Commercial Broilers Exposed to Aflatoxin B<sub>1</sub>: Efficacy of a Commercial Mycotoxin Binder on Internal Organ Weights, Biochemical Traits and Mortality

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Abstract  The aim of this study was to determine the binding capacity of a commercial mycotoxin binder (BINDER) for aflatoxin B<sub>1</sub> (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<sub>1</sub>, 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<sub>1</sub>, 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<sub>1</sub> 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<sub>1</sub> (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<sub>1</sub> in diet on digestibility of DM and nitrogen (N) per hen/day. At 0.6 and 1.2 mg AFB<sub>1</sub>/kg diet, the apparent metabolizable energy (AME) was however found to be reduced in their
against rheumatoid arthritis) [26]. In another study, it has been reported that AFB1 in diet increases the activity of pancreatic chymotrypsin, amyase and lipase. Pancreatic trypsin was not affected by AFB1 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 AFB1/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 of search for natural antioxidants especially 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%), bisdemethoxycurcumin (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], thrombospessive [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 AFB1.

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 AFB1 contaminated diets has been shown to greatly reduce the bioavailability of aflatoxin in the gastrointestinal tract [5]. The liver is the target organ of AFB1 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 AFB1, and also the place which most residues accumulate [28]. The AFB1 is primarily biotransformed 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].

Eralson 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 B1.

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 B1 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 B1 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 AFB1. Diets were prepared without addition of aflatoxin and binder as Control (group 1); 600 ppb Aflatoxin B1 (group 2); 0.2% of binder (group 3) and 600ppb Aflatoxin B1 + 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 AFB1 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 B1, BRONHOPEST B1 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 B1, BRONHOPEST B1 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, %)

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

1The 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 (a-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.

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 B₁ 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 membrane 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.
Aflatoxin B<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub>. Supplementation of curcumin in the diet normalized the altered activities of LDH and ALT induced by AF. At molecular level, curcumin significantly reduced AFB<sub>1</sub>-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<sub>1</sub>-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<sub>1</sub> 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, 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].

Table 2. Visceral Organ weights (g/kg live weight) of broilers fed Aflatoxin B<sub>1</sub> and Mycotoxin Binder (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Thymus</th>
<th>Spleen</th>
<th>bursa of Fabricius</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4.18±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.77±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.62±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.42±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.51±0.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AF&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.16±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.95±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.10±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.45±0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.53±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BINDER&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.02±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.83±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.38±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.61±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AF+BINDER&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.08±0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.22±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.42±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.54±0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.64±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values within a row with different superscript letters (a to d) were significantly different (P<0.05). 1CON (Control), 2AF (Aflatoxin B<sub>1</sub> at 600ppb level), 3BINDER (mycotoxin binder at 0.2% level) and 4AF+BINDER (Aflatoxin B<sub>1</sub> 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<sub>1</sub> and Mycotoxin Binder (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Cholesterol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON&lt;sup&gt;1&lt;/sup&gt;</td>
<td>97.33±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.05±0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.33±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AF&lt;sup&gt;2&lt;/sup&gt;</td>
<td>143.52±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.52±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.29±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BINDER&lt;sup&gt;3&lt;/sup&gt;</td>
<td>99.27±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.84±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.18±0.93&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AF+BINDER&lt;sup&gt;4&lt;/sup&gt;</td>
<td>114.29±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.27±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.13±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values within a row with different superscript letters (a to d) were significantly different (P<0.05). 1CON (Control), 2AF (Aflatoxin B<sub>1</sub> at 600ppb level), 3BINDER (mycotoxin binder at 0.2% level) and 4AF+BINDER (Aflatoxin B<sub>1</sub> 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<sub>1</sub> and Mycotoxin Binder (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Week I (day 7)</th>
<th>Week II (day 14)</th>
<th>Week III (day 21)</th>
<th>Week IV (day 28)</th>
<th>Week V (day 35)</th>
<th>Week VI (day 42)</th>
<th>Total (day 0-42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0</td>
<td>1.66</td>
<td>0</td>
<td>1.66</td>
<td>0</td>
<td>3.32±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.64±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AF&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>3.33</td>
<td>1.66</td>
<td>1.66</td>
<td>6.66</td>
<td>13.31±0.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BINDER&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71±0.015&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AF+BINDER&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0</td>
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<td>0</td>
<td>1.66</td>
<td>1.25±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

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

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 chemoprotective 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 AFB1 accumulation and metabolism, liver is also the main site where AFB1 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 AFB1. In such animals, lymphoid cell depletion in thymus, spleen, and bursa of Fabricius has been described [50]. Thus one explanation of immune-toxicity of AFB1, 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 chemopreventive 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.
and mortality of broilers fed with Aflatoxin B₁. Results obtained from this study concludes that besides the adverse and negative effects of aflatoxin B₁ 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.

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REFERENCES


