Efficacy of Turmeric (Curcuma longa), Containing a Known Level of Curcumin, and a Hydrated Sodium Calcium Aluminosilicate to Ameliorate the Adverse Effects of Aflatoxin in Broiler Chicks

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ABSTRACT A 3-wk feeding study was conducted to evaluate the efficacy of turmeric (Curcuma longa) powder (TMP), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate (HSCAS; Improved Milbond–TX, IMTX, an adsorbent, Milwhite Inc., Houston, TX) to ameliorate the adverse effects of aflatoxin B1 (AFB1) in broiler chicks. Four pen replicates of 5 chicks each were assigned to each of 7 dietary treatments, which included the basal diet not containing TMP, HSCAS, or AFB1 (control); basal diet supplemented with 0.5% food grade TMP that contained 1.48% total curcuminoids (74 mg/kg); basal diet supplemented with 0.5% HSCAS; basal diet supplemented with 1.0 mg/kg AFB1; basal diet supplemented with 0.5% TMP and 1.0 mg/kg AFB1; basal diet supplemented with 0.5% HSCAS and 1.0 mg/kg AFB1; and basal diet supplemented with 0.5% TMP, 0.5% HSCAS, and 1.0 mg/kg AFB1. The addition of TMP to the AFB1 diet significantly (P < 0.05) improved the weight gain of chicks, and the addition of HSCAS to the AFB1 diet significantly (P < 0.05) improved feed intake and weight gain, and reduced relative liver weight. The addition of TMP or HSCAS and TMP with HSCAS ameliorated the adverse effects of AFB1 on some of the serum chemistry parameters (total protein, albumin, cholesterol, calcium). Further, decreased antioxidant functions in terms of level of peroxides, superoxide dismutase activity, and total antioxidant concentration in liver homogenate due to AFB1 were also alleviated by the inclusion of TMP, HSCAS, or both. The reduction in the severity of hepatic microscopic lesions due to supplementation of the AFB1 diet with TMP and HSCAS demonstrated the protective action of the antioxidant and adsorbent used in the present study.

Key words: aflatoxin B1, aluminosilicate, curcumin, broiler, turmeric

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INTRODUCTION

Aflatoxins (AF), a class of mycotoxins produced by the fungi Aspergillus parasiticus and Aspergillus flavus are major contaminants of common feed ingredients used in poultry rations (Smith et al., 1995). Aflatoxin B1 (AFB1) is the most biologically active form of AF and causes poor performance, liver lesions, and immunosuppression in poultry (Kubena et al., 1993; Ledoux et al., 1998). One of the negative effects of AFB1 is cell damage, release of free radicals, and lipid peroxidation (Surai, 2002). Adsorbents have been employed to ameliorate the toxic effects of AFB1 in poultry diets, and certain aluminosilicate binders have shown beneficial effects (Phillips et al., 1988; Ledoux et al., 1998). Because lipid peroxidation plays a major role in the toxicity of AF, a protective effect of antioxidants is possible (Galvano et al., 2001). Plant compounds like coumarins, flavonoids, and curcuminoids have inhibitory action on biotransformation of AF to their active epoxide derivatives (Lee et al., 2001). Turmeric (Curcuma longa), a medicinal plant native to the Asian subcontinent, is known to possess antimicrobial and antioxidant properties. The powder of dried roots and rhizomes of turmeric is used as one of the spices in Indian curries and other cuisine. The curcuminoids, yellowish pigments present in turmeric powder, have shown protective effects against AFB1 (Soni et al., 1997). The most recent dietary approach to prevent mycotoxicoses in poultry is the combined use of antioxidants and adsorbents (Surai, 2002). The objectives of this study were to evaluate the efficacy of using turmeric powder (TMP), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate (HSCAS) singly or in combination to ameliorate aflatoxicosis in broiler chicks and to demonstrate that the inclusion of TMP and HSCAS in poultry diets would not negatively affect the performance of chicks.

MATERIALS AND METHODS

Experimental Design and Birds

One hundred forty 1-d-old (Cobb × Cobb) male broiler chicks were purchased from a commercial hatchery (Hoover’s Hatchery Inc., Rudd, IA), weighed, wing banded,
and assigned to pens in stainless steel chick batteries based on initial body weights. Chicks were maintained on a 24-h continuous light schedule and allowed ad libitum access to feed and water during the 21-d study. The animal care and use protocol was reviewed and approved by the University of Missouri–Columbia Animal Care and Use Committee. A completely randomized design was used with 4 pen replicates of 5 chicks assigned to each of 7 dietary treatments. Mortality was recorded as it occurred, and birds were inspected daily for any health-related problems.

### Diets

The corn-soybean meal based basal diet (mash form) was formulated to meet or exceed the nutritional requirements of chicks from hatch to 3 wk as recommended by the NRC (1994), using individual ingredients obtained from the University feed mill (Table 1). Trace mineral and vitamin premixes (0.2%) were added to the basal diet but no commercial antioxidant was included in the basal diet. Dietary treatments evaluated included the basal diet not containing TMP, HSCAS, or AFB1 (control); basal diet supplemented with 0.5% food grade TMP containing 1.48% total curcuminoids (74 mg/kg); basal diet supplemented with 0.5% HSCAS (Improved Milbond–TX, Milan, TX); basal diet supplemented with 1.0 mg/kg AFB1 by including ground Aspergillus parasiticus (NRRL 2999) culture material containing 760 mg/kg AFB1, 28 mg/kg AFB2, 320 mg/kg AFG1, and 10 mg/kg AFG2; basal diet supplemented with 0.5% TMP and 1.0 mg/kg AFB1; basal diet supplemented with 0.5% HSCAS and 1.0 mg/kg AFB1; and basal diet supplemented with 0.5% TMP, 0.5% HSCAS, and 1.0 mg/kg AFB1.

Dietary AF (B1, B2, G1, G2) concentrations were confirmed by analysis. In brief, feed samples were extracted with acetonitrile and water (86:14), and an aliquot of the extract was passed through a puriTox TC-M160 cleanup column and suitably diluted with water before analysis using HPLC with cobra cell post column derivatization with fluorescence detection at 365 nm excitation and 440 nm emission. All diets were screened by the method of Rottinghaus et al. (1982, 1992) for the presence of citrinin, T-2 toxin, vomitoxin, zearalenone, fumonisins, and ochratoxin A, before the start of the experiment and found to be below detection limits for these mycotoxins.

### Sample Collection

On d 21, birds were weighed by pen and total feed consumption recorded for each pen. Average feed intake and weight gain were corrected for mortality while calculating feed conversion for each pen. Eight chicks (4 replicates of 2 chicks each) from each treatment were selected randomly, killed with carbon dioxide, and blood collected via cardiac puncture for serum chemistry analysis. Liver weight of each bird was recorded, and a piece of liver tissue (2 to 3 g) was collected, rinsed with ice-cold phosphate buffered saline (pH 7.4) containing 0.16 mg of heparin per mL to prevent blood clot formation. The liver tissue was quickly preserved in a preweighed centrifuge tube under ice-cold conditions for assay of antioxidant status. Liver tissue samples from 6 birds from each treatment were fixed in 10% neutral buffered formalin for histopathologic evaluation.

### Serum Chemistry and Liver Antioxidant Status

Blood was centrifuged at 1,400 × g at 8°C for 30 min (Sorvall, RC 3 B plus) and serum separated and preserved at −20°C until submitted for biochemical analysis. Serum samples were analyzed for total protein, albumin, cholesterol, uric acid, gamma glutamyl transferase (EC 2.3.2.2), Ca, and P using an auto analyzer (Kodak Ektachem Analyzer, Eastman Kodak Co., Rochester, NY).

Liver tissue was diluted with ice-cold phosphate buffered saline (pH 7.4) without heparin at a ratio of 1:9, homogenized in a homogenizer (Tekmar, SDT 1810, Cincinnati, OH), and centrifuged (10,000 × g, 4°C, 15 min). The clear supernatant was aspirated into vials and preserved in different aliquots at −80°C until antioxidant status was determined. The parameters measured included total antioxidant concentration, lipid peroxide, aqueous peroxide, total protein, superoxide dismutase (SOD; EC 1.15.1.1), and catalase (EC 1.11.1.16) using assay kits (Sigma Diagnostics, Sigma Chemical Co., St. Louis, MO).

| Table 1. Ingredient composition of basal ration |
|-----------------|-----------|
| Ingredient     | Composition (%) |
| Corn            | 53.38      |
| Soybean meal    | 34.61      |
| Corn oil        | 5.89       |
| Pork meal       | 3.54       |
| Dicalcium phosphate | 1.03     |
| Limestone       | 0.75       |
| Salt            | 0.41       |
| DL-Methionine   | 0.19       |
| Trace mineral mix | 0.10     |
| Selenium PM     | 0.05       |
| Vitamin mix     | 0.05       |
| Copper sulfate  | 0.004      |
| Total           | 100.0      |

1Trace mineral mix provided (mg/kg of diet): manganese, 110 mg from MnSO4; iron, 60 mg from FeSO4·7H2O; zinc, 110 mg from ZnSO4; iodine, 2 mg from ethylenediamine dihydroiodide.

2Selenium premix provided 0.2 mg of Se/kg of diet from Na2SeO3.

3Vitamin mix supplied (per kg of feed): vitamin A (retinyl acetate), 8,800 IU; cholecalciferol, 3,855 ICU; vitamin E (α-tocopheryl acetate), 14 IU; niacin, 55 mg; calcium pantothenate, 17 mg; riboflavin, 6.6 mg; pyridoxine, 2.2 mg; manadione sodium bisulphite, 1.7 mg; folic acid, 1.4 mg; thiamin mononitrate, 1.1 mg; biotin, 0.2 mg; cyanocobalamine, 11 μg.

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**Histopathology**

Fixed liver samples were trimmed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for microscopic examination. The lesions were recorded using a score system of 1 to 4 (1 = liver unremarkable; 2 = mild aflatoxicosis lesions; 3 = moderate aflatoxicosis lesions; 4 = severe aflatoxicosis lesions).

**Total Curcuminoid Analysis**

Turmeric powder was analyzed for total curcuminoid content, including curcumin, bisdemethoxycurcumin, and demethoxycurcumin. Procedures used in the current study were adapted from Jayaprakasha et al. (2002). Briefly, 10 g of turmeric powder was extracted with 50 mL of methanol. After extraction, hexane was discarded, and turmeric powder was dried and finely ground. One gram of hexane extracted powder was re-extracted with 20 mL of methanol for 2 h. An aliquot of the extract was transferred to a microcentrifuge tube and centrifuged at 26,450 × g for 5 min. One milliliter of the supernatant was removed and diluted with 4 mL of methanol. Total curcuminoid content (curcumin, bisdemethoxycurcumin, and demethoxycurcumin) was determined by HPLC.

The HPLC system consisted of a Hitachi Model L-7100 liquid chromatograph pump equipped with a Hitachi Model L-7400 UV detector, Hitachi Model L-7200 autosampler, 250 × 4.6 mm HyperSil reverse-phase C18 column (5 μm particle size) (Phenomenex), Hitachi D-7000 data acquisition interface, and Concert Chrom software at a detection wavelength of 425 nm. The mobile phase was a 5:55:50 mixture of methanol:acetonitrile:2% acetic acid with a flow rate of 1 mL/min. Because bisdemethoxycurcumin and demethoxycurcumin standards are not readily available commercially, they were estimated by comparing their peak areas to that of the standard curcumin peak area. Total curcuminoid content of TMP was determined by totaling the concentration of the individual pigments.

**Statistical Analysis**

Data were analyzed as a 1-way ANOVA by the general linear model procedures of SAS (SAS Institute, 1996). Pens were used as the experimental unit for performance, liver weight, serum chemistry, and antioxidant data. The means for treatments showing significant differences in the ANOVA were compared using Fisher’s protected least significant difference procedure at a significance level of 0.05.

**RESULTS AND DISCUSSION**

**Performance of Broiler Chicks**

Chicks fed TMP or HSCAS alone had similar feed intake, body weight gain, and feed conversion as control chicks (Table 2). Compared with controls, chicks fed 1 mg/kg AFB1 had significantly lower feed intake and weight gain. Addition of 0.5% TMP, containing 1.48% total curcuminoid (74 mg/kg), to the AFB1 diet increased feed intake (906 vs. 858 g) but significantly improved weight gain (746 vs. 662 g) in chicks, suggesting antioxidant protection by TMP. The addition of 0.5% HSCAS to the AFB1 diet improved both feed intake and body weight gain, and the performance was comparable with that of control chicks. The addition of TMP to the diet containing both AFB1 and HSCAS did not result in further improvement in chick performance when compared with chicks fed the diet containing both AFB1 and HSCAS, indicating that the adsorbent (HSCAS) was more effective in ameliorating the toxic effects of AFB1 than the antioxidant (TMP).

Compared with control chicks, relative liver weight was increased in chicks fed the diet containing AFB1 alone. Although the reduction in liver weight in chicks fed the combination of TMP and AFB1 diet was not significant compared with liver weight of chicks fed AFB1 alone, it was comparable with that of control chicks, indicating partial hepatoprotection due to the feeding of TMP. Supplementation of HSCAS to the AFB1 diet was more effective than TMP in reducing the toxic effects of AFB1 in the liver.

Reduced feed intake, lower body weight gain, and heavier livers observed in chicks fed AFB1 alone are consistent with previous reports on the performance depressor effects of AFB1 (Kubena et al., 1990; Ledoux et al., 1998). The addition of TMP to the AFB1 diet partially improved the performance of chicks in the present study. Feeding curcumin (5 μg/d) for 14 d to AF intoxicated ducklings, reversed fatty changes, necrosis, and biliary hyperplasia in the liver (Soni et al., 1992). Curcumin, the major antioxidant ingredient of turmeric, is known to inhibit the biotransformation of AFB1 to aflatoxicol in liver (Lee et al., 2001) and is also responsible for its anti-mutagenic and anticarcinogenic action (Chun et al., 1999). Recently, Emadi and Kermanshahi (2007) fed broiler chicks turmeric powder (0.25, 0.5, 0.75%) from hatch to 49 d and concluded that turmeric might have some positive effects on liver enzymes by reducing alanine amino transferase and alkaline phosphatase activities that directly or indirectly reflect a healthier liver status in the birds. However, the authors did not report the curcumin content of the turmeric used in their study; therefore, it is difficult to draw comparisons to the present study. In the present study, HSCAS at 0.5% of the diet almost completely ameliorated the adverse effects of AFB1. The silica binders have been shown to bind AFB1 in the digestive tract, making them unavailable for gut absorption and allowing harmless passage through the animal (Phillips et al., 1990). The supplementation of the AFB1 diet with a combination of both TMP and HSCAS did not result in any further benefits as compared with either TMP or HSCAS alone, suggesting that the concentration of curcuminoids (74 mg/kg) supplied by the level of TMP (0.5%) employed in this study was too low to exert a more potent antioxidant action. Total curcuminoid content of the TMP used...
Table 2. Performance and relative liver weight of chicks fed diets containing antioxidant, adsorbent, and aflatoxin

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Feed intake (g/chick)</th>
<th>Body weight gain (g/chick)</th>
<th>Feed conversion</th>
<th>Relative liver weight (% body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,047 a</td>
<td>840 a</td>
<td>1.24</td>
<td>2.74 b</td>
</tr>
<tr>
<td>Control + 0.5% TMP</td>
<td>1,005 a</td>
<td>791 ab</td>
<td>1.26</td>
<td>2.96 ab</td>
</tr>
<tr>
<td>Control + 0.5% HSCAS</td>
<td>1,023 b</td>
<td>805 a</td>
<td>1.27</td>
<td>2.79 b</td>
</tr>
<tr>
<td>Control + 1.0 mg/kg AFB 1</td>
<td>858 b</td>
<td>662 a</td>
<td>1.29</td>
<td>3.37 a</td>
</tr>
<tr>
<td>Control + 0.5% TMP + 1.0 mg/kg AFB 1</td>
<td>906 b</td>
<td>746 b</td>
<td>1.21</td>
<td>3.09 ab</td>
</tr>
<tr>
<td>Control + 0.5% HSCAS + 1.0 mg/kg AFB 1</td>
<td>1,030 a</td>
<td>817 ab</td>
<td>1.26</td>
<td>2.76 b</td>
</tr>
<tr>
<td>Control + 0.5% TMP + 0.5% HSCAS + 1.0 mg/kg AFB 1</td>
<td>1,047 a</td>
<td>822 a</td>
<td>1.27</td>
<td>2.97 ab</td>
</tr>
</tbody>
</table>

Pooled SEM 32 24 0.028 0.14

*a–cMeans with different superscripts in a column differ significantly (P < 0.05).

1Means represent 4 pens per treatment, 4/5 birds per pen. AFB 1 = aflatoxin B 1; TMP = turmeric powder; HSCAS = hydrated sodium calcium aluminosilicate.

2Means represent 4 pens per treatment, 2 birds per pen.

Table 3. Serum chemistry of chicks fed diets containing, antioxidant, adsorbent, and aflatoxin

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Cholesterol (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>GGT2 (U/L)</th>
<th>Ca (mg/dL)</th>
<th>P (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.4 ab</td>
<td>1.0 a</td>
<td>121 ab</td>
<td>6.2</td>
<td>13.8</td>
<td>10.5 ab</td>
<td>8.1</td>
</tr>
<tr>
<td>Control + 0.5% TMP</td>
<td>2.2 bc</td>
<td>0.9 ab</td>
<td>128 b</td>
<td>5.8</td>
<td>12.0</td>
<td>10.6 a</td>
<td>8.0</td>
</tr>
<tr>
<td>Control + 0.5% HSCAS</td>
<td>2.5 a</td>
<td>1.0 a</td>
<td>121 ab</td>
<td>4.8</td>
<td>15.6</td>
<td>10.4 ab</td>
<td>7.9</td>
</tr>
<tr>
<td>Control + 1.0 mg/kg AFB 1</td>
<td>1.4 e</td>
<td>0.9 b</td>
<td>90 b</td>
<td>6.5</td>
<td>14.5</td>
<td>9.1 e</td>
<td>7.0</td>
</tr>
<tr>
<td>Control + 0.5% TMP + 1.0 mg/kg AFB 1</td>
<td>1.8 d</td>
<td>0.8 c</td>
<td>107 b</td>
<td>5.9</td>
<td>15.0</td>
<td>9.2 e</td>
<td>7.5</td>
</tr>
<tr>
<td>Control + 0.5% HSCAS + 1.0 mg/kg AFB 1</td>
<td>2.0 d</td>
<td>0.8 b</td>
<td>112 b</td>
<td>5.3</td>
<td>12.8</td>
<td>9.8 ab</td>
<td>7.1</td>
</tr>
<tr>
<td>Control + 0.5% TMP + 0.5% HSCAS + 1.0 mg/kg AFB 1</td>
<td>2.2 bc</td>
<td>0.9 ab</td>
<td>128.6 a</td>
<td>5.8</td>
<td>13.9</td>
<td>10.1 ab</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Pooled SEM 0.08 0.05 6 0.89 0.86 0.29 0.39

*a–eMeans with different superscripts in a column differ significantly (P < 0.05).

1Means represent 4 pens per treatment, 2 birds per pen. AFB 1 = aflatoxin B 1; TMP = turmeric powder; HSCAS = hydrated sodium calcium aluminosilicate; GGT = gamma glutamyl transferase.

2One unit of activity is the amount of enzyme that catalyzes the liberation of 1 μM of p-nitroaniline per min at 25°C.

Serum Chemical Parameters

Feeding chicks AFB 1 (1.0 mg/kg) resulted in significantly reduced serum total protein, albumin, cholesterol, and Ca levels (Table 3). Supplementation of TMP to the AFB 1 diet increased the total protein and cholesterol levels compared with chicks fed AFB 1 alone. The inclusion of HSCAS in the AFB 1 diet also improved total protein, albumin, and cholesterol values compared with those chicks fed AFB 1 alone. Serum concentrations of uric acid, phosphorus, and glutamyl transferase were not affected by dietary treatment. The reduced levels of total protein, albumin, cholesterol, and Ca are indicative of the toxic effect of AFB 1 on hepatic and renal tissues and are consistent with previous literature reporting aflatoxicosis (Murry, 1982; Abdel-Wahhab and Aly, 2003). The positive effect of TMP and HSCAS on serum values (total protein, albumin, cholesterol) demonstrated their ameliorative effect against AFB 1, with TMP providing antioxidant protection and HSCAS decreasing the amount of AFB 1 absorbed. Similarly, supplementation of plant extracts of cumin (Nigella sativa) and clove (Syzygium aromaticum) to rat diets containing AFB 1 overcame the negative effect of AFB 1 on serum chemistry (Abdel-Wahhab and Aly, 2005). The toxic effects of AF in cockerels were reversed with administration of an alcoholic extract of African nutmeg (Flora and Taiwo, 2004). The protective effects of aluminosilicates against AFB 1 in terms of serum chemistry have been reported previously (Kubena et al., 1990; Ledoux et al., 1998).

Liver Antioxidant Status

Lipid peroxide level was increased in liver homogenate of chicks fed AFB 1 (Table 4). Supplementation of the AFB 1 diet with TMP or HSCAS or their combination reduced (P < 0.05) peroxide levels. Compared with controls, SOD activity was higher in chicks fed the diet supplemented with TMP, and chicks fed the AFB 1 diet supplemented with TMP or HSCAS. In contrast, the activity of catalase was reduced in the groups fed AFB 1 alone and the combination of TMP and AFB 1 as compared with that of the control, HSCAS, and HSCAS with AFB 1-fed groups. Further detailed studies are needed to explain the possible mechanism of low catalase activity with TMP supplementation. The overall antioxidant concentration in liver homogenates was statistically similar but numerically

in the present study was found to be 1.48% as compared with reported values of 2.5 to 7.0% for different samples of turmeric powder available on the market (Sasikumar et al., 2004; Tayyem et al., 2006).
higher in the group fed the combination of TMP and AFB₁. In general, the antioxidant data suggest that TMP supplementation along with AFB₁ stimulated the antioxidant system in the liver for counteracting the oxidative damage caused by AFB₁. The lower level of peroxides in the groups fed a combination of HSCAS and AFB₁ indicated minimum or no cell damage in the liver because enough AFB₁ was adsorbed onto the HSCAS to reduce gut AFB₁ concentrations to at or below the maximum tolerable level for birds of this age. Aflatoxin B₁ is known to cause lipid peroxidation in liver (Shen et al., 1994) and is a potent carcinogen that forms adducts with DNA and induces cellular oxidative damage (Imlay and Linn, 1988). An increase in peroxide level in liver due to the feeding of AFB₁ to rats was associated with a decrease in activity of SOD, catalase, glutathione peroxidase, and reductase (Rastogi et al., 2001), and further supplementation of root extracts of Picrorhiza kurroa and seeds of Silybum marianum ameliorated the effects of AFB₁ and reversed peroxide and antioxidant enzymes to control levels. Rosmarinic acid, a phenolic compound present in Boraginaceae species of plants (sage, basil, mint), reduced free radical oxygen formation and apoptosis of human hepatoma cells induced by AFB₁ (Renuzzi et al., 2004). The carbonyl functional group of curcuminoids of turmeric was responsible for its antimutagenic and anticarcinogenic action (Chun et al., 1999). Further, curcumin has a strong inhibitory action on superoxide anion generation (Iqbal et al., 2003) and biotransformation of AFB₁ to aflatoxicol in liver (Lee et al., 2001). Supplementation of turmeric is known to reduce AFB₁–DNA adduct formation through modulation of cytochrome P 450 function (Soni et al., 1997). The above findings explain a mode of action of curcumin as an antioxidant, and the results of the present study suggest that curcumin may need to be supplemented at levels higher than the 74 mg/kg used in the current study to achieve maximum protection against 1.0 mg/kg AFB₁.

**Histopathology**

Mild to moderate lesions were observed in liver sections of birds fed dietary treatments with AFB₁. Hepatic lesions included biliary hyperplasia, mild perportal swelling, and vacuolar degeneration of hepatocytes, and mild to severe periportal heterophil or mononuclear cell infiltration, or both. The mean liver lesion score for different groups was 1.0 (unremarkable) for diets without AFB₁, 2.5 (mild to moderate) for the diet with AFB₁ alone, 2.0 (mild) for the TMP and AFB₁ combination diet, and 1.3 to 1.5 (normal or mild lesions) for diets containing a combination of HSCAS and AFB₁ or a combination of HSCAS, TMP, and AFB₁, respectively. A total of 5 birds (1 control; 1 AFB₁; 2 TMP and AFB₁; 1 HSCAS) died during the course of the study. Necropsy of these birds did not reveal any diet-related gross lesions.

Necropsy lesions observed in the liver are similar to those reported previously in birds fed AFB₁ (Hoerr, 1997; Ledoux et al., 1998). The decline in the severity of lesions in groups fed combinations of HSCAS and AFB₁ indicates that most of the AFB₁ was neutralized in the gut and not absorbed into the hepatic system. Reduction in the severity of lesions due to TMP supplementation of the AFB₁ diet is consistent with the serum chemistry data and antioxidant status in the liver.

From this study, it is concluded that supplementation of a diet containing AFB₁ (1.0 mg/kg) with turmeric powder containing 74 mg/kg curcumin improved the antioxidant status and partially protected against the adverse effects of AFB₁, suggesting that higher levels of curcumin may be required for maximum efficacy. Inclusion of HSCAS (0.5%) in the diet containing AFB₁ (1.0 mg/kg) almost completely prevented the toxic effects of AFB₁. The combined inclusion of TMP (0.5%) and HSCAS (0.5%) to the diet with AFB₁ (1.0 mg/kg) did not result in a further amelioration of the toxic effects of AFB₁ when compared with supplementation with TMP or HSCAS alone.

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