International Journal of Agriculture and Forestry 2014, 4(5): 351-358
DOI: 10.5923/j.ijaf.20140405.02

Commercial Broilers Exposed to Aflatoxin B_1: Efficacy of a Commercial Mycotoxin Binder on Internal Organ Weights, Biochemical Traits and Mortality

Hedayati M.[1,*], M. Manafi[1], M. Yari[1], S. V. Mousavipour[2]

[1]Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran
[2]Private Section Veterinary Clinician, Qom, Iran

Abstract The aim of this study was to determine the binding capacity of a commercial mycotoxin binder (BINDER) for aflatoxin B_1 (AF) and the efficacy of the binder to reduce the adverse effects of AF on broilers fed AF. Two hundred and Forty 1-d-old unsexed broilers (Ross 308) were maintained in chick batteries and allowed ad libitum access to feed and water. A completely randomized design was used with 3 replicate pens of 20 chicks assigned to each of 5 dietary treatments from hatch to 42 d. Dietary treatments included the following: A) control (CON), with no binder or AF, B) AF supplemented at the rate of 0.6ppm, C) 0.2% binder, and D) 0.6ppm AF supplemented with 0.2% binder. On d 42, 8 chicks from each treatment were killed by cervical dislocation and samples of visceral organ weight, cholesterol, HDL, LDL and mortality percentage were recorded. Results showed that weights of Thymus and Kidney had not been affected and Spleen, Liver and Pancreas and cholesterol, HDL and LDL were affected by incorporation of AF in the diet. The addition of binder to the affected parameters could restore some of these adverse effects significantly. Incorporation of only binder had reached the mortality into zero.

Keywords Aflatoxin B_1, Mycotoxin binder, Visceral Organs, Cholesterol, LDL, HDL, Broilers

1. Introduction

In 2012, approximately 82.9 million metric tons of broilers were produced. Nutrition plays as the most important issue in rearing poultry. As per the FAO, 25% of World's cereals are contaminated with mycotoxin. Mycotoxins are an increasingly discussed issue and among them, aflatoxin is the most dangerous and poisonous one in poultry. The toxigenic strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* produce a group of secondary metabolites such as aflatoxins, which are known to be potent mutagenic, carcinogenic, teratogenic, hepatotoxic, immunosuppressive and also inhibit several metabolic systems [1]. Aflatoxins are fungal metabolites found as contaminants in a wide range of food and agricultural products. Aflatoxin B_1, the most commonly occurring aflatoxin, is a potent mutagen and hepatocarcinogen to a wide range of animal species [2]. Chronic exposure to aflatoxins may not only significantly alter productivity and animal farming trends, but may also impose a risk to the consumer from direct exposure to aflatoxin-contaminated food commodities [3]. Formations of these toxins are linked to fungal growth and the environment in which the grains/cereals are stored (especially relative humidity and temperature). Fungal growth and subsequent mycotoxin production in stored grains can be inhibited by physical methods (aeration, cooling, modified atmospheres, etc.) or by fungi stats of which the propionic, acetic and sorbic acids are the most commonly used [4]. These toxins have been incriminated as the cause of high mortality in poultry and livestock and some cases of death in human being [5]. Thus foods contaminated with these toxigenic fungi and presence of aflatoxin is a major concern, which has received worldwide attention due to their deleterious effects on human and animal health as well as their importance in international food trade [6].

Aflatoxin B_1 is widely believed to result in mal-absorption syndrome regarding macro nutrients and also in reduced activity of digestive enzymes [7]. However, many reports contrary to this notion are available. For instance, Nelson *et al.* [8] did not find any effect of AFB_1 (natural contamination of corn with *A. flavus*) on dry matter (DM) and amino acid digestibility and energy utilization in chicken. Applegate *et al.* [9] did not find any effect of 0.6, 1.2 and 2.5 ppm AFB_1 in diet on digestibility of DM and nitrogen (N) per hen/day. At 0.6 and 1.2 mg AFB_1/kg diet, the apparent metabolizable energy (AME) was however found to be reduced in their

* Corresponding author:
mhvet@chmail.ir (Hedayati M.)
Published online at http://journal.sapub.org/ijaf

study. Regarding the activity of pancreatic enzymes, Matur *et al.* [10] found higher amylase and chymotrypsin activity, while lower lipase activity after exposure of Ross 308 female birds to 0.1 mg AFB_1/kg diet (at 427 to 457 days age). The activity of trypsin in pancreas was not affected by AFB_1 treatment. These results, except for reduction in lipase activity, are supported by earlier work of Richardson and Hamilton [11] on layers. These authors reported that 4 ppm AFB_1 in diet increases the activity of pancreatic chymotrypsin, amylase and lipase. Pancreatic trypsin was not affected by AFB_1 in their study and the noted changes in the pancreatic secretions were also not reflected in the lipid content of the feces. Contrary to these two reports, Osborne and Hamilton [12] noted lower activity of pancreatic amylase, trypsin, lipase, RNAse, and DNAse when broilers were exposed to 1.25 and 2.5 mg AFB_1/kg diet.

The use of numerous plant extracts, spices and their constituents may provide an alternative way to prevent fungal growth and aflatoxin formation [13]. It is believed that the extracts of certain spices and herbs of medicinal importance exhibit antifungal property. These natural antifungal agents can be potentially exploited in controlling the growth of fungi and consequently inhibiting aflatoxin formation [14]. The addition of antioxidants is a method of increasing the shelf-life, especially of lipids and lipid containing foods. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene, have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic [15]. Therefore, the importance for search of natural antioxidants especially of plant origin has greatly increased in recent years [16].

Curcumin (diferuloylmethane), a polyphenol, is an active principle of the perennial herb *Curcuma longa* (commonly known as turmeric). The yellow-pigmented fraction of turmeric contains Curcuminoids, which are chemically related to its principal ingredient, curcumin. The major Curcuminoids present in turmeric are curcumin I (77%), demethoxycurcumin (curcumin II) (17%), bisdemethoxycur cumin (curcumin III) (3%) and the recently identified cyclocurcumin. Throughout the Orient, it has traditionally been used to good therapeutic effect, particularly as an anti-inflammatory [17] and many of its therapeutic effects have been confirmed by modern scientific research. Such effects include antioxidant [18], antiinflammatory [19-21], anticarcinogenic and antimicrobial [22], hepatoprotective [23], thrombosuppressive [24], cardiovascular (i.e., as protection against myocardial infarction) [24], hypoglycemic [25] and antiarthritic (i.e., as protection against rheumatoid arthritis) [26]. In another study, it has been reported that turmeric and curcumin, inhibited mutation frequency by more than 80 percent. Dietary administration of turmeric (0.05 per cent), garlic (0.25 per cent), curcumin and ellagic acid (0.005 per cent each) to rats significantly reduced the number of gamma glutamyl transpeptidase-positive foci induced by AFB_1.

One of turmeric's components is curcumin, a type of phytochemical known as a polyphenol. Research findings suggest that phytochemicals, which are the chemicals found in plants, appear to help prevent disease. As the bioactive component of turmeric, curcumin is readily absorbed for use by the body. The addition of products containing minerals from clay products to AFB_1 contaminated diets has been shown to greatly reduce the bioavailability of aflatoxin in the gastrointestinal tract [5]. The liver is the target organ of AFB_1 in broilers and is characterized by a severe hepatic enlargement and fatty infiltration [27]. The liver and kidney are the main organs involved in the detoxification of AFB_1, and also the place which most residues accumulate [28]. The AFB_1 is primarily biotransformated in the liver by cytochrome P-450 associated enzymes, which generate hydroxylated metabolites [2].

Diatomaceous earth refers to a naturally-formed sedimentary mineral coming from the remains of what were once oceanic unicellular shells and algae known as diatoms. Diatoms, the ocean's "spiny honeycombs," are over 30 million years old, and were formed when these microscopic algae-like plants died and remained compounded in the earth's surface as skeletal remains [29]. These organisms, much like a mollusc emits lime-carbonate, had the ability to emit silica. Scientists refer to these clay-like, chalky remains as diatomite [30].

Clays are natural adsorbents chemically made of silicates or aluminosilicates. They include a large range of products such as hydrated sodium calcium aluminosilicates (HSCAS), phyllosilicates (of which montmorillonite or magnesium hydrated HSCAS is one of the major compounds in this group), bentonite and zeolite (the latter two are clays of volcanic origin). Silica is also known as Diatomaceous Earth, made up of 84% Silicon Dioxide (Silica). Bentonite clay carries a uniquely strong negative ionic charge which causes it to “magnetically” attract any substance with a positive ionic charge (i.e., bacteria, toxins, metals, etc.). These substances are both adsorbed (sticking to the outside like Velcro) and absorbed (drawn inside) by the clay molecules. The clay`s immediate action upon the body is directly on the digestive channel. This involves the clay actually binding with the toxic substances and removing them from the body with the stool. It performs this job with every kind of toxin, including those from the environment, such as heavy metals, and those that occur naturally as by-products of the body`s own health processes, such as metabolic toxins. The clay and the adsorbed toxins are both eliminated together; this keeps the toxins from being reabsorbed into the bloodstream [28].

Due to limited forage supplies, more poor quality feeds, such as those high in nitrates, lignin, ash, and mycotoxins will be fed. Feeding extra minerals can help mitigate the negative effects of feeding poor quality forages. Clearly much of the pioneering work with mycotoxin binders was done with silicates and specifically with the HSCAS material. These binders have the property of adsorbing organic substances either on their external surfaces or within their inter-laminar spaces, by the interaction with/or substitution of the exchanged cations within these spaces. Therefore, mycotoxins can be adsorbed into this porous structure and be

trapped by elementary, electric charges. However, clay and zeolitic minerals, which comprise a broad family of diverse aluminosilicates, are not produced equally and thus; do not possess the same physical properties [13].

Eralsan *et al.* [30] reported a moderate increase in the albumin: globulin ratio of broilers by addition of 0.3 per cent hydrated sodium bentonite in aflatoxin mixed feed of broilers. They also reported that histopathological finding in liver sections of broiler fed aflatoxin plus hydrated sodium bentonite indicated a non-protective effect of this adsorbent. Due to their montomorillonite content, bentonites swell and form thixotropic gels, as result of their ion exchange capabilities, they are widely used as mycotoxin sequestering agent [31]. Eralson *et al.* [32] reported the effectiveness of sodium bentonite in reliving the damages due to the presence of aflatoxins (1ppm) in 45 day old broiler chickens.

The aims of the current study were to determine the activity of the commercial mycotoxin binder containing curcuminoids, minerals and enzymes and also to evaluate the protective effects of the binder on internal organ weights, biochemical traits and mortality of broilers fed with Aflatoxin B_1.

2. Materials and Methods

This experiment was planned and carried out in the Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran with objective of evaluating the internal organ weights, biochemical traits and mortality of broilers fed with aflatoxin B_1 and a mycotoxin binder.

Experimental design, housing, management and test diet

240 day-old unsexed Ross 308 strain of broiler chicks were wing banded, weighed and randomly spread in a completely randomized experimental design with four treatments and three replications of twenty chicks in each. Each replicate group of chicks was housed in an independent pen, conventional deep litter house. Chicks in all the replicates were kept up to six weeks of age under uniform standard conditions. Brooding was done till three weeks of age. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided *ad libitum* feed and water throughout the study. Feeding of test diets commenced at first day of age and continued till the termination of experiment at six weeks of age. The temperature was maintained at 30±1°C in the first week and reduced by 2.5°C per week to 21°C. From day one until day 4, the lighting schedule was 24 hour. At days 14-42 the dark time was gradually increased to 4 hour. Diets were prepared to meet the nutrient requirements of commercial broilers during the starter (0-2wks), grower (2-4wks) and finisher (4-6 wks) periods. The composition of diets was adopted from NRC, [33] and is presented in Table 1. The basal diet was formulated using commonly available feed ingredients which were screened for AF prior to the formulation of diets and it is found to be around 280ppb in normal feed. The Aflatoxin B_1 was procured from Sigma Aldrich, USA and diluted to reach to the required level of administration. The experimental diets were prepared by adding required quantity of aflatoxin to arrive at the level of 600ppb of AFB_1. Diets were prepared without addition of aflatoxin and binder as Control (group 1); 600 ppb Aflatoxin B_1 (group 2); 0.2% of binder (group 3) and 600ppb Aflatoxin B_1 + 0.2% of binder (group 4). Niltox, the mycotoxin binder used in this study is a unique composition of minerals (extra purified clay containing diatomaceous earth mineral), antioxidants (Curcuminoids extracted from Turmeric) and enzymes (Epoxidase and Esterase), a property product of Zeus Biotech Limited, Mysore, India. It is claimed that incorporation of this product in poultry diets would effectively prevent DNA adduct formation and cellular damages in the biological systems through degrading peroxides, amides and lacto rings in non-polar toxins such as aflatoxins. This study was undertaken to evaluate the efficacy of a mycotoxin binder for counteracting AFB_1 in experimentally contaminated broiler breeder diets.

Vaccination schedule

The local office of Iranian Veterinary Organization has proposed the required vaccination which is modulated by the veterinarian of Department of Animal Science, Malayer University, as below:

vaccination for Newcastle Disease (ND) virus happened three times: first spray at one days old of chicken in breeder farm, second on the 13th day as B_1, BRONHOPEST B_1 SPF (VETERINA®, Zagreb, Croatia) and (CEVA®, Libourne, France) in drinking water and their booster on 20th day as clone-30 (HIPRAVIAR® CLON, Amer, Spain) through drinking water. Vaccination against Bronchitis virus happened in two times as the following: first spray at commencement of the experiment and it's booster in drinking water on the 10th day, both as H-120 (CEVA®, Libourne, France). Vaccination against Infectious Bronchitis (IB) virus happened in two times: first on day 16 and the second on the 23th day, both as Gambo-l (CEVA®, Libourne, France) in drinking water. The sera were applied to HI test in 28 days, to determine Ab to NDV. In titers lower than 5, the booster B_1, BRONHOPEST B_1 SPF (VETERINA®, Zagreb, Croatia) was administrated in drinking water for broilers.

Studied parameters

Visceral organ weights

Upon obtaining the permission of Ethical Committee of the University, at the end of the trial, six birds from each replicate which were closed to average weight of each replicate were sacrificed by cutting the jugular vein method and blood samples were individually collected in 10-mL heparinized tubes and stored on ice cubes for further hematology analysis. The visceral were then opened and the thymus, spleen, bursa of Fabricius, liver, kidney and pancreas detached and weighed on digital top pan electronic balance (0.1g accuracy) and the later three weighed on

monopan balance (1mg accuracy). The weights were adjusted to one kg live weight (g/kg BW) and treatment wise means were calculated.

Biochemical parameters

The collected blood samples were analysed for cholesterol, LDL and HDL using automatic analyser (Boehringer Mannheim Hitachi 704 automatic analyser, Japan). The methodology and the set of reagents used in respect of each parameter were as per the recommendations of the manufacturer of the analyser system. Data are presented as means of each treatment.

Mortality

The number of dead birds in each replicate was recorded to calculate the mortality percentage. The dead birds were subjected to post-mortem examination to identify the cause of death. The weekly per cent mortality up to 6th week was computed.

Table 1. Ingredients and composition of the basal diets (as-fed basis, %)

Ingredients (%)	Starting diet (0-2wk)	Growing diet (2-4wk)	Finishing diet (4-6wk)
Corm	59.00	67.36	72.01
Soybean meal	33.74	28.63	24.46
Soybean oil	1.56	0.65	0.56
Calcium carbonate	0.60	0.67	0.63
Dicalcium phosphate	1.41	1.02	0.84
Oyster shell	0.66	0.66	0.63
Common salt	0.30	0.30	0.30
Vit. And Min. Permix[1]	0.50	0.50	0.50
DL-Methionine	0.13	0.06	0.02
Lysine – HCL	0.09	0.14	0.05
Calculated analysis			
ME (Kcal/kg)	2900	2950	3000
Crude protein (%)	20.84	18.43	16.87

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (all-trans-retinal); Vit. D3 (cholecalciferol), 2,000 IU; vitamin E, 20 IU (α-tocopherol); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg and antioxidant, 250 mg.

Statistical analysis

The total experimental data were statistically analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS®) software [34]. Overall data were analyzed using one way ANOVA test. Duncan multiple comparisons range test with 0.05 significance level was employed for comparison of the means [35].

3. Results and Discussion

The data on visceral organ weights of broilers fed aflatoxin, toxin binder and their combinations at 42 day of age are shown in Table 2. Results 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. The binder alone fed treatment also has shown a significantly ($P<0.05$) lesser in Spleen weight of broilers. The bursa of Fabricius weight has increased in AF treatment and by addition of toxin binder, the weight is decreased. The binder alone fed group is also significantly ($P<0.05$) decreased, when compared with control group. The Liver weight has also been increased in AF fed group, In AF+BINDER group, the liver weight is decreased to even below the control group level. The Kidney weight has not been affected in different treatments. The Pancreas weight had been decreased significantly ($P<0.05$) in AF group and by adding binder to AF group remained unchanged.

The data on the effects of aflatoxin B_1 and mycotoxin binder on broilers at 42 days of age on selected biochemical parameters are shown in Table 3. Results showed that cholesterol level has been increased significantly ($P<0.05$) by addition of AF into the diet. While adding binder into AF, the cholesterol level had decreased significantly ($P<0.05$), when compared with AF group. The binder alone group has been remained unchanged, when compared with control group. The LDL of broilers fed AF has been increased significantly ($P<0.05$) and by addition of binder to AF group, no statistical changes has been observed. Addition of binder alone into the diet has showed no changes when compared with control group. The HDL levels of broilers fed AF had been increased significantly ($P<0.05$), compared with control group and by addition of binder, no changes were observed, when compared with AF group. The HDL level in binder alone fed group has been increased significantly ($P<0.05$), when compared with control group.

The findings of this trial on mortality percentage of broilers fed different dietary treatments at 6 weeks of age are shown in Table 4. The mortality rate found to be 3.32, 13.31, 0.00 and 1.25% in control, AF, binder and AF+BINDER treatment groups, respectively.

The clear effects of aflatoxin on the broilers have been well documented previously by Manafi *et al.,* [13]. The increase in serum enzyme levels noted during aflatoxicosis can be interpreted as the sequelae of hepatocyte degeneration or damage to the cell membrance and subsequent leakage of enzyme into the circulation [7]. The main causes for the increased mortality are reduced feed intake, altered protein metabolism, altered enzymatic activity and decreased nutrient utilization and absorption.

Table 2. Visceral Organ weights (g/kg live weight) of broilers fed *Aflatoxin B_1* and *Mycotoxin Binder* (Mean±SE)

Treatment groups	Thymus	Spleen	bursa of Fabricius	Liver	Kidney	Pancreas
CON[1]	4.18±0.24[a]	1.77±0.14[a]	1.48±0.01[b]	27.62±0.58[b]	7.42±0.18[a]	3.51±0.65[ab]
AF[2]	4.16±0.35[a]	1.06±0.24[d]	1.95±0.27[a]	31.10±0.18[a]	7.45±0.59[a]	2.53±0.24[b]
BINDER[3]	4.02±0.31[a]	1.36±0.06[b]	1.46±0.27[b]	25.83±0.64[c]	7.38±0.17[a]	3.61±0.17[a]
AF+BINDER[4]	4.08±0.89[a]	1.22±0.17[c]	1.43±0.29[b]	24.42±0.18[c]	7.54±0.69[a]	2.64±0.14[b]

Mean values within a row with different superscript letters (a to d) were significantly different (P<0.05). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]BINDER (mycotoxin binder at 0.2% level) and [4]AF+BINDER (Aflatoxin B_1 and mycotoxin binder at 600ppb and 0.2% levels, respectively). SEM: Standard Error of the Means.

Table 3. Biochemical parameters of broilers fed *Aflatoxin B_1* and *Mycotoxin Binder* (Mean±SE)

Treatment groups	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
CON[1]	97.33±0.48[c]	40.05±0.85[b]	66.33±0.57[c]
AF[2]	143.52±0.36[a]	66.52±0.44[a]	83.29±0.19[a]
BINDER[3]	99.27.±0.31[c]	42.84±0.17[b]	72.18±0.93[b]
AF+BINDER[4]	114.29±0.35[b]	64.27±0.14[a]	82.13±0.71[ab]

Mean values within a row with different superscript letters (a to d) were significantly different (P<0.05). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]BINDER (mycotoxin binder at 0.2% level) and [4]AF+BINDER (Aflatoxin B_1 and mycotoxin binder at 600ppb and 0.2% levels, respectively). SEM: Standard Error of the Means.

Table 4. Mortality rates (%) of chicks fed *Aflatoxin B_1* and *Mycotoxin Binder* (Mean±SE)

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)	Total (day 0-42)
CON[1]	0	1.66	0	0	1.66	0	3.32±0.25[b]
AF[2]	0	0	3.33	1.66	1.66	6.66	13.31±0.94[a]
BINDER[3]	0	0	0	0	0	0	0.00±0.00[d]
AF+BINDER[4]	0	0	0	0	0	1.66	1.25±0.23[c]

Mean values within a row with different superscript letters (a to d) were significantly different (P<0.05). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]BINDER (mycotoxin binder at 0.2% level) and [4]AF+BINDER (Aflatoxin B_1 and mycotoxin binder at 600ppb and 0.2% levels, respectively). SEM: Standard Error of the Means.

Aflatoxin B_1 is metabolized in the liver which is having a high level of metabolizing enzymes and induces damage to this organ even leading to hepatocarcinogenesis [36]. Maurice *et al.* [37] observed significant reduction in plasma proteins when broilers were fed with 50 to 100ppb of AFB_1 through oral route for a period of 0 to 3 weeks.

The current mycotoxin binder used in this study is consisted of curcumin, minerals from clay sources and enzymes. Plant compounds like curcuminoids have inhibitory action on biotransformation of AF to their active epoxide byproducts. Turmeric (*Curcuma longa*), a medicinal plant native to the Asian subcontinent, is known to possess antimicrobial and antioxidant properties. The curcuminoids, yellowish pigments present in turmeric powder, have shown protective effects against AFB_1. Supplementation of curcumin in the diet normalized the altered activities of LDH and ALT induced by AF. At molecular level, curcumin significantly reduced AFB (1)-N (7)-guanine adduct excretion in the urine, DNA adduct in the liver and albumin adduct in the serum [38]. Supplementation of turmeric powder in diets in chicks fed AF contaminated diets improves the antioxidant, biotransformation, and immune system genes in livers of chicks.

Supplementation of turmeric (curcumin) to AF intoxicated ducklings were effective in reducing the liver damage through reduction in AFB_1-DNA adducts formation, and modulation of cytochrome P 450 activity. Addition of turmeric powder (0.5%) containing 1.4 per cent of total curcuminoid to an AFB_1 contaminated chick diet increased the activity of SOD and reduced the peroxide leveling homogenates of broiler chicks [39]. It is found that both turmeric and curcumin inhibited the aflatoxin induced toxicity in ducklings, which have been faced a lower BW due to AF and curcumin could partially reverse this adverse effect. In this study, the other studied parameters like WBC, GPT and ALP remained unaltered [38].

Turmeric was significantly increased total serum proteins at (6^{th} and 8^{th} weeks) and albumin at (8^{th} week) while, AST, cholesterol and triacylglycerol were significantly decreased. In a study on broiler given a diet mixed with turmeric for 45 days. Supplementation of ducklings with turmeric after aflatoxin treatment increased the total serum proteins level than that of (aflatoxicated birds) group. This might be due to the antioxidant effect of them against aflatoxin or due to that turmeric increase the total serum proteins level. In another study, Curcumin admixed with the diet (0.5% w/w)

decreased serum cholesterol by about 21% and LDL-cholesterol by 42.5%, but it increased serum HDL by 50%. In G6, turmeric was reverted the reduction in (total proteins and albumin) levels and the elevation in (ALT, AST, cholesterol and triacylglycerol) which induced by AF as turmeric opposed the AF hepatotoxicity [4].

Obtained data evidenced the protective effect of turmeric against the oxidant adverse effect induced by AF. Curcumin's antioxidant properties might not be only due to its chemical nature as a free radical scavenger, but also due to its ability to induce GSH clinked defense mechanisms against oxidative stress as well as increases in the activity of γ-glutamyl cysteinyl synthase, the rate limiting step in glutathione synthesis. Moreover, induces de novo synthesis of GSH by stimulating the activity and gene expression of glutamate-cysteine ligase [29]. Turmeric as it increases the synthesis of glutathione as the antioxidant mechanisms against any xenobiotic including aflatoxin.

Clay materials have the capability to bind molecules of certain sizes and configurations and have been used effectively to decrease effects of AF contaminated grains in poultry. It is postulated that the clays formed a complex with the toxin thus preventing the absorption of AF across the intestinal epithelium. Due to their montmorillonite content, clay minerals swell and form thixotropic gels as result of their ion exchange capabilities and are widely used as mycotoxin sequestering agent (Duarte and Smith, 2005). Eralson *et al.* [32] reported the effectiveness of sodium bentonite in relieving the damages due to the presence of aflatoxins (1.00ppm) in 45 day old broiler chickens.

Santurio *et al.* [40] evaluated the protective effects of clays in the prevention of aflatoxicosis and concluded that sodium bentonite partially neutralized the effects of AF in broiler chicks when included at 5.00g/kg in the diet. In a similar study, Rosa *et al.* [41] reported moderate protective effect of 0.3 per cent bentonite against the development of aflatoxicosis in broilers.

According to Surai, [42] and Weiss, [43], the antioxidant system in the body mainly involves reducing agents (tocopherol, ascorbic acid, glutathione, and carotenoids), peroxidases (glutathione peroxidase, catalase), enzymes (peptidases, proteases, vitamin A) and superoxide dismutase (SOD). The most common functional chemical groups which have radical scavenging properties are hydroxyl (phenolics), sulfhydryl (cysteine, glutathione), and amino groups (uric acid, spermine). Antioxidative phenolics include tocopherol, catechins, ubiquinone and synthetic compounds (Butylated hydroxyanisole, Butylated Hyrdoxytoluene).

There is sufficient evidence to suggest that antioxidants ameliorate oxidative stress during mycotoxicosis by reducing the level of free radicals. Several natural (vitamins, pro-vitamins, carotenoids, polyphenols, and micronutrients) and synthetic compounds seem to be chemo protective against common mycotoxins. However, most data available on the above aspect are from *in vitro* studies. The antioxidant activity of phenolics is influenced by alkyl and hydroxyl groups which can enhance their reactivity to neutralize lipid radicals.

It has been reported that spices owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins. All antioxidants are not suitable for adding to diets due to stability concerns, solubility, and interaction with other feed components [44]. The enzymes are believed to break the functional atomic group of the mycotoxin molecule and thereby render them nontoxic [45]. Enzymes viz., carboxylesterase present in the microsomal fraction of the liver, esterase and epoxidase are being tried for their practical applicability in the field conditions [46].

Compared with the current study, the adsorbents used in studies by Ledoux *et al.* [47] and Shi and Davis, [48] were effective in ameliorating the toxic effects of aflatoxin on relative liver and kidney weights. Similar to the current study, Kubena *et al.* [49] also did not observe any benefits to relative organ weights using 0.25% of an adsorbent. The liver is considered the target organ during aflatoxicosis in poultry because the relative liver weight is significantly increased by lower levels of aflatoxin compared with any other organ [27] and becomes pale with rounded margins [47].

Besides being the primary organ of AFB_1 accumulation and metabolism, liver is also the main site where AFB_1 is metabolized and where the metabolites bind with nucleic acids and proteins. Kidneys also take part in detoxification of aflatoxins and are also among the organs where most of the aflatoxin residues are detected.

It is a general observation that size of lymphoid organs is not normal in birds exposed to AFB_1. In such animals, lymphoid cell depletion in thymus, spleen, and bursa of Fabricius has been described [50]. Thus one explanation of immune-toxicity of AFB_1, as also proposed by Azzam and Gabal, [51], 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 [52].

While investigating the mechanisms of curcumin's chemo preventive effects, in another study, curcumin's effect on serum cholesterol and lipid peroxide levels in 10 healthy volunteers. Daily administration of curcumin (500 mg) for 7 days led to a significant 33% decrease in serum lipid peroxides, a 29% increase in serum HDL cholesterol, and a nearly 12% decrease in total serum cholesterol. Together, these striking findings suggest a potential chemopreventive role for curcumin in arterial disease. In concordant with these findings are results of another study in which curcumin (10mg) administered twice a day for 28 days lowered serum LDL and increased serum HDL levels in patients with atherosclerosis [52].

4. Conclusions

The purpose of this study was to introduce the activity of curcuminoids, minerals and enzymes, as a mycotoxin binder in broilers on internal organ weights, biochemical traits

and mortality of broilers fed with Aflatoxin B_1. Results obtained from this study concludes that besides the adverse and negative effects of aflatoxin B_1 in the diet on visceral organ weights, some biochemical parameters and mortality of broilers with a commercial mycotoxin binder can rule its beneficial effects to some extent. However, there is insufficiency in the marks of its beneficial effects in nutrient digestibility and gut function of broilers.

ACKNOWLEDGEMENTS

This study was funded by Directorate of Research, Malayer University, Malayer, Iran.

REFERENCES

[1] Li, Y., Liu, Y.H., Yang, Z.B., Wan, X.L. and Chi, F., 2012. The efficacy of clay enterosorbent to ameliorate the toxicity of aflatoxin B_1 from contaminated corn (*Zea mays*) on hematology, serum biochemistry, and oxidative stress in ducklings. J. Appl. Poult. Res. 21:806–815.

[2] Biehl, M.L., and W. B. Buck. 1987. Chemical contaminants: Their metabolism and their residues. J. Food Prot. 50:1058–1073.

[3] Ramos, A. J. and E. Hernandez. 1996. In vitro aflatoxin adsorption by means of montmorillonite silicate. A study of adsorption isotherms. Anim. Feed Sci. Technol. 62:263–269.

[4] Manafi, M. and Khosravinia, H., 2013. Effects of Aflatoxin on the Performance of Broiler Breeders and Its Alleviation by Herbal Mycotoxin Binder. Journal of Agricultural Science and Technology. 15: 55-63.

[5] Phillips, T.D. 1999. Dietary clay in the chemoprevention of aflatoxin- induced disease. Toxicol. Sci. 52:118–126.

[6] Mishra, H.N. and Das, C., 2003. A review on biological control and metabolism of aflatoxin. Critical Rev. Food Sci. Nutr., 43: 245-264.

[7] Devegowda, G. and Murthy, T.N.K. 2005. Mycotoxins: Their effects in poultry and some practical solutions. In The Mycotoxin Blue Book; Diaz, D.E., Ed.; Nottingham University Press: Nottingham, UK, pp. 25–56.

[8] Nelson, T.S., Johnson, Z.B., Kirby, L.K. and Beasley, J.N. 1982. Digestion of dry matter and amino acids and energy utilization by chicks fed molded corn containing mycotoxins. Poult. Sci., 61, 584–585.

[9] Applegate, T.J., Schatzmayr, G., Pricket, K., Troche, C. and Jiang, Z. 2009. Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. Poult. Sci., 88, 1235–1241.

[10] Matur, E., Ergul, E., Akyazi, I., Eraslan, E. and Cirakli, Z.T. 2010. The effects of *Saccharomyces cerevisiae* extract on the weight of some organs, liver, and pancreatic digestive enzyme activity in breeder hens fed diets contaminated with aflatoxins. Poult. Sci., 89, 2213–2220.

[11] Richardson, K.E. and Hamilton, P.B. 1987. Enhanced production of pancreatic digestive enzymes during aflatoxicosis in egg-type chickens. Poult. Sci., 66, 640–644.

[12] Osborne, D.J. and Hamilton, P.B. 1981. Decreased pancreatic digestive enzymes during aflatoxicosis. Poult. Sci., 60, 1818–1821.

[13] 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.

[14] Škrinjar, M., A. Mandić, A. Mišan, M. Sakač, Lj. Šarić and M. Zec. 2009. Effect of mint (*Mentha piperita L.*) and caraway (*Carvum carvi L.*) on the growth of some toxigenic Aspergillus species and aflatoxin B_1 production. Proc. Nat. Sci, Matica Srpska Novi Sad 116: 131-139.

[15] Madhavi, D.L. and D.K. Salunkhe. 1995. Antioxidants, In: Food Additive Toxicology, J.A. Maga and A.T. Tu, eds. Marcel Dekker, New York. pp. 89-177 of 542 pp.

[16] Najafi, S. and Taherpour K. 2014. Effects of Dietary Ginger (*Zingiber Officinale*), Cinnamon (*Cinnamomum*), Synbiotic and Antibiotic Supplementation on Performance of Broilers. J. Anim. Sci. Adv., 2014, 4(1): 658-667.

[17] Hara-Kudo, Y., A. Kobayashi, Y. Sugita-Konishi and K. Kondo. 2004. Antibacterial activity of plants used in cooking for aroma and taste. J. Food Protect. 67: 2820–2824.

[18] Moreira, M.R., A.G. Ponce, C.E. Del Valle and S.I. Roura. 2005. Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT–Food Sci. Tech. 38, 5: 565–570.

[19] Gutierrez, J., C. Barry-Ryan and P. Bourke. 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol. 124: 91-97.

[20] Gutierrez, J., C. Barry-Ryan and P. Bourke. 2009. Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. Food Microbiol. 26: 142-150.

[21] Glass, K.A. and E.A. Johnson. 2004. Antagonistic effect of fat on the antibotulinal activity of food preservatives and fatty acids. Food Microbiol. 21: 675–682.

[22] Juven, B.J., J. Kanner, F. Schved and H. Weisslowicz. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. App. Bacteriol. 76: 626–631.

[23] Lopez, P., C. Sanchez, R. Batlle and C. Nerin. 2005. Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. J. Agr. Food Chem. 53: 6939–6946.

[24] Škrinjar M., I. Kolarović, G. Dimić and O. Jovanović. 2000. Pojava plesni i nekih mikotoksina u sirovinama korišćenim u konditorskoj industriji. Žito hleb 27, 1: 9-14.

[25] Škrinjar, M. 2005. Uljarice – mogući nosioci mikotoksina. Uljarstvo 36, 1-2: 47-50.

[26] Šarić, Lj. and M. Škrinjar. 2008. Share of aflatoxigenic moulds from genera Asspergillus and Penicillium in mycopopulations isolated from spices for meat processing industry. Proceedings for Natural Sciences 114: 115-122.

[27] Huff, W. E., L. F. Kubena, R. B. Harvey, D. E. Corrier, and H.

H. Mollenhauer. 1986. Progression of aflatoxicosis in broiler chickens. Poult. Sci. 65:1891–1899.

[28] Hussain, Z., M. Z. Khan, A. Khan, I. Javed, M. K. Saleemi, S. Mahmood, and M. R. Asi. 2010. Residues of aflatoxin B_1 in broiler meat: Effect of age and dietary aflatoxin B_1 levels. Food Chem. Toxicol. 48:3304–3307.

[29] Grant, P.G. and T.D. Phillips. 1998. Isothermal adsorption of aflatoxin B_1 on HSCAS clay. J. Agric. Food Chem. 46: 599-605.

[30] Eralsan, G., D. Essz, M. Akdogan, F. Sahindokuyucu and L. Altrintas. 2005. The effects of aflatoxin and sodium bentonite and alone on some blood electrolyte levels in broiler chickens. Turk Veterinerlik ve Hayvanclk Dergisi. 29: 601-605.

[31] Duarte, E.D. and T.K. Smith. 2005. Mycotoxin blue book. Nottingham University press. pp. 323-339.

[32] Eralson, G., Essz, D., Akdogan, M., Karoz, E., Oncu, M. and Ozyldz, Z., 2006. Efficacy of dietary sodium bentonite against subchronic exposure to dietary aflatoxin in broilers. Bulletin of the veterinary Institute in Puawy. 50: 107-112.

[33] NRC, National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Sci., Washington.

[34] SAS. Institute. 2000. SAS® User's Guide: Statistics. Version & Edition. SAS Institute Inc., Cary, NC, USA.

[35] Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics. 11:1-42.

[36] Hsieh, D.P.H., 1985. An assessment of liver cancer risk posed by aflatoxin B_1 in western world. In: Trichothecenes and other mycotoxins. 11: 369-383.

[37] Maurice, D.V., Bodine, A.B. and Roher, N.J., 1983. Metabolical effects of low level of aflatoxin B_1 on broiler chicks. App. Environ. Microbiol., 45: 980-984.

[38] Soni, K.B., A. Rajan and R. Kuttan. 1992. Reversal of aflatoxin induced liver damage by turmeric and curcumin. Cancer Letters, 66: 115- 121.

[39] Gowda, N.K.S., Ledoux, D.R., Rottinghaus, G.E., Bermudez, A.J. and Chent, Y.C., 2008. Efficacy of Turmeric (Curcuma longa), containing a known level of Curcumin, and Hydrated Sodium Calcium Aluminosilicates to ameliorate the adverse effects of aflatoxin in broiler chicks. Poult. Sci., 87: 1125-1130.

[40] Santurio, J.M., Mallmannl, C.A., Rosa, A.P., Appel, G., Heer, A., Dageforde, S. and Bottcher, M., 1999. Effect of sodium bentonite on the performance and blood variables of broiler chickens intoxicated with aflatoxin. Br. Poult. Sci., 40: 115-119.

[41] Rosa, C.A.R., Miazoo, R., Magnoli, C., Salvano, M., Chicchiera, S.M., Ferrero, S., Saenz, M. Carvalho, E.C.Q., and Dalcero, A. 2001. Evaluation of the efficacy of bentonite from the south Argentina to ameliorate the toxic effects of aflatoxin in broilers. Janerio Institute de Veterrinaria, Brazil.

[42] Surai, P.F. 2001. Natural antioxidants in avian nutrition and reproduction. 1st ed. Nottingham University press, U.K.

[43] Weiss, W.P. 2002. Amntioxidants nutrients and milk quality. In: Roche symposium, pacific Northwest nutrition conference, Vancouver, British Columbia, Canada. 67–103.

[44] Shahidi, F., Janitha, P. K., and Wanasundara, P. D. (1992). Phenolic antioxidants. Critical Reviews of Food Science & Nutrition, 32(1).

[45] Kumar, A.A., S.K. Chanda, A.T. Rao, P.C. Biscoi, and P.K. mishra. 1993. Clinico-pathological changes in experimental aflatoxicosis in quails. Indian J. Poult. Sci., 28:150-153.

[46] Pasteiner, S. 1997. Copying with mycotoxin contaminated feed stuffs. Feed Int., 18: 12-16.

[47] LeDoux, J. E. 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23:155–184.

[48] Shi, C. and Davis, M. 1999. Pain pathways involved in fear conditioning measured with fear-potentiated startle: Lesion studies. J. Neurosci. 19:420–430.

[49] Kubena, L.F., R.B. Harvey, R.H. Bailey, S.A. Buckley and G.E. Rottinghaus. 1998. Effects of a hydrated sodium calcium aluminosilicate (T-BindTM) on mycotoxicosis in young broilers chickens. Poult. Sci., 77: 1502-1509.

[50] 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.

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

[52] Carrillo, M.C., Monti, J.A., Grosman, M.E. and Rodriguez Garay, E.A. 1985. Effect of pH on aflatoxin B_1 transfer in the everted rat jejunum. Toxicol. Lett., 27, 35–44.