Effects of turmeric (Curcuma longa) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin

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ABSTRACT The objective of the present study was to evaluate the efficacy of curcumin, an antioxidant found in turmeric (Curcuma longa) powder (TMP), to ameliorate changes in gene expression in the livers of broiler chicks fed aflatoxin B1 (AFB1). Four pen replicates of 5 chicks each were assigned to each of 4 dietary treatments, which included the following: A) basal diet containing no AFB1 or TMP (control), B) basal diet supplemented with TMP (0.5%) that supplied 74 mg/kg of curcumin, C) basal diet supplemented with 1.0 mg of AFB1/kg of diet, and D) basal diet supplemented with TMP that supplied 74 mg/kg of curcumin and 1.0 mg of AFB1/kg of diet. Aflatoxin reduced (P < 0.05) feed intake and BW gain and increased (P < 0.05) relative liver weight. Addition of TMP to the AFB1 diet ameliorated (P < 0.05) the negative effects of AFB1 on growth performance and liver weight. At the end of the 3-wk treatment period, livers were collected (6 per treatment) to evaluate changes in the expression of genes involved in antioxidant function [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST)], biotransformation [epoxide hydrolase (EH), cytochrome P450 1A1 and 2H1 (CYP1A1 and CYP2H1)], and the immune system [interleukins 6 and 2 (IL-6 and IL-2)]. Changes in gene expression were determined using the quantitative real-time PCR technique. There was no statistical difference in gene expression among the 4 treatment groups for CAT and IL-2 genes. Decreased expression of SOD, GST, and EH genes due to AFB1 was alleviated by inclusion of TMP in the diet. Increased expression of IL-6, CYP1A1 and CYP2H1 genes due to AFB1 was also alleviated by TMP. The current study demonstrates partial protective effects of TMP on changes in expression of antioxidant, biotransformation, and immune system genes in livers of chicks fed AFB1. Practical application of the research is supplementation of TMP in diets to prevent or reduce the effects of aflatoxin in chicks fed aflatoxin-contaminated diets.

Key words: aflatoxin B1, turmeric, gene expression, broiler, liver

INTRODUCTION

Aflatoxins (AF) are contaminants of feed used in poultry rations and are produced by the fungi Aspergillus parasiticus and Aspergillus flavus (Smith et al., 1995). The most biologically active form of AF is aflatoxin B1 (AFB1) and it is responsible for decreased performance, liver lesions, and immunosuppression in poultry (Kubena et al., 1993; Ledoux et al., 1999). Aflatoxin B1 causes cell damage, free radical production, and lipid peroxidation (Surai, 2002). Increased lipid peroxide levels and decreased antioxidant enzyme levels have been observed in AFB1-treated rats (Rastogi et al., 2001). Protection against oxidative damage is provided by a group of substances called antioxidants that inhibit reactions of free radicals, such as reactive oxygen species (ROS). Because lipid peroxidation plays a major role in AF toxicity, a protective effect of antioxidants against aflatoxicosis is possible (Galvano et al., 2001). Supplementation of antioxidants (picroliv and silymarin) ameliorated the effects of AFB1 and prevented an increase in antioxidant enzymes caused by AFB1 (Rastogi et al., 2001). Plant compounds such as coumarins, flavonoids, and curcuminoids have been shown to inhibit the biotransformation of AF to their...
epoxide metabolites, which are more genotoxic than the parent compound (Lee et al., 2001). Reports have shown that the curcuminoid yellowish pigments present in the powder of dried roots and rhizomes of turmeric (Curcuma longa; TMP) have protective effects against AFB1 (Soni et al., 1997). A recent approach to prevent aflatoxicosis in poultry is the use of antioxidants in the diet (Surai, 2002). Several studies have reported that TMP is beneficial against aflatoxicosis at the level of the animal, but to date, no study has been published that reports on the beneficial effects of TMP on hepatic gene expression of broiler chicks fed AF. Therefore, the objective of the present study was to evaluate the effects of TMP, containing a known level of curcuminoids, on the expression of hepatic genes involved in antioxidant function [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST)], biotransformation [epoxide hydro-lase (EH), cytochrome P450 1A1 and 2H1 (CYP1A1 and CYP2H1)], and the immune system [interleukin 6 and 2 (IL-6 and IL-2)] in broiler chicks fed AF. The present study is an extension of our earlier work evaluating the efficacy of TMP and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of TMP, containing a known level of curcuminoids, to the method described by Gowda et al. (2008).

MATERIALS AND METHODS

Experimental Design and Birds

Eighty 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 for 21 d. Chicks were maintained on a 24-h continuous light schedule and allowed ad libitum access to feed and water. 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 4 dietary treatments. Mortality was recorded and birds were inspected daily for any health-related problems.

Diets

The basal diet was a commercial-type corn-soybean meal diet formulated to meet or exceed the nutritional requirement of growing chicks as recommended by the NRC (1994). Dietary treatments evaluated included the following: A) basal diet containing neither TMP nor AFB1 (control), B) basal diet supplemented with 0.5% food-grade TMP that supplied 74 mg of total curcuminoids/kg of diet, C) basal diet containing 1.0 mg of AFB1/kg of diet, and D) basal diet containing 1.0 mg of AFB1/kg of diet plus 0.5% TMP that supplied 74 mg of total curcuminoids/kg of diet. Aflatoxin B1 was supplied by Aspergillus parasiticus (NRRL 2999) culture material containing 760 mg/kg of AFB1. Dietary AFB1 concentrations were confirmed by HPLC analysis, and all diets were screened by the methods of Rottinghaus et al. (1982, 1992) for the presence of AFB1, citrinin, T-2 toxin, vomitoxin, zearalenone, fumonisins, and ochratoxin A, before the start of the experiment. Total curcuminoid content, including curcumin, bisdemethoxy-curcumin, and demethoxycurcumin, was determined by the method described by Gowda et al. (2008).

Sample Collection

On d 21, all birds were weighed and feed consumption was recorded for each pen. Average feed intake and BW gain were determined. Liver weight of each bird was recorded, and liver tissue (6 per treatment) was collected randomly, with at least 1 bird representing each pen. Collected liver samples were snap-frozen in liquid nitrogen and stored at −80°C for real-time PCR analysis.

Statistical Analysis

Data were analyzed by the GLM procedures of SAS (SAS Institute, 1996). The means for treatments showing significant differences in the ANOVA were compared using Fisher’s protected least significant difference procedure at a significance based on the 0.05 level of probability.

RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR

Ribonucleic acid was extracted from the liver samples (6 samples per treatment) using a RNeasy Midi Kit (Qiagen Inc., Valencia, CA; Settivari et al., 2006), purified using DNase-1 (Ambion Inc., Austin, TX) and phenol:chloroform:isoamyl alcohol (25:24:1), and concentrated using Microcon YM30 filters (Millipore Corp., Bedford, MA) as described previously (Settivari et al., 2006). The quality and integrity of the purified RNA was checked through agarose gel electrophoresis and the quantity was measured using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE; Flanagan, 2005). The purified RNA samples were preserved at −80°C until used. Two-step quantitative real-time PCR (qRT-PCR) was used to measure expression patterns of genes involved in antioxidant function (CAT, SOD, GPx, GST), biotransformation (EH, CYP1A1, CYP2H1), and the immune system (IL-6 and IL-2). From each chick liver, 10 µg of total RNA was reverse-transcribed using Stratascript RT (Stratagene, La Jolla, CA) with oligo dT (5 µg/µL; IDT DNA, Coralville, IA) and random hexamers (5 µg/µL; IDT DNA).
Then, 6.25 ng of cDNA was added to a 25-µL PCR reaction to get a final concentration of 0.25 ng/µL of cDNA in a SYBR green assay (Applied Biosystems, Foster City, CA). Forward and reverse primer final concentrations were 100 nM in the SYBR green assay. Primers were designed using the Primer3 program (Rozen and Skaletsky, 2000) with an annealing temperature of 60°C and amplification size of less than 250 bp (Table 1). Glyceraldehyde phosphate dehydrogenase (GAPDH) was used as the endogenous control gene in the qRT-PCR experiments. Thermal cycling was carried out with an ABI Prism 7500 sequence detection system (Applied Biosystems) using factory default conditions (50°C, 2 min; 95°C, 10 min; and 40 cycles at 95°C, 15 s; 60°C, 1 min). Each gene was measured in triplicate and the formation of single PCR products was confirmed using melting curves. Negative controls, which consisted of all of the components of the qRT-PCR mix except cDNA, were used for all primers. The relative quantification of gene expression changes were recorded after normalizing for GAPDH gene expression computed by using the \( 2^{-\Delta \Delta C_t} \) method (user manual 2, ABI Prism 7700 SDS). In the \( 2^{-\Delta \Delta C_t} \) analysis, the threshold cycle (CT; cycle number at which the expression exceeds threshold level) from control birds was used as a calibrator sample. Statistical analyses of the data were performed by comparing birds fed AF with control birds for each gene using a 2-tailed t-test with unequal group variance.

### RESULTS

#### Performance of Broiler Chicks

Data on average feed intake, weight gain, and relative liver weight are presented in Table 2. Chicks fed TMP alone had similar feed intake and BW gain as control chicks. Chicks fed 1 mg of AFB1/kg of diet had significantly \((P < 0.05)\) lower feed intake and BW gain compared with controls. Addition of 0.5% TMP, containing 74 mg/kg of total curcuminoids, to the AFB1 diet numerically increased feed intake and significantly \((P < 0.05)\) improved BW gain of chicks. Relative liver weight was increased \((P < 0.05)\) in chicks fed the diet containing AFB1 alone compared with control chicks. Although the reduction in liver weights of chicks fed the combination of TMP and AFB1 diet was not statistically significant \((P > 0.05)\) from chicks fed AFB1 alone, it was comparable to that of controls.

#### Changes in Gene Expression of Antioxidant, Biotransformation, and Immune System Genes

The expression profiles of genes involved in antioxidant function (CAT, SOD, GPx, GST), biotransformation (EH, CYP1A1, CYP2H1), and the immune system (IL-6 and IL-2) in all treatment groups were measured using the qRT-PCR technique. There was no statis-

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**Table 1. Primer sequences \((5'\rightarrow3')\) used in real-time PCR**

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size (bp)</th>
</tr>
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<tbody>
<tr>
<td>Catalase</td>
<td>CAT</td>
<td>GGGGAGCTGTTTTACTGCAAG</td>
<td>TTTCCATGGGCTATGGAATT</td>
<td>139</td>
</tr>
<tr>
<td>Carnitine palmitoyl transferase 1A</td>
<td>CPT 1A</td>
<td>ATCCCCAGCTGAGCTAGTCTCTCT</td>
<td>GAGCTTCTGCCATTTTGGAG</td>
<td>116</td>
</tr>
<tr>
<td>Cytochrome P450 1A</td>
<td>CYP1A1</td>
<td>CACTTTCTGGCTGCTTCCTGT</td>
<td>GGTGCTTCTCAGTGTCGCC</td>
<td>125</td>
</tr>
<tr>
<td>Cytochrome P450 2H1</td>
<td>CYP2H1</td>
<td>ATCCCCATCATGGGAAATGT</td>
<td>TCAGGCTACACGGACCA</td>
<td>137</td>
</tr>
<tr>
<td>Epoxide hydratase</td>
<td>EH</td>
<td>AAAGGGCACAGAGCCTGAGACA</td>
<td>CCTCAGTGGTCGCTGTAAT</td>
<td>128</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>GPx</td>
<td>TTGIAAACATCAGGAGGCCAA</td>
<td>TGCGCCAAGCATCCTGTAAG</td>
<td>140</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>SOD</td>
<td>AGGGGGCTCACAGTGCTTCC</td>
<td>CCACTTGGGCAAGTGCAAG</td>
<td>122</td>
</tr>
<tr>
<td>Glyceraldehyde-3 phosphate</td>
<td>GAPDH</td>
<td>CCTCTCTGGCAGAAGTCCAGA</td>
<td>GAACATCAAATGGGCAGAT</td>
<td>128</td>
</tr>
<tr>
<td>Interleukin 2</td>
<td>IL2</td>
<td>TGCAAGCTGTACCTGAGGAGAGA</td>
<td>CTTGATCATCCATTCCGGTGT</td>
<td>135</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>IL6</td>
<td>GACTGGAGAGAGGAGGAGGAGGAGA</td>
<td>CGCACGCGGAACTCTTCTT</td>
<td>128</td>
</tr>
<tr>
<td>Glutathione S-transferase-α</td>
<td>GSTα</td>
<td>GCCTGACTTTCAGTCTTTGGT</td>
<td>CGCAAGTTGACTTCATCTT</td>
<td>131</td>
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</tbody>
</table>

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**Table 2. Effects of aflatoxin B1 (AFB1; 1 mg/kg) and turmeric powder (0.5%; 74 mg/kg of total curcuminoids) on chick performance and relative liver weight**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feed intake(^1) (g/chick)</th>
<th>BW gain(^1) (g/chick)</th>
<th>Liver weight(^2) (% BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (control)</td>
<td>1.047(^a)</td>
<td>8.40(^b)</td>
<td>2.75(^b)</td>
</tr>
<tr>
<td>B (AFB1)</td>
<td>0.858(^a)</td>
<td>6.62(^a)</td>
<td>3.37(^a)</td>
</tr>
<tr>
<td>C (AFB1 + TMP)</td>
<td>0.906(^b)</td>
<td>7.46(^b)</td>
<td>3.00(^b)</td>
</tr>
<tr>
<td>D (TMP)</td>
<td>1.005(^ab)</td>
<td>7.91(^ab)</td>
<td>2.96(^b)</td>
</tr>
<tr>
<td>SEM</td>
<td>0.36</td>
<td>0.26</td>
<td>0.13</td>
</tr>
</tbody>
</table>

\(^a\) Means within a column with no common superscripts differ significantly \((P < 0.05)\).

\(^1\) Means represent 4 pens of 5 chicks each per treatment.

\(^2\) Means represent 4 pens of 3 chicks each per treatment.

\(^3\) TMP = turmeric powder.
tical difference \((P > 0.05)\) in gene expression among the 4 treatment groups for CAT and IL-2 genes (data not shown). Turmeric alone in the diet increased \((P < 0.05)\) the expression of hepatic SOD, GPx, and EH genes \((P < 0.05)\) and decreased the expression of IL-6 and CYP1A1 genes (Figures 1, 2, 3, 4, 5). Decreased expression of SOD, EH, and GST genes due to AFB1 was alleviated by the inclusion of TMP in the AFB1 diet \((P < 0.05; \text{Figures 1, 4, 6})\). Increased expression of CYP1A1, IL-6, and CYP2H1 genes due to AFB1 was prevented \((P < 0.05)\) by the addition of TMP to the AFB1 diet (Figures 3, 5, 7).

**DISCUSSION**

**Performance of Broiler Chicks**

Reduced feed intake, BW gain, and increased liver weights observed in chicks fed AFB1 alone are consistent with previous reports on the negative effects of AFB1 on performance (Kubena et al., 1990; Ledoux et al., 1999; Yarru et al., 2009). The addition of TMP containing 74 mg/kg of curcuminoids to the AFB1 diet improved the performance of chicks in the current study. Earlier reports suggest that TMP might have protective actions on the liver of broiler chicks (Emadi and Kermanshahi, 2007). Feeding TMP (that supplied 5 µg of curcumin per day) for 14 d to AFB1-intoxicated ducklings reversed fatty changes, necrosis, and biliary hyperplasia (Soni et al., 1992). In the current study, although the reduction in liver weights of chicks fed the combination of TMP and AFB1 diet was not statistically significant when compared with liver weight of chicks fed AFB1 alone, it was comparable to that of controls, which suggests that TMP gave partial hepatoprotection.

**Effects of AF and Turmeric on the Expression of Antioxidant Genes**

Aflatoxin B1 causes lipid peroxidation in liver (Shen et al., 1994), induces oxidative damage in the liver cells, forms DNA adducts, and thus acts as a potent car-
cinogen (Inlay and Linn, 1988). Supplementation of root extracts of *Picrorhiza kurroa* and seeds of *Silybum marianum* prevented the effects of AFB1 by improving the performance of chicks, reducing the formation of peroxides, and returning antioxidant enzymes to control levels (Rastogi et al., 2001). Rosmarinic acid, a phenolic compound present in the *Boraginaceae* species of plants, reduced free radical production in human hepatoma cells induced by AFB1 (Rezulli et al., 2004). Further, earlier reports suggested that curcumin inhibits superoxide anion generation (Iqbal et al., 2003). Recently, Emadi and Kermanshahi (2007) fed broiler chicks TMP (0.25, 0.5, 0.75%) from hatch to 49 d and stated that turmeric might have some positive effects on liver enzymes that directly or indirectly reflect a healthier liver. The above findings demonstrate the possible mode of action of TMP as an antioxidant.

**SOD.** Superoxide dismutase catalyzes the conversion of superoxide anions to hydrogen peroxide and is one of the primary enzymatic defenses against ROS. In the present study, hepatic gene expression of SOD was downregulated in chicks fed a diet containing AFB1 versus the control diet (Figure 1). This downregulation in SOD gene expression could result in accumulation of superoxide anions within mitochondria, leading to oxidative stress, and thereby impairing vital cellular functions (Marczuk-Krynicka et al., 2003). Curcumin is known to augment antioxidant status especially through SOD (Cheng et al., 2005). This could probably be the reason for the increased hepatic gene expression of SOD in chicks fed TMP compared with controls. Birds fed the combination of AFB1 and TMP had greater (*P* ≤ 0.05) expression levels for SOD gene expression compared with those fed only AFB1 (Figure 1). These results suggest protective effects of curcinoids in TMP in improving SOD gene expression in liver of chicks fed AFB1.

**CAT and GPx.** Antioxidant enzymes, such as CAT within the peroxisomes and cytosolic GPx, are involved in the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, into water and molecular oxygen (Liska, 1998). Hydrogen peroxide is unstable and forms a hydroxyl free radical; its breakdown on the inner mitochondrial membrane and within the mitochondrial matrix causes site-specific damage of critical matrix targets. The decomposition of hydrogen peroxide outside the mitochondria may cause damage to the outer mitochondrial membrane itself, which suggests that the breakdown of hydrogen peroxide to the hydroxyl radical is highly detrimental.

Compared with controls, the expression of the GPx gene was not significantly decreased in birds fed AFB1 (Figure 2). However, compared with controls, expression of the GPx gene was increased in birds fed TMP alone, and in those fed the combination of AFB1 and TMP. This suggests that although AFB1 did not affect GPx expression, the addition of TMP to the AFB1 diet...
improved overall antioxidant protection in birds fed AFB1 and could enhance the ability of the bird to convert highly reactive hydrogen peroxide to water. There was no statistical difference in gene expression among the 4 treatment groups for the CAT gene.

**GSTα.** Conjugation of reactive xenobiotic metabolites with glutathione is an important step in detoxification and is mediated by GSTα. An overload of xenobiotics may deplete glutathione through conjugation activities, thereby contributing to oxidative stress (Percival, 1997). Decreased hepatic gene expression of GSTα, as observed in birds fed AFB1 in this study, could limit the ability of the hepatic tissue to conjugate reactive metabolites. Decreased expression of the GSTα gene due to AFB1 was alleviated by the inclusion of TMP in the AFB1 diet (Figure 6). Results of the present study are consistent with earlier reports with regards to the protective effects of TMP in aflatoxicosis (Son et al., 1997; Osawa, 2007). The beneficial effects have been explained by the induction of antioxidant enzymes and thereby protection against oxidative stress (Osawa, 2007).

There are important interactions among the activities of several antioxidant enzymes and various ROS and cellular reactions, all of which could be responsible for some of the observations in the present study. Decreased expression of SOD and GST genes in chicks fed AFB1 is additive with respect to oxidative damage. Nonenzymatic decomposition of hydrogen peroxide involving transition metals, such as iron, in a Fenton-type reaction can be more damaging to the cell than the production of the hydroxyl radical species (Scandalios, 1997). Furthermore, increased levels of hydrogen peroxide within the cells reduce SOD activity (Marczuk-Krynicka et al., 2003), thereby increasing superoxide levels within the cell.

**Effects of AF and Turmeric on the Expression of Biotransformation Genes**

Primary hepatic detoxification processes include xenobiotic biotransformation (phase I metabolism) and the subsequent conjugation of the resulting metabolites (phase II metabolism), making them more water-soluble and available for excretion from the body. The microsomal cytochrome P450 (CYP)-dependent monooxygenase system in the liver plays an essential role in phase I metabolism (Akahori et al., 2005). The CYP enzymes are associated with several biological interactions involving hydroxylation, epoxidation, oxygenation, dehydrogenation, nitrogen dealkylation, and oxidative deamination (Hari Kumar and Kuttan, 2006). Cytochrome P450-mediated reactions can also generate ROS.

**CYP.** The major CYP enzymes involved in hepatic metabolism of AF in poultry are the CYP1A1 (Klein et al., 2003) and CYP2H1 isoforms (Hamilton et al., 1993). The gene CYP1A1 is known to activate certain promutagens to their carcinogenic forms (Haas et al., 2006; Hari Kumar and Kuttan, 2006). In the present study, an increase in the expression of hepatic CYP1A1 and CYP2H1 genes was observed in chicks fed AFB1 (Figures 3 and 7). Overexpression of these CYP isoforms has been shown to induce chronic oxidative stress by generating more ROS, possibly leading to hepatocellular injury and death (Hari Kumar and Kuttan, 2006). It is evident from the results of the present study that transcriptional activation of CYP1A1 and CYP2H1 isoforms, in response to AFB1, has the potential to increase oxidative stress. In addition, these CYP isoforms are involved in biotransformation of AFB1 to the highly genotoxic metabolite AF 8, 9-epoxide (Klein et al., 2003). The CYP isoforms oxidize AFB1 into 2 metabolites: the reactive intermediate, AF 8, 9-epoxide (AFBO) and AFM1. Because of the importance of AFBO and AFM1 in the toxicity of AFB1, Yip and Coulombe (2006) concluded that CYP isoforms play an important role in the well-known hypersensitivity of turkeys to AFB1. Klein et al. (2003) conducted a study to determine if the inclusion of butylated hydroxytoluene, an antioxidant in the diet, has any chemoprotective effects in birds fed AFB1. They observed decreased activity of hepatic microsomal CYP1A1 as well as conversion of AFB1 to the putative genotoxic metabolite, AFBO, compared with controls. In agreement with the above findings, in the current study, curcuminoids decreased CYP1A1 (Figure 3) and CYP2H1 (Figure 7) gene expression in chicks fed the AFB1 + TMP combination compared with chicks fed the control diet, suggesting chemoprotective effects.

**EH.** All aerobic cells contain a battery of defenses to prevent cellular oxidative damage associated with ROS or active metabolites generated by CYP, or both (Tiedge et al., 1998). The toxic metabolite, AF 8, 9-epoxide, is detoxified by EH (Tiemersma et al., 2001) and GST enzymes (Tiemersma et al., 2001; Klein et al., 2003). Because genes coding for CYP isoforms were upregulated in birds fed AFB1, compared with controls in the present study, there is a greater chance for formation of more toxic intermediate metabolites such as AF 8, 9-epoxide. Furthermore, downregulation of the detoxification genes (EH and GSTα) could reduce the ability of the bird to detoxify AFBO and other reactive substances resulting in accumulation of those active metabolites inside the bird, which could lead to various toxicological and carcinogenic effects. Decreased expression of EH (Figure 4) and GST genes and increased expression of CYP1A1 and CYP2H1 genes due to AFB1 were alleviated by TMP inclusion in the AFB1 diet. Also, there was an increase in the expression of EH gene in chicks fed TMP alone compared with controls, which indicates that TMP improves the detoxification ability in chicks fed AFB1.
Effects of AF and Turmeric on the Expression of Interleukins

Interleukins are a group of cytokines that are important components of the immune system. They play a physiological role in inflammation and a pathological role in systemic inflammatory states and are now well recognized (Tayal and Kalra, 2007). Any imbalance in cytokine production and dysregulation of a cytokine process could result in various pathological disorders (Tayal and Kalra, 2007). Aflatoxin exerts part of its immunosuppressive effects through these cytokines. Hinton et al. (2003) investigated the effects of AFB$_1$ on interleukin 1 (IL-1), IL-2, and IL-6 production in male rats and found an increase in the production of IL-1 and IL-6. Dugyala and Sharma (1996) used male mice to test the effects of AFB$_1$ (0.7 mg of AFB$_1$/kg) on the gene expression of IL-1α and IL-2. They reported that AFB$_1$ significantly suppressed the levels of IL-1α protein and mRNA and protein levels of IL-2.

IL-6 and IL-2. Interleukin 6 is a proinflammatory cytokine and IL-2 stimulates T-cell growth and cytotoxic and killer-cell activities. The effect of AFB$_1$ on IL-2 gene expression was measured by Han et al. (1999) in murine thymocytes using real-time PCR. They observed a decrease in the transcriptional levels of IL-2 in these cells. In the present study, we observed no statistical difference in gene expression among the 4 treatment groups for the IL-2 gene (data not shown). There was an increase in the expression of the IL-6 gene in chicks fed AFB$_1$ (Figure 5). Similarly, Hinton et al. (2003) observed an increase in the production of IL-6 in rats fed AFB$_1$. Furthermore, Dugyala and Sharma (1996) observed similar induction in mRNA levels of IL-6; however, they noticed a reduction in the corresponding protein level and they speculated that AFB$_1$ treatment decouples the correlation between transcription and translational controls of IL-6. In the current study, TMP alone decreased the expression of the IL-6 gene possibly because of its anti-inflammatory action (Thangapazham et al., 2006). Moreover, the increased expression of the IL-6 gene caused by AFB$_1$ was prevented by TMP inclusion in the AFB$_1$ diet.

Current findings suggest that AFB$_1$ at 1.0 mg/kg of the diet has detrimental effects on growth performance and liver weight of chicks. This level of AF also decreased hepatic gene expression of SOD, GST, and EH and increased the gene expression of IL-6, CYP1A1, and CYP2H1. Turmeric alone had beneficial effects on the expression of antioxidant (SOD and Gpx), biotransformation (CYP1A1 and EH), and IL-6 genes. Turmeric powder inclusion in the AFB$_1$ diet improved bird performance and prevented the negative effects on the expression of genes associated with antioxidant (SOD and GSTs), immune (IL-6), and detoxification (CYP1A1, CYP2H1, and EH) mechanisms in livers of chicks fed AFB$_1$. However, no significant difference was observed in the expression of CAT, an important antioxidant gene, and the IL-2 gene among the 4 treatment groups. The present study demonstrates that supplementation of TMP (0.5%), that supplied 74 mg/kg of curcuminoids, to a diet containing AFB$_1$ (1.0 mg/kg) gave partial protection against the adverse effects of AFB$_1$, suggesting that higher levels of TMP may be required for maximum efficacy.

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