in improving and increasing the level of leucocytic cells especially lymphocytes and neutrophils.

3.6.2. Phagocytosis:

Effect of chemical or biological mycotoxin binder supplementation on phagocytic activity and index of broiler chicken is presented in table (10). Statistical analysis of the obtained data indicated that mycotoxin binder supplementation significantly ($P \leq 0.05$) increased phagocytic activity and index of broiler chicken compared with control group fed on the basal diet without mycotoxin binder supplementation. Feed contamination by AFB1 reduced phagocytic activity and index compared with broiler chicken group fed on the basal diet without AFB1 contamination, moreover, it was observed that chemical or biological mycotoxin binder supplementation with AFB1 feed contamination significantly ($P \leq 0.05$) increased phagocytic activity and index compared with broiler chick group fed on the contaminated diet without mycotoxin binder supplementation. Regarding types of mycotoxin binder (chemical or biological) supplementation without or with AFB1 contamination, it can be concluded that biological binder more immune stimulant in broiler chicken compared with chemical mycotoxin binder source. Our data is in the line with Ibrahim et al. (2000) who detected that addition of binder was significantly effective in ameliorating the negative effect of mycotoxin on the percentage and mean of phagocytosis of broiler chicken.

Table (6): Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 contamination on calcium and phosphorus level of broiler chicken.

Parameter	Types of mycotocin adsorbant supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Calcium (mg/dl)	9.90±0.57^{B}	8.75±0.59^{C}	10.00±0.30^{A}	9.30±0.26^{B}	9.33±0.55^{B}	10.83±1.99^{A}
Phosphorus (mg/dl)	2.63±0.15^{C}	3.37±0.15^{A}	3.07±0.48^{B}	3.57±0.44^{A}	3.13±0.28^{B}	3.30±0.21^{AB}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (7): Effect of mycotoxin binder supplementation without or with aflatoxin contamination on kidney and liver function related blood serum parameters of broiler chicken.

Parameters	Types of mycotocin binder supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Urea (mg/dl)	53.08±7.27^{A}	45.93±4.48^{C}	54.28±1.69^{A}	48.23±1.67^{B}	48.15±2.82^{B}	45.33±2.62^{C}
Uric acid (mg/dl)	5.83±0.07^{B}	5.93±0.09^{B}	5.93±0.03^{A}	6.07±0.03^{A}	6.00±0.10^{AB}	5.87±0.03^{B}
Creatinine (mg/dl)	2.17±0.60^{A}	2.05±0.71^{AB}	1.65±0.35^{B}	2.81±0.45^{A}	1.90±0.56^{B}	2.57±0.57^{A}
GOT (µ/l)	13.50±2.75^{C}	14.25±3.99^{C}	17.75±3.68^{B}	16.00±2.12^{B}	19.25±7.28^{A}	13.00±2.74^{C}
GPT (µ/L)	26.00±7.71^{C}	34.25±6.14^{B}	31.00±6.52^{B}	26.75±9.20^{C}	38.25±2.43^{A}	34.00±4.67^{B}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (8): Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 contamination on serum lipids level of broiler chicken.

Item	Types of mycotocin adsorbant supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Triglycerides (mg/dl)	204.70±4.44^{B}	211.50±1.07^{A}	207.60±0.89^{AB}	212.83±1.03^{A}	209.93±1.63^{A}	210.83±2.16^{A}
TCHO (mg/dl)	204.47±3.32^{B}	197.03±1.81^{C}	197.03±3.70^{C}	213.00±4.31^{A}	205.20±2.15^{B}	206.93±3.07^{B}
HDL (mg/dl)	51.37±1.20^{B}	53.70±1.10^{A}	54.13±2.19^{A}	46.80±1.73^{C}	50.20±1.42^{B}	49.73±1.10^{B}
LDL (mg/dl)	112.16±4.06^{B}	101.03±3.06^{C}	101.38±4.99^{C}	123.63±5.75^{A}	113.01±2.19^{B}	115.03±4.19^{B}
VLDL (mg/dl)	40.94±0.89^{B}	42.30±0.21^{AB}	41.52±0.18^{AB}	42.57±0.21^{A}	41.99±0.33^{AB}	42.17±0.43^{A}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

3.6.3. Antibody production:

Effect of chemical or biological mycotoxin binder supplementation without or with aflatoxin B1 feed contamination on antibody titer production against Newcastle disease vaccine of broiler chicken is presented in table (11). Statistical analysis of the obtained data revealed that no significant ($P \geq 0.05$) difference between antibody titer of different groups at 21th day of broiler age, while at 35 and 42 days of age chemical (based on organic acids) or biological (based on yeast cell wall, some enzume and β-glucan) mycotoxin binder supplementation significantly ($P \leq 0.05$) increased antibody titre angainst Newcastle disease vaccine when compared with broiler chicken group fed on the basal diet without mycotoxin binder supplementation.

Moreover, AFB1 contamination significantly ($P \leq 0.05$) reduced antibody titer at 35 to 42 days of broiler age when compared with broiler chick group fed on the basal diet without AFB1 contamination. On the other hand, chemical or biological mycotoxin binder supplementation with aflatoxin B1 feed contamination significantly increased antibody titer against Newcastle disease vaccine at 35 or 42 days of broiler age compared with broiler chicks group fed on the contaminated diet without mycotoxin binder supplementation.

This reduction in titer values is cleared induction of immune depression effects of aflatoxin on humoral antibody response. The reduction of antibody titer could be due to inhibition of DNA and protein synthesis by aflatoxin through impairment of amino acid transport and mRNA transcription resulting in low level of antibody (Gupta et al., 2003). Also, the responses on antibody of birds fed AF (humoral immunity) are clearly indicated in current study. The enquiry about immune-toxicity sensitivity of new generations of commercial broilers remains unanswered. Furthermore, there is proof about biphasic nature of AFB_1 on humoral immunity. It is worthy to mention that humoral immune feedback of birds might rise and decline depends upon the dose and duration of aflatoxin exposure (Manafi et al., 2012).

Mycotoxin binder supplementation improved clearly antibody titer production in this study was supported by Hedayati et al. (2014) indicated that inclusion of binder alone and in combination with AF had increased the ND values significantly ($P<0.05$), when compared with control group. The positive effect of mycotoxin binder with AF observed in the present study is in agreement with the findings of Ibrahim et al. (2000) and Pasha et al. (2007). The advantage of feeding algae extract and live yeast (biological) binder in the present study is in agreement with that of Raju et al. (2005) who reported that spirulina at 0.02 per cent level in the diet improved cellular immune response while no effect was seen in broilers fed with 300 AF.

3.7. Immune organs:

Effect of mycotoxin binder supplementation without or with aflatoxin feed contamination on weight of immune organs in broiler chicken is presented in table 12. It was observed that chemical or biological mycotoxin adsorbant supplementation significantly increased spleen, bursa and thymus gland weight while, biological binder reduced spleen and bursa relative weight compared with broiler chicken fed on the basal diet without supplement. On the other hand, AFB1 feed contamination significantly ($P \leq 0.05$) reduced bursa and thymus gland weight and relative weight compared with broiler chicken group fed on basal diet without AFB1 contamination, howeve, aflatoxin contamination increased spleen weight of broiler chicken.

Chemical or biological mycotoxin adsorbant supplementation with AFB1 feed contamination restore bursa and thymus gland weight and relative weight to nearly the normal control while reduced spleen weight and relative weight compared with broiler chicken group fed on contaminated diet without mycotoxin supplementation. Significant increases in the relative spleen weight of broilers exposed to aflatoxin-contaminated diets have also been reported by Bailey et al. (2006) and Shi et al. (2006). On the other hand, our data are in contrast with Hedayati et al. (2014) showed that Thymus weight has not been affected by any dietary treatments. The weight of Spleen has been decreased significantly ($P<0.05$) in AF fed group and while adding binder to the AF diet, these reduction could significantly ($P<0.05$) restored.

It is a general observation that size of lymphoid organs is not normal in birds exposed to AFB1. In such animals, lymphoid cell depletion in thymus, spleen, and bursa of Fabricius has been described (Shivachandra et al., 2003). Thus one explanation of immune-toxicity of AFB1, as also proposed by Azzam and Gabal (1998), could be inhibition of antibody production through the toxin's effects on lymphocytes leading to enhanced turnover of serum antibodies and consequently to decreased antibody half-life (Carrillo et al., 1985).

Table (9): Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 contamination on differential leucocytic counts of broiler chicken.

Item	Types of mycotocin adsorbant supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Neutrophils	26.40±1.29^{B}	31.18±0.89^{A}	30.55±1.29^{A}	15.00±2.65^{C}	16.25±1.49^{C}	14.00±0.58^{C}
Eosinophils	13.38±2.05^{A}	11.75±1.32^{B}	8.53±0.66^{CD}	9.00±0.41^{C}	8.00±0.41^{CD}	7.25±0.25^{D}
Basophils	5.88±0.74^{C}	5.05±1.00^{C}	4.20±0.87^{D}	7.75±0.48^{A}	6.75±0.25^{B}	5.50±0.29^{C}
Monocytes	12.35±2.25^{A}	10.65±2.15^{B}	9.25±2.42^{B}	7.50±0.87^{C}	5.50±0.50^{D}	4.75±0.25^{D}
Lymphocytes	42.00±2.39^{E}	41.38±3.25^{E}	47.48±1.50^{D}	60.75±1.32^{C}	63.50±0.65^{B}	68.50±0.29^{A}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (10): Effect of mycotoxin binder supplementation without or with aflatoxin contamination on phagctosis of broiler chicken.

Parameters	Types of mycotocin binder supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Phagctic activity%	21.00±0.41^{b}	23.25±0.25^{b}	26.25±0.75^{a}	18.25±0.48^{d}	23.00±0.41^{b}	27.00±0.41^{a}
Phagocytic index	2.50±0.29^{d}	3.75±0.25^{c}	5.75±0.48^{b}	2.25±0.25^{d}	3.75±0.25^{c}	7.00±0.41^{a}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (11): Effect of mycotoxin binder supplementation without or with aflatoxin contamination on antibody titer of broiler chicken.

Age/day	Types of mycotocin binder supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
21	3.67±1.76^{a}	3.33±1.86^{a}	3.33±1.20^{a}	3.67±2.33^{a}	3.67±0.88^{a}	3.67±0.88^{a}
35	4.00±1.53^{c}	4.67±0.88^{b}	6.67±0.33^{a}	1.67±0.33^{e}	2.33±0.33^{d}	2.33±0.33^{d}
42	2.33±0.33^{b}	3.00±0.58^{a}	2.67±0.67^{a}	0.67±0.33^{d}	1.67±0.33^{c}	3.00±0.58^{a}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (12): Effect of mycotoxin binder supplementation without or with aflatoxin contamination on some immune organs weight of broiler chicken.

Parameters	Types of mycotocin binder supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Spleen weight (g)	0.62±0.09^{d}	1.51±0.25^{a}	0.68±0.07^{c}	0.89±0.28^{b}	0.69±0.08^{c}	0.69±0.08^{c}
Spleen relative weight	0.04±0.01^{a}	0.07±0.01^{a}	0.03±0.00^{a}	0.07±0.02^{a}	0.04±0.01^{a}	0.03±0.00^{a}
Bursa weight (g)	4.48±0.21^{b}	5.22±0.31^{a}	5.04±0.60^{a}	3.57±0.33^{c}	4.99±0.38^{b}	3.80±0.24^{c}
Bursa relative weight	0.30±0.01^{b}	0.24±0.00^{b}	0.19±0.02^{c}	0.27±0.01^{a}	0.28±0.01^{a}	0.17±0.01^{c}
Thymus gland weight (g)	1.17±0.35^{c}	3.68±0.21^{a}	3.85±0.47^{a}	0.77±0.15^{d}	1.74±0.47^{c}	2.76±0.21^{b}
Thymus gland relative weight	0.08±0.02^{c}	0.17±0.01^{a}	0.14±0.01^{a}	0.06±0.01^{c}	0.09±0.02^{c}	0.12±0.01^{b}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (13): Effect of mycotoxin binder supplementation without or with AFB1 feed contamination on some carcass traits of broiler chicken.

Parameters	Types of mycotocin binder supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
Dressing %	71.84±1.35 ^{E}	72.51±0.96^{D}	79.26±1.28^{A}	66.69±1.57^{F}	73.38±1.29^{C}	75.74±0.95^{B}
Liver weight (g)	25.23±1.74^{f}	47.10±0.82^{B}	56.42±0.55^{A}	39.82±0.38^{e}	31.47±4.72^{D}	43.53±1.43^{C}
Liver relative weight	1.70±0.13^{B}	2.15±0.10^{A}	2.14±0.07^{A}	2.03±0.07^{B}	1.75±0.20^{B}	1.94±0.13^{AB}
Gizzard weight (g)	25.45±1.09^{E}	34.06±0.75^{C}	46.84±0.62^{A}	23.90±1.24^{E}	31.35±4.10^{D}	37.03±1.49^{B}
Gizzard relative weight	1.72±0.11^{A}	1.55±0.06^{B}	1.78±0.04^{A}	1.84±0.05^{A}	1.74±0.16^{A}	1.64±0.01^{B}
Heart weight (g)	5.77±0.44^{C}	9.80±0.55^{B}	13.23±0.42^{A}	4.50±0.32^{D}	6.82±0.72^{C}	8.77±0.51^{B}
Heart relative weight	0.39±0.01^{BC}	0.44±0.01^{B}	0.50±0.01^{A}	0.34±0.01^{C}	0.38±0.03^{BC}	0.39±0.03^{BC}
Kidney weight (g)	11.37±0.39^{C}	13.19±0.88^{A}	13.12±0.31^{A}	11.99±0.39^{C}	12.68±0.42^{B}	12.46±0.47^{B}
Kidney relative weight	0.77±0.03^{A}	0.60±0.00^{B}	0.50±0.01^{C}	0.87±0.02^{A}	0.71±0.01^{A}	0.55±0.02^{C}
Proventriculus Wt (g)	6.39±0.27^{B}	8.61±0.29^{A}	7.97±0.58^{A}	5.90±1.01^{B}	6.89±0.47^{B}	6.19±0.23^{B}
Proventriculus relative Wt	0.43±0.03^{A}	0.39±0.02^{B}	0.30±0.01^{C}	0.44±0.05^{A}	0.39±0.01^{B}	0.27±0.01^{C}

Values are means ± standard error. Means within the same row of different litters are significantly different at (P≤0.05).

Table (14): Effect of mycotoxin binder supplementation without or with aflatoxin contamination on Aflatoxin residue in liver tissue (ppb) of broiler chicken.

Items	Types of mycotocin adsorbant supplementation					
	Control	Chemical	Biological	Control	Chemical	Biological
	Without aflatoxin feed contamination			With aflatoxin feed contamination		
AFB1 (ppb)	1.2±0.09^{c}	ND	ND	14.5±1.34^{a}	4.6±0.44^{b}	3.3±0.32^{b}

ND = not detectable aflatoxins. Values are expressed as mean ± standard errors. Means in the same row (a-c) with different letters significantly differ at (p≤0.05).

Table (15): Effect of chemical or biological mycotoxin binder supplementation on AFB1 content in the ingesta and fecal excretion of broiler chicken.

AFB_1 (ppb)	Types of mycotocin adsorbant supplementation		
	Control	Chemical	Biological
	With aflatoxin feed contamination		
Ileum content	10.6±2.5^{c}	45.1±3.9^{b}	60.5±5.1^{a}
Cecal content	15.8±2.1^{c}	49.2±6.2^{b}	56.6±6.6^{a}

Values are expressed as mean ± standard errors. Means in the same row (a-c) with different letters significantly differ at (p≤0.05).

To tell the truth, the precise mechanisms of even immunosuppression during aflatoxicosis are not clearly understood despite more than 50 years of research on the field of toxins. However, it is believed that the susceptibility of the immune system to mycotoxin immunosuppression comes from the vulnerability of the continually proliferating and differentiating cells that subsidize in immune-mediated activities and regulate the multifaceted communication chain between cellular and humoral components. This immunosuppression effect may be established as depressed T- or B-lymphocyte activity, suppressed antibody production and impaired macrophage/neutrophil-effector activities. The immune system is primarily responsible for defense against attacking organisms. Inhibited immune activity by mycotoxins may ultimately decrease resistance to infectious diseases, reactivate chronic infections and/or decrease vaccine and drug efficacy (Corrier, 1991& Surai and Dvorska, 2001).

3.8. Some carcass traits:

Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 feed contamination on some carcass traits are presented in table (13). Statstical analysis of the obtained data revealed that chemical or biological mycotoxin supplementation significantly improved dressing percentage of broiler chicken compared with control. On the other hand AFB1 feed contamination without mycotoxin supplementation significantly reduced dressing percentage of broiler compared with control, while chemical or biological mycotoxin supplementation with AFB1 feed contamination allivate the toxic effect and restore dressing percentage to the normal value.

Regarding liver weight and relative weight, it was observed that chemical or biological mycotoxin binder supplementation significantly increased liver weight and relative weight compared with control while AFB1 feed contamination without mycotoxin binder supplementation increased liver weight when compared with control. Moreover, chemical or biological mycotoxin binder supplementation with AFB1 feed contamination counteract the adverse effect of AF on liver weight.

The findings herein demonstrated that the absolute and relative weight of the liver, kidney and gizzard in control positive group which fed on AFB1 contaminated diet were significantly ($p<0.05$) different from blank control negative (group 1), An increase in organs weight and their relatives weight owing to reduction in body weight. Parallel to our results those of **Ortatatli et al. (2005)** who found an increase in the absolute and relative weights of liver, kidney and gizzard of birds fed on ration containing aflatoxin indicating the hepto and nephrotoxicity of aflatoxins. Liver is considered the target organ for aflatoxin B1 because it is the organ where most aflatoxins are bioactivated to the reactive 8, 9-epoxide form, which is known to bind DNA and proteins, damaging the liver structures and increasing liver weight (Miazzo et al., 2005; Bailey et al., 2006 and Pasha et al., 2007). The increase in the liver weight could be attributed to increased lipid deposits in the liver due to impaired fat metabolism (Hsieh, 1979). The hepatic lipidosis is primarily mediated through inhibition of phospholipids synthesis and cholesterol. This in-turn affects the transportation of lipid from the liver (Manegar et al., 2010).

3.9. Aflatoxin residue in liver tissue:

The amount of AFB1 recovered from liver tissues given in table 14. AFB_1 was not detected in the liver of broilers fed on the basal diet with chemical (based on orqanic acids) or biological (based on S. cervisae, yeast cell wall, fungal and plant extract) mycotoxoin binder. Among other treatments, AFB_1 level was the highest in group fed on the basal diet with feed AFB1 contamination (14.5 ppb). Compared to broiler chicks group fed on AFB1 contaminated diet without supplement, supplementing chemical or biological mycotoxin binder to drinking water markedly reduced the AFB_1 concentration by about 68.3% and 77.2% respectively. The higher AFB_1 residue in liver of broiler chicken fed on aflatoxin contaminated diet agrees with Herzallah et al. (2014) where the residual level of AFB_1 was increased in liver of broiler chicks maintained for 6 weeks on AFB_1 contaminated diets of 384.5 µg/kg AFB_1 or 128.9 µg/kg AFB_1. Feeding aflatoxin (0.5ppm) to broiler chicken for 0-6 weeks of age resulted in liver tissue residue (Kumar and Balachandran, 2015). Also, aflatoxin B_1 residues were detected in liver tissues of naturally contaminated corn fed to broilers (Yang et al., 2012). But Hussain et al. (2016) reported that no AFB_1 residues were detected in the liver after 50 ppb AFB1but residues above the permissible threshold (>2.0 ng/g) were only detected in liver tissues of groups fed 400 ppb and 800 ppb AFB_1 in the diet.

AFB_1 residues in liver not only affect the performance and health of broiler, but also impair the

health of the broiler product consumers as aflatoxins accumulate in edible parts of poultry, so it is necessary to control the quality of poultry products and analyze aflatoxin residues in different tissues of birds considering public health and safety. The results of our study show significantly decreased residue levels of AFB_1 incorporated chemical or biological adsorbent in the drinking water for broiler fed on AFB_1 contaminated diet. The protective effects of yeast cell wall and organic acids of the used products from aflatoxins may be due to their specific biotransformation of aflatoxins in the intestinal tract, which leads to the reduction of aflatoxins absorbed by the intestinal tract and, consequently, a decrease in aflatoxin residues in the liver. Moreover, it can be concluded that biological mycotoxin binder higher effective to bind AFB1 than chemical one.

3.10. Toxin excretion:

Amount of AFB1 in the ileal and cecal content presented in table 15. It was observed that chemical or biological mycotocin binder supplementation significantly ($P \leq 0.05$) increased AFB1 in the ileal and cecal content by about (325.5% and 211.4%) and (470.8% and 258.2%) respectively compared with broiler chicks group fed on AFB1 feed contamination without supplementation. These show that additional amount of AFB1 excreted in the fecal matter due to chemical or biological mycotoxin supplementation in the drinking water for broiler chicken. This is in agreement with in vivo findings by (Silvia Gratz, 2007) suggesting that probiotic administration only increased fecal excretion of AFB1 within 24 hours of AFB1 dosage, but not at later time points. However, the ultimate goal is to reduce AFB1 bioavailability in the intestinal tract, and an increase in fecal AFB1 excretion in the presence of probiotics at any time point is convincing evidence that this goal can be achieved. Also these data are in agreement with (Salgado-Tránsito et al., 2011) concluded that supplementation with aqueous citric acid (CA) substantially reduced the pH of the diets according to the added content, this effect is interesting because the acidic treatment reduced the aflatoxin concentration in the feed up to 92%. The chromatograms of acidified samples may provide support for detoxification activity. It also suggests that the molecule structure in post-treated aflatoxin contaminated samples changes, the lactone ring may be opened. Thus, detoxification initially involves the formation of the b-keto acid structure (catalyzed by de acidic medium), followed by hydrolysis of the lactone ring yielding the AFD1 molecule, derivated from decarboxylation of the lactone ring-opened form of AFB1 (Méndez-Albores et al, 2005).

Comparing the efficacy of chemical and biological mycotoxin to bind AFB1 in the broiler chicken intestinal tract, it was observed that additional 15.4ppband 7.4ppb AFB1 are excreted in ileal and cecal content due to biological mycotoxin binder supplementation more than chemical binder. Numerous strategies for the detoxification/inactivation of mycotoxin-contaminated feed have been proposed; however, methods to detoxify AF-contaminated feed on a large scale and in a cost-effective manner are not available. A new approach to detoxify AF is the use of YCW in the diet. A few species of yeast are commercially used. Saccharomyces cerevisiae is one of the most widely commercialized species and one of the most effective adsorbents. S. cerevisiae, whose biological value is high, is rich in protein (40-45%) and in vitamin B complex (Çelyk et al., 2003). These YCW products are already used as prebiotics, which improve the performance of broiler chickens, stimulate the immune system, contribute to intestinal integrity, and compete with pathogenic microorganisms in the intestinal lumen (Flemming and Freitas, 2005 & Keller et al., 2012).

3.11. Mortality percentage:

The findings of this trial on mortality percentage of broilers fed different dietary treatments at different experimental periods are shown in table (16). It was observed that chemical or biological mycotoxin binder supplementation reduced broiler chicken mortality compared with control group. On the other hand AFB1 feed contamination increased chicken mortality compared with broiler chicken group fed on basal diet without aflatoxin feed contamination. Moreover, chemical or biological mycotoxin binder supplementation with AFB1 feed contamination highly reduced chicks mortality compared to broiler chicken group fed on contaminated diet without mycotoxin binder supplementation. However, it was observed that biological mycotoxin binder more effective to allivate the toxic effect of mycotoxicosis in broiler chicken. The clear effects of aflatoxin on the broilers have been well documented previously by Manafi (2010). The main causes for the increased mortality are altered protein metabolism, altered enzymatic activity and

decreased nutrient utilization and absorption. These data are in harmony with those obtained by (Hedayati et al., 2014) showed AF contamination increased broiler mortality and incorporation of only binder had reached the mortality into zero.

3.12. European production efficiency factor (EPEF):

Effect of chemical or biological mycotoxin binder supplementation without or with AFB1 feed contamination on EPEF of broiler chicken is presented in table (17). The obtained data indicated that chemical (based on organic acids) or biological (based on enzyme, yeast, fungal and plant extract with glucomannan) mycotoxin binder supplementation highly improved EPEF of broiler compared with control. On the other hand, it was observed that AF1 feed contamination reduced EPEF of broiler chicken compared with control while, AFB1 feed contamination with chemical or biological mycotoxin binder supplementation reduced EPEF of broiler chicken compared with broiler chicks group fed the same mycotoxin binder without AFB1 feed contamination.

The highest EPEF value was obtained by broiler chicken group fed on the basal diet with biological mycotoxin binder supplementation (359.9), followed by broiler chicken group fed on the basal diet with chemical mycotoxin binder supplementation (314.9), while the lowest EPEF was obtained by broiler chicken fed on AFB1 contaminated diet without mycotoxin binder supplementation (146.3).

CONCLUSION:

The obtained data indicated that chemical (based on organic acids) or biological (based on yeast cells, gluomannan, plant and fungal extract) mycotoxins binder supplementation significantly ($P \leq 0.05$) improved growth performance, feed efficiency parameters and immune response of broiler chicken. The presence of aflatoxins in diets could induce growth performance, immune response and liver function in broilers. Both chemical and biological binder suppressed the deleterious effects of aflatoxins on growth, immunity and hepatic functions. The ameliorative effects of biological product are better than chemical one as it is more immune stimulant in broiler chicken compared with chemical mycotoxin binder source. .

Table (16): Effect of mycotoxin binder supplementation without or with aflatoxin contamination mortality of broiler chicken.

Period/week	Types of mycotocin adsorbant supplementation					
	Control	**Chemical**	**Biological**	**Control**	**Chemical**	**Biological**
	Without aflatoxin feed contamination			**With aflatoxin feed contamination**		
1 – 0	1	0	0	0	0	0
2 – 1	0	1	0	1	1	0
3 – 2	0	0	0	0	0	0
4 – 3	0	1	0	1	1	1
5 – 4	1	0	1	3	2	0
6 – 5	1	1	0	3	0	1
6 – 0	3	2	1	8	4	2
Mortality%	8.6	5.7	2.9	22.9	11.4	5.7

Table (17): Effect of mycotoxin binder supplementation without or with aflatoxin contamination on EPEF of broiler chicken.

Items	Types of mycotocin adsorbant supplementation					
	Control	**Chemical**	**Biological**	**Control**	**Chemical**	**Biological**
	Without aflatoxin feed contamination			**With aflatoxin feed contamination**		
EPEF	225.9	314.9	359.9	146.3	291.7	298.1
EPEF relative to control	100	139.4	159.3	64.8	129.1	131.9

4. REFERENCES

Abbès, S., Ouanes, Z, Salah-Abbes, J., Houas, Z., Oueslati, R., Bacha, H., O. Othman. 2006. The protective effect of hydrated sodium calcium aluminosilicate against haematological, biochemical and pathological changes induced by Zearalenone in mice. Toxcion. 47:567-574.

Abbès, S., Z. Ouanes, J. Salah-Abbes, Z. Houas, R. Oueslati, H. Bacha and O. Othman. 2006. The protective effect of hydrated sodium calcium aluminosilicate against haematological, biochemical and pathological changes induced by Zearalenone in mice. Toxcion. 47:567-574.

Abdalla O.A., Ahmed TH.I. and Manal M. A. 2012. Pathological and biochemical changes induced by aflatoxin in chickens and atrial for treatment using lactobacillus acidophilus.Assiut Vet. Med. J. 58: 133.

Abdel-Wahhab, M.A. and Aly, S.E. 2005. Antioxidant property of Nigella sativa (black cumin) and Syzygium aromaticum (clove) in rats during aflatoxicosis. J. Appl. Toxicol. 25: 218–223.

Ali, E.J. 2014. Comparative study between some additives on immune response of infectious bursal disease vaccine in broiler fed diet with AF-contaminated poisons. IJSN, 5, 113-120.

AOAC. 1990. Official methods of analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, Virginia, USA, p. 1200.

Aravind, K.L., Patil, V.S., Devegowda, G., Umakantha B. and Ganpule S.P. 2003. Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally-contaminated feed on performance, serum biochemical and haematological parameters in broilers. Poult. Sci., 82: 570–576

Azizpour, A., and Moghadam, N. 2015. Effects of Yeast Glucomannan and Sodium Bentonite on the Toxicity of Aflatoxin in Broilers.RevistaBrasileira de CiênciaAvícola, 17(SPE), 7-13.

Azzam, A.H. and Gabal, M.A. (1998). Aflatoxin and immunity in layer hens. Avian Pathol., 27, 570–577.

Bagherzadeh Kasmani, F.; Karimi Torshizi, M.A.; Allameh, A.; Shariatmadari, F. A. 2012. novel aflatoxin-binding Bacillus probiotic: Performance, serum biochemistry, and immunological parameters in Japanese quail. Poult. Sci., 91, 1846–1853.

Bailey, R.H., Latimer, G.W., Barr, A.C., Wigle, W.L., Haq, A.U., Balthrop, J.E., Kubena, L.F. 2006. Efficacy of MNT clay (NovaSil Plus) for protecting full-term broilers from aflatoxicosis. J Appl Poult Res, 15, 198-206.

Bligh, E.G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Brugh, M. A. J. 1978. A simple method for recording and analysing serological data. Avian Dis., 22: 362-365.

Carrillo, M.C., Monti, J.A., Grosman, M.E. and Rodriguez Garay, E.A. 1985. Effect of pH on aflatoxin B1transfer in the everted rat jejunum. Toxicol. Lett., 27, 35–44.

Çelyk K., Denly M., Savas T. 2003. Reduction of toxic effects of aflatoxin by using baker yeast in growing broiler chicken diets. R Bras Zootec 32, 615-619.

Che, Z., Liu, Y., Wang, H., Zhu, H., Hou, Y., Ding, B. 2011. The protective effects of different mycotoxin adsorbents against blood and liver pathological changes induced by moldcontaminated feed in broilers. Asian-Aust J Anim Sci, 24, 2, 250-257.

Corrier, D.E. (1991). Mycotoxicosis: mechanisms of immunosuppression. Veterinary Immunology and Immunopathology, 30: 73–87.

Dalvi, R. R., and Ademoyero, A. A. 1984. Toxic effects of aflatoxin B1 in chickens given feed contaminated with Aspergillus flavus and reduction of the toxicity by activated charcoal and some chemical agents. Avian Dis. 28:61–69.

Denli, M., Okan, F., Doran, F. 2004. Effect of CLA on the performance and serum variables of broiler chickens intoxicated with AFB1. South Afr J Anim Sci, 34, 97-103.

Do, J.H. and Choi, D.K. 2007. Aflatoxins: Detection, toxicity, and biosynthesis. Biotechnol. Bioprocess Eng, 12, 585–593.

Dowling T.S. 1997. Fumonisins and its toxic effects. Cereal Foods World 42, 13-15.

Fan, Y.; Zhao, L.; Ma, Q.; Li, X.; Shi, H.; Zhou, T.; Zhang, J.; Ji, C. 2013. Effects of Bacillus subtilis ANSB060 on growth performance, meat quality and aflatoxin residues in broilers fed moldy peanut meal naturally contaminated with aflatoxins. Food Chem. Toxicol, 59, 748–753.

Fernandez, A., Verde, M. T. and Gascon, M. 1994. Aflatoxin and its metabolites in tissues from laying hens and broiler chickens fed a contaminated diet. J. Sci. Food Agric. 65:407–414.

Flemming J.S. and Freitas R.J.S. 2005. Avaliação do efeito de prebióticos, probióticos e promotor de crescimento na alimentação de frangos de corte. Arch Vet Sci 10, 41-47.

Food and Agriculture Organization of the United Nations (FAO), 2001. Manual on the Application of the HACCP System in Mycotoxin Prevention and Control; FAO: Rome, Italy.

Gargees, M.T. and Shareef A.M. 2008. Mycofix® ameliorative effect on Newcastle disease antibody production in broiler chickens during aflatoxicosis. Iraqi J. Vet. Sci., 22: 29-34.

Gargees, M.T. and Shareef A.M. 2009. Reducing liver aflatoxin M1 residues in chicks with Mycofix® Plus 3.0 during aflatoxicosis. Iraqi J. Vet. Sci., 23: 37-44.

Giacomini, L., Fick, F., Dilkin, P., Mallmann, C.A. 2006. Efeitos toxicológicos das aflatoxinas sobre o desempenho e plumagem de frangos. Cienc Rural 36, 234-239.

Girish, C. K. and T. K. Smith. (2008): Effects of feeding blends of grains naturally contaminated with fusarium

mycotoxins on small intestinal morphology of Turkeys. Poult. Sci. 87:1075- 1082.

Guan, S.; Ji, C.; Zhou, T.; Li, J.; Ma, Q.; Niu, T. 2008. Aflatoxin B1 degradation by Stenotrophomonas maltophilia and other microbes selected using coumarin medium. Int. J. Mol. Sci. 9, 1489–1503.

Gupta, K.; Ramneek, B.; and Singh, A. 2003. Immunomodulatory effects of aflatoxicosis and infectious bursal disease vaccination in broilers. Ind. Vet. j., 80: 78-80.

Hanson, S.W.F. and Olly, J. 1963. Fat extraction. (Cited by Pearson's chemical analysis of foods). 8th Edn.

Hassan, R., S. El-Kadi, and M. Sand, 2015. Effect of some organic acids on some fungal growth and their toxins production. Int. J. Adv. Biol, 2.

Hassan, R., Sand M. and El-Kadi S. 2012. Effect of some organic acid on fungal growth and their toxin production. J. Agric. Chem. And Biotechn., 3(9): 391-397.

Hedayati, M., Manafi, M., Yari, M., Mousavipour, S. V. 2014. Commercial Broilers Exposed to Aflatoxin B1: Efficacy of a Commercial Mycotoxin Binder on Internal Organ Weights, Biochemical Traits and Mortality. International Journal of Agriculture and Forestry, 4(5): 351-358.

Herzallah, S., Al-Ameiri, N., and Al-Dmoor, H. 2014. Meat and organs quality of broilers chickens fed diet contaminated with B1 aflatoxin. Global Veterinaria, 12, 376-80.

Hsieh, D.P.H. 1979. Basic metabolic effects of mycotoxins interactions of mycotoxins in animal production. Proceeding of Symposium, National Academy of Sciences. Washington, DC, pp: 43-55.

Hussain Z., Rehman H., Manzoor S., Tahir S., Mukhtar M. 2016. Determination of liver and muscle aflatoxin B1 residues and select serumchemistry variables during chronic aflatoxicosis.

Ibrahim, I.K., Shareef, A.M., Al-Joubry, K.M.T. 2000. Ameliorative effects of SB on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Res Vet Sci 69, 119-122.

Kawahara, E., Ueda, T., Nomura, S. 1991. In vitro phagocytic activity of white spotted shark cells after injection with Aeromoas salmonicida extraceular products. Gyobyo , Kenkyu, Japan, 26(4):213-214.

Keller K.M., Oliveira A.A., Almeida T.X., Keller L.A.M., Queiroz B.D., Nunes L.M.T., Cavaglieri L.R. and Rosa C.A.R. 2012. Efeito de parede celular de levedura sobre o desempenho produtivo de frangos intoxicados com aflatoxina B1. Rev Bras Med Vet 34,101-105.

Kumar, C. T. A., and Balachandran, C. 2015. Tissue Residues of Citrinin and Aflatoxin in the Broiler Chicken.Indian Vet. J, 92(1), 88-89.

Line, J.E.; Brackett, R.E.; Wilkinson, R.E. 1994. Evidence for degradation of aflatoxin B1 by Flavobacterium aurantiacum. J. Food Prot. 57, 788–791.

Lodhi, G.N., Singh, D. and Ichponani, L. 1976. Variation in nutrients content of feeding stuffs rich in protein and reassessment of chemical methods of metabolizable energy estimation for poultry. J. Agric. Sci. 69;634-639.

Lucky, 1977. Handbook of histopathologic and histochemical staining. 3rd Ed., Buterworth London . Lysenko, O (1961): Pseudomonas on attempt at general classification. J. of Microbiol. 25, 379.

Manafi, M. 2010. Effect of AFlatoxicosis in Hatching Eggs Quality and Immune Status in Broiler Breeders. In: World Poult. Sci. J. (Spp.), 66: 704-705.

Manafi, M., Murthy, H.N.N., Swamy, N. 2012. Evaluation of different mycotoxin binders on aflatoxicosis in broiler breeders induced with AFB1: Effects on biochemical and immunological parameters. American-Eurasian J Agric Environ Sci, 12, 429-433.

Manegar, G.A., Shambulingappa, B.E. and Ananda, K.J. 2010. Studies on tolerance limit of aflatoxin in commercial broilers. Libyan Agric. Res. Center J. Inter., 1(3): 177-181.

Maxine, M. and Benjamin, B.S. 1985. Outline of Veterinary Clinical Pathology. 3rd edition, Colorado State Univ., Printed in India at Rekha printers PVT. LTD., New Delhi-110020.

Méndez-Albores A., Arámbula-Villa G., Loarca-Piña M.G.F., Castaño-Tostado E., Moreno-Martínez E. 2005. Safety and efficacy evaluation of aqueous citric acid to degrade B-aflatoxins in maize. Food Chem Toxicol, 43, 233-238.

Miazzo, R., Peralta, M.F., Magnoli, C., Salvano, M., Ferrero, S., Chiacchiera, S.M., Carvalho, E.C.Q., Rosa, C.A.R., Dalcero, A. 2005. Efficacy of SB as a detoxifier of broiler feed contaminated with AF and fumonisin. Poult Sci, 84, 1-8.

Mykkänen H., Zhu H. L., Salminen E., Juvonen R. O., Ling W. H., Ma J., Polychronaki N., Kemilainen H., Mykkänen O., Salminen S., El-Nezami H. 2005. Fecal and urinary excretion of aflatoxin B1 metabolites (AFQ1, AFM1 and AFB-N-7-guanine) in young Chinese males. Int J Cancer 115:879-884.

Ortatatli, M., Oguz, H., Hatipoglu, F., Karaman, M. 2005. Evaluation of pathological changes in broilers during chronic AF (50 and 100 ppb) and CLI exposure. Res Vet Sci, 78, 61-68.

Pasha, T.N., Farooq, M.U., Khattak, F.M., Jabbar, M.A., Khan, A.D. 2007. Effectiveness of SB and two commercial products as AF absorbents in diets for broiler chickens. Anim Feed Sci Technol, 132, 103-110.

Peir, A.C., Heddleston K.L., Cysew ski S.J.and Patterson J.M. 1972. Effect of aflatoxin on immunity in turkeys. II. Reversal of impaired resistance to bacterial infection by passive transfer of plasma. Avian Dis., 16: 381-387.

Raju, M.V.L.N., S.V. Rama RAO, Radhika K. and Chawak M.M. 2005. Dietary supplementation of Spirulina and its

effects on broiler chicken exposed to aflatoxicosis, Indian J. Poult. Sci., 40: 36-40.

Randhir, S. and Pradhan, K. 1981. Forage evaluation. First published, Printox, New Dalhi, Dhawan printing works

Sainsbury, D. 1984. Systems of management. In: Sainsbury, D (Ed.), Poultry health and management. (2nd Edn.), London, Granada Publishing Ltd., PP: 102-104.

Salgado-Tránsito, L. Del Río-García, J.C. Arjona-Román, J.L. Moreno-Martínez, E. Méndez-Albores, A. 2011. Effect of citric acid supplemented diets on aflatoxin degradation, growth performance and serum parameters in broiler chickens. *Archivos de Medicina Veterinaria*, 43: 215-222.

Santin, E., Paulillo, A.C., Maiorka, A., Nakagui, L.S.O., Macari, M., Silva, A.V.F., Alessi, A.C. 2003. Evaluation of the efficacy of SCE cell wall to ameliorate the toxic effects of AF in broilers. Int J Poult Sci, 2, 341-344.

SAS. 2004. Statistical analysis system, 5th ed. SAS Institute, Cary, NC, USA.

Schalm, O.W. 1986. Veterinary hematology. 4th Ed., Lea and Febigez, Philadelphia

Shakhashiri, B.Z., 2008. Acetic Acid and Acetic Anhydride. Department of Chemistry, University of Wisconsin. Available at: http://scifun.chem.wisc.edu/ Chemweek/pdf/AceticAcid.

Shareef , A. M. and Aziz, L. S. (2012). Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Res. Vet. Sci., 69, 119–122.

Shi, Y. H., Xu, Z. R., Feng, J. L. and Wang, C. Z. 2006. Efficacy of modified montmorillonite nanocomposite to reduce the toxicity of aflatoxin in broiler chicks. Anim. Feed Sci. Technol. 129:138–148.

Shivachandra, S.B., Sah, R.L., Singh, S.D., Kataria, J.M. and Manimaran, K. 2003. Immunosuppression in broiler chicks fed aflatoxin and inoculated with fowl adenovirus serotype-4 (FAV-4) associated with hydropericardium syndrome. Vet. Res. Commum., 27, 39–51.

Silvia Gratz, 2007. Aflatoxin Binding by Probiotics Experimental Studies on Intestinal Aflatoxin Transport, Metabolism and Toxicity. Kuopio University Publications D. Medical Sciences 404. 85 p.

Smith, J. E., Solomons G., Lewis C.and Anderson J. G. 1995. Role of mycotoxins in human and animal nutrition and health. Nat. Toxins 3:187–192.

Suksombat, W., Suksombat, P. and Mirattanaphraip, R. 2011. Effect of commercial or bovine yeasts on the performance and blood variables of broiler chickens intoxicated with aflatoxins. World Academy of Science, Engineering and Technology, 58, 664–668.

Surai, P.F. and Dvorska, J.E. 2001. Dietary organic selenium and egg: from improvement in egg quality to production of functional food. Proceedings of the IX Symposium on the Quality of Eggs and Egg Products, Kusadasi, Turkey, pp. 163–160.

Takatasy, G. Y. 1955. The use of 100 M in serological and virological micro methods.

Van Kessel, T.F.M. and Hiang-Chek, N. 2004. Aflatoxin binders-how to get the best value for money. Int. Poult. Prod., 12(4): 33-35.

Verma, J., B. K. Swain, and Johri, T. S. 2002. Effect of various levels of aflatoxin and ochratoxin A and combinations thereof on protein and energy utilisation in broilers. J. Sci. Food Agric. 82:1412–1417

Yang, J.; Bai, F.; Zhang, K.; Bai, S.; Peng, X.; Ding, X.; Li, Y.; Zhang, J.; Zhao, L. 2012. Effects of feeding corn naturally contaminated with aflatoxin B1 and B2 on hepatic functions of broilers. Poult. Sci. 91, 2792–2801.

Yildirim, E., Yalcinkaya, I., Kanbur, M., Çinar, M. and Oruc, E. 2011. Effects of yeast glucomannan on performance, some biochemical parameters and pathological changes in experimental aflatoxicosis in broiler chickens. Revue Méd. Vét., 2011, 162, 8-9, 413-420.

Yunus, A.W.; Razzazi-Fazeli, E.; Bohm, J. 2011. Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. Toxins, 3, 566–590.

Zhang, W.; Xue, B.B.; Li, M.M.; Mu, Y.; Chen, Z.H.; Li, J.P.; Shan, A. 2014. Screening a strain of Aspergillus niger and optimization of fermentation conditions for degradation of aflatoxin B1. Toxins, 6, 3157–3172.

Zhao, J., Shirley, R.B., Dibner, J.D. Uraizee, F., Officer, M., Kitchell, M., Vazquez-Anon, M., Knight, C.D. 2010. Comparison of HSCAS and yeast cell wall on counteracting aflatoxicosis in broiler chicks. Poult Sci, 89, 2147-2156.

Zhao, L.H.; Guan, S.; Gao, X.; Ma, Q.G.; Lei, Y.P.; Bai, X.M.; Ji, C. 2011. Preparation, purification and characteristics of an aflatoxin degradation enzyme from Myxococcus fulvus ANSM068. J. Appl. Microbiol, 1.