Antioxidant efficacy of curcuminoids from turmeric (Curcuma longa L.) powder in broiler chickens fed diets containing aflatoxin B1

Nisarani K. S. Gowda1*, David R. Ledoux2, Goerge E. Rottinghaus2, Alex J. Bermudez2 and Yin C. Chen2

1National Institute of Animal Nutrition and Physiology, Bangalore 560030, India
2Fusarium/Poultry Research Laboratory, University of Missouri, Columbia, MO 65211, USA

(Received 6 November 2008 – Revised 21 May 2009 – Accepted 28 May 2009 – First published online 17 August 2009)

A 3-week-feeding study (1–21 d post-hatch) was conducted to evaluate the efficacy of total curcuminoids (TCMN), as an antioxidant, to ameliorate the adverse effects of aflatoxin B1 (AFB1) in broiler chickens. Turmeric powder (Curcuma longa L.) that contained 2.55% TCMN was used as a source of TCMN. Six cage replicates of five chicks each were assigned to each of six dietary treatments, which included: basal diet; basal diet supplemented with 444 mg/kg TCMN; basal diet supplemented with 1.0 mg/kg AFB1; basal diet supplemented with 74 mg/kg TCMN and 1.0 mg/kg AFB1; basal diet supplemented with 222 mg/kg TCMN and 1.0 mg/kg AFB1; basal diet supplemented with 444 mg/kg TCMN and 1.0 mg/kg AFB1. The addition of 74 and 222 mg/kg TCMN to the AFB1 diet significantly (P<0.05) improved weight gain and feed efficiency. Increase (P<0.05) in relative liver weight in birds fed AFB1 was significantly reduced (P<0.05) with the addition of 74, 222 and 444 mg/kg TCMN to the AFB1 diet. The inclusion of 222 mg/kg TCMN ameliorated the adverse effects of AFB1 on serum chemistry in terms of total protein, albumin and γ-glutamyl transferase activity. The decreased antioxidant functions due to AFB1 were also alleviated by the inclusion of 222 mg/kg TCMN. It is concluded that the addition of 222 mg/kg TCMN to the 1.0 mg/kg AFB1 diet demonstrated maximum antioxidant activity against AFB1.

Aflatoxin B1: Antioxidants: Curcuminoids: Free radicals: Turmeric powder

Aflatoxins, a class of mycotoxins produced by the fungi Aspergillus parasiticus and Aspergillus flavus, are major contaminants of common feed ingredients used in poultry rations (1). Poor on-farm storage of feeds is a primary reason that contaminants of common feed ingredients used in poultry for aflatoxicosis in farm animals (2). Aflatoxin B1 (AFB1) is the most biologically active form of AF and causes poor performance, liver lesions and immunosuppression in poultry (3,4). Negative effects of AFB1 include cell damage, release of free radicals and lipid peroxidation (5). Since lipid peroxidation plays a major role in the toxicity of AF, a protective effect of antioxidants is possible (6,7). Synthetic antioxidants like butylated hydroxytoluene are known to reduce hepatic lesions caused by AFB1 through modulating the detoxification system (8). Plant compounds like coumarins, flavonoids and curcuminoids have inhibitory action on biotransformation of AF to their epoxide-active derivatives (9). Turmeric (Curcuma longa L.), 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 known to possess antimicrobial and antioxidant properties. The powder of dried roots and rhizomes of turmeric are used as one of the spices in Indian curries and other cuisine. The total curcuminoids (TCMN), yellowish pigments present in turmeric powder, have shown antioxidant/protective effects against AFB1 (10,11). Most antioxidants have a dose-dependent efficacy against free radicals and hence need to be evaluated accordingly (12). The objectives of the present study were to evaluate the antioxidant efficacy of graded levels of TCMN in ameliorating aflatoxicosis in broiler chicks, and to demonstrate that the inclusion of TCMN in poultry diets would not negatively affect the performance of chicks.

Materials and methods

Experimental design and birds

One hundred and eighty-day-old (Ross × Ross) male broiler chicks were purchased from a commercial hatchery, weighed, wing banded and assigned to cages in stainless steel chick batteries based on initial body weights. The chicks were maintained on a 24-h continuous light schedule and allowed ad libitum access to feed and water from hatch to day 21. The temperature of the shed ranged between 35–36°C in the beginning and 30–31°C towards the end of the experiment. The animal care and use protocol was reviewed and approved by the University of Missouri–Columbia Animal Care and Use Committee. A completely randomised design was used with six cage replicates of five chicks assigned to each of six dietary treatments. Mortality was recorded as it occurred and birds were inspected daily for any health-related problems.

Diets

A maize–soyabean meal-based basal diet (mash form) was formulated to meet or exceed the nutritional requirements of chicks.

Abbreviations: AFB1, aflatoxin B1; ppb, parts per billion; TCMN, total curcuminoids.

* Corresponding author: Nisarani K. S. Gowda, fax +918025711420, email nksgowda@rediffmail.com
broiler starters (1–21 d post-hatch) as recommended by the National Research Council\(^{(13)}\) (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: basal diet containing neither TCMN nor AFB\(_1\) (control); basal diet supplemented with 444 mg/kg TCMN; basal diet supplemented with 1·0 mg/kg AFB\(_1\) by including ground aflatoxin culture material that contained 760 mg/kg of AFB\(_1\) produced on rice using Aspergillus parasiticus (NRRL 2999)\(^{(14,15)}\); basal diet supplemented with 1·0 mg/kg AFB\(_1\) and 74 mg/kg TCMN; basal diet supplemented with 1·0 mg/kg AFB\(_1\) and 222 mg/kg TCMN; basal diet supplemented with 1·0 mg/kg AFB\(_1\) and 444 mg/kg TCMN. Commercially available food grade turmeric powder (Curcuma longa L.) containing an analysed TCMN content of 2·55 % was used as a source of antioxidant. The graded levels of TCMN and 1·0 mg/kg AFB\(_1\) were selected in the present study based on our previous findings\(^{(11)}\).

Dietary AFB\(_1\) concentrations were confirmed by analysis\(^{(15)}\). In brief, feed samples were extracted with acetonitrile–water (86:14), and an aliquot of this extract was passed through a puriTox TC-M160 cleanup column (Trilogy Analytical Laboratory, Inc., Washington, MO, USA) and suitably diluted with water before analysis using HPLC with Kobra cell (R-Biopharm, Inc., Marshall, MI, USA) post-column derivatisation with fluorescence detection at 365 nm excitation and 440 nm emission. All diets were screened for the presence of citrinin, T-2 toxin, vomitoxin, zearalenone, fumonisins and ochratoxin A\(^{(16,17)}\) before the start of the experiment and were found to be negative. The detection limits for these mycotoxins were as follows: aflatoxin B\(_1\), 10 parts per billion (ppb); ochratoxin A, 50 ppb; zearalenone, 500 ppb; vomitoxin, 500 ppb; citrinin, 500 ppb; T-2 toxin, 1000 ppb and fumonisins, 500 ppb.

**Sample collection**

On day 21, all birds were weighed by cage and total feed consumption recorded for each cage. Average feed intake was corrected for mortality when calculating feed conversion for each cage by considering the total bird days. Twelve birds (six replicates of two birds each) from each treatment were selected randomly, weighed and euthanised with carbon dioxide, and blood was collected via cardiac puncture for serum chemistry analysis. Liver weight of each bird was recorded, and a piece of liver tissue (2–3 g) was collected, rinsed with ice cold PBS (pH 7·4) containing 0·16 mg per ml heparin to prevent blood clot formation. The liver tissue was quickly preserved in a pre-weighed centrifuge tube under ice-cold conditions for assay of antioxidant status.

**Serum chemistry and liver antioxidant status**

Blood was centrifuged at 1400 g at 8°C for 30 min (Sorvall, RC 3 B plus) and serum was separated and preserved at −20°C until submitted for biochemical analysis. Serum samples were analysed for glucose, total protein, albumin, globulin, γ-glutamyl transferase (EC 2.3.2.2), aspartate aminotransferase (EC 2.6.1.1), uric acid and Ca using an auto analyser (Kodak Ektachem Analyser, Eastman Kodak Co., Rochester, NY, USA).

Liver tissue was diluted with ice cold PBS (pH 7·4) without heparin at a ratio of 1:9, homogenised in a homogeniser (Tekmar, SDT 1810, Cincinnati, OH, USA) and centrifuged (10000 g, 4°C, 15 min). The clear supernatant was aspirated into vials and preserved in several aliquots at −80°C until antioxidant status was determined. The parameters measured included total antioxidant concentration, lipid peroxide, aqueous peroxide, superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.16) and total protein using assay kits (Sigma Diagnostics, Sigma Chemical Co., St Louis, MO, USA).

**Total curcuminoid analysis**

Turmeric rhizome powder was analysed for curcumin, bisdeme-thoxycurcumin, and demethoxycurcumin and totalled to calculate the TCMN content\(^{(18)}\). Briefly, 10 mg turmeric powder was extracted with 50 ml hexane. After extraction, hexane was discarded, and turmeric powder was dried and finely grounded. One gram of hexane extracted powder was re-extracted with 20 ml 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 microlitre of the supernatant was removed and diluted with 4 ml methanol. Total curcuminoid content (curcumin, bisdemethoxycurcumin and demethoxy curcumin) 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 commercially available, they were estimated by comparing their peak areas to that of the standard curcumin peak area. Three major peaks appeared in the chromatogram very close to each other. Based on the retention time of curcumin, the third of the three peaks, the other two peaks in the chromatogram were assigned to bisdemethoxycurcumin and demethoxy curcumin. Since all three have the same chromophore present, they should adsorb similarly in the UV; therefore, their total area was quantitated against the areas of standards of curcumin. Total curcuminoid content of the turmeric powder was determined by totalling the concentration of the individual pigments.

Statistical analysis
Data were analysed by one-way ANOVA using the general linear model procedures of Statistical Analysis System® (SAS Institute, Cary, NC, USA)). Cages were used as the experimental unit for all parameters. 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.

Results
Performance and liver weights of birds fed dietary treatments are summarised in Table 2. Feeding a diet with 444 mg/kg TCMN alone had no effect on growth with birds performing as well as the controls. Similarly, 444 mg/kg TCMN did not affect relative liver weights that were comparable to those of control birds. Compared with controls, birds fed 1.0 mg/kg AFB1 had significantly lower feed intake and weight gain. Addition of TCMN (74 and 222 mg/kg) to the AFB1 diet had no effect on feed intake, but significantly increased weight gain and improved feed conversion when compared with birds fed AFB1 alone. Compared with controls, relative liver weight was increased significantly in birds fed AFB1. The addition of all levels of TCMN significantly ameliorated the increase in relative weight of liver observed in birds fed AFB1 alone, but relative liver weights were still heavier than those of control birds. The mortality included two birds in group fed AFB1 alone and one bird in group fed AFB1 with 444 mg/kg TCMN.

Serum biochemical parameters
Feeding diets containing 1.0 mg/kg AFB1 to broiler chickens significantly reduced total protein, albumen, globulin and Ca levels, and increased the activities of γ-glutamyl transferase, aspartate aminotransferase and uric acid content in the serum (Table 3). Supplementation of 222 mg/kg TCMN to the AFB1 diet significantly improved the serum values of total protein, albumen, globulin, γ-glutamyl transferase and aspartate aminotransferase compared with chickens fed AFB1 alone or those fed AFB1 plus 74 or 444 mg/kg TCMN. Birds fed diets containing TCMN with or without AFB1 had significantly lower serum glucose concentrations compared to controls.

Liver antioxidant status
Lipid peroxide and aqueous peroxide levels were significantly increased in liver homogenates of birds fed AFB1 (Table 4). Supplementation of the AFB1 diet with 74 and 222 mg/kg TCMN significantly improved antioxidant status in the liver in terms of total antioxidant concentration, superoxide dismutase and catalase activity and level of peroxides; however, 222 mg/kg TCMN was more effective compared to birds fed the control diet or the diet with AFB1 (Table 4). Although the highest concentration of total antioxidants was observed in liver homogenates of birds fed the diet containing 444 mg/kg TCMN and AFB1, antioxidant protection was not evident in terms of levels of peroxides and superoxide dismutase and catalase activities.

Discussion
Performance of broiler chickens
Performance of birds fed AFB1 alone is in agreement with earlier reports of performance-depressing effects of AFB1. Supplementation of TCMN at 74 and 222 mg/kg to the AFB1 diet partially improved the performance of chickens in...
Effects of diets with total curcuminoid (TCMN) and aflatoxin on serum chemistry of broilers

Table 3. Effect of diets with total curcuminoid (TCMN) and aflatoxin on serum chemistry of broilers*

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Glucose (mg/l)</th>
<th>Total protein (g/l)</th>
<th>Albumin (g/l)</th>
<th>Globulin (g/l)</th>
<th>GGT (U/l)†</th>
<th>AST (U/l)‡</th>
<th>Uric acid (mg/dl)</th>
<th>Ca (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet: control</td>
<td>2480a</td>
<td>26.1a</td>
<td>10.2a</td>
<td>16.0a</td>
<td>13.0a</td>
<td>221b</td>
<td>60.1ab</td>
<td>10.6a</td>
</tr>
<tr>
<td>Basal diet + 444 ppm TCMN</td>
<td>2010bc</td>
<td>25.0a</td>
<td>9.6ab</td>
<td>15.4ab</td>
<td>12.2b</td>
<td>229b</td>
<td>47.1b</td>
<td>10.1ab</td>
</tr>
<tr>
<td>Basal diet + 1.0 ppm AFB1</td>
<td>2230ab</td>
<td>17.2c</td>
<td>6.2d</td>
<td>13.3c</td>
<td>15.9c</td>
<td>312a</td>
<td>65.2c</td>
<td>9.6c</td>
</tr>
<tr>
<td>Basal diet + 1.0 AFB1 + 74 ppm TCMN</td>
<td>1910c</td>
<td>21.0bc</td>
<td>8.0cd</td>
<td>13.0bc</td>
<td>13.5bc</td>
<td>263ab</td>
<td>58.3ab</td>
<td>9.9c</td>
</tr>
<tr>
<td>Basal diet + 1.0 ppm AFB1 + 222 ppm TCMN</td>
<td>1990c</td>
<td>22.2ab</td>
<td>9.2abc</td>
<td>13.1bc</td>
<td>11.9b</td>
<td>251ab</td>
<td>54.2ab</td>
<td>9.6c</td>
</tr>
<tr>
<td>Basal diet + 1.0 AFB1 + 444 ppm TCMN</td>
<td>2090bc</td>
<td>19.0d</td>
<td>8.1bcd</td>
<td>12.4d</td>
<td>13.3ab</td>
<td>310a</td>
<td>59.3ab</td>
<td>9.8c</td>
</tr>
</tbody>
</table>

**SEM** 103.2 1.3 0.61 0.8 0.94 27.75 6.1 0.20

GOT, γ-glutamyl transferase; AST, aspartate amino transferase; ppm, parts per million; AFB1, aflatoxin B1.

† One unit of activity is the amount of enzyme that catalyses the liberation of 1 μmol of π-nitroaniline per min at 25°C.

‡ One unit of activity is the amount of enzyme that converts 1.0 μmol of α-ketoglutarate to L-glutamate in the presence of L-aspartic acid per min at pH 7.5 at 37°C.

Table 4. Effect of diets with total curcuminoid (TCMN) and aflatoxin on antioxidant status in liver of broilers*

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Total antioxidant (mM/ml)</th>
<th>Lipid peroxides (μM/ml)</th>
<th>Aqueous peroxide (μM/ml)</th>
<th>SOD (U/mg protein)†</th>
<th>Catalase (U/mg protein)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet: control</td>
<td>21.1c</td>
<td>0.20c</td>
<td>0.13b</td>
<td>0.72d</td>
<td>20.6ab</td>
</tr>
<tr>
<td>Basal diet + 444 ppm TCMN</td>
<td>44.2a</td>
<td>0.16a</td>
<td>0.09a</td>
<td>0.81ab</td>
<td>21.7a</td>
</tr>
<tr>
<td>Basal diet + 1.0 ppm AFB1</td>
<td>17.8c</td>
<td>0.36c</td>
<td>0.20a</td>
<td>0.84bc</td>
<td>18.4c</td>
</tr>
<tr>
<td>Basal diet + 1.0 AFB1 + 74 ppm TCMN</td>
<td>25.2a</td>
<td>0.22c</td>
<td>0.12bc</td>
<td>0.86bc</td>
<td>19.6c</td>
</tr>
<tr>
<td>Basal diet + 1.0 AFB1 + 222 ppm TCMN</td>
<td>33.1c</td>
<td>0.18bc</td>
<td>0.10cd</td>
<td>1.01a</td>
<td>20.4ab</td>
</tr>
<tr>
<td>Basal diet + 1.0 AFB1 + 444 ppm TCMN</td>
<td>45.4c</td>
<td>0.30c</td>
<td>0.14bc</td>
<td>0.92bc</td>
<td>20.7ab</td>
</tr>
</tbody>
</table>

**SEM** 0.95 0.015 0.008 0.03 1.01

SOD, superoxide dismutase; ppm, parts per million; AFB1, aflatoxin B1.

† One unit of activity is the amount of enzyme that catalyses the liberation of 1 μM of hydrogen peroxide per min at 25°C.

‡ One unit of activity is the amount of enzyme that inhibits the rate of reaction by 100%.

* Means represent twelve observations per treatment.

**Serum biochemical parameters**

The reduced levels of total protein, albumin, globulin, Ca and increased level of γ-glutamyl transferase, aspartate aminotransferase and uric acid are indicative of the toxic effects of AFB1 on hepatic and renal tissue, and the findings are in agreement with previous reports of aflatoxicosis(25,26). The positive effect of TCMN on serum values demonstrated its ameliorating effect against AFB1, as the curcuminoid pigments of turmeric powder possess antioxidant activity against oxidative stress caused by free radicals(27). Similarly, plant extracts of cumin (Nigella sativa), clove (Syzygium aromatica) and African nutmeg (Monodora myristica) have also been shown to have protective properties against AFB1 in both rats and chickens(28,29). The reduction in serum glucose levels in birds supplemented with TCMN demonstrated a hypoglycemic effect, and this finding is in agreement with

the present study. Curcumin, the major pigment in TCMN of turmeric, is known to protect the liver against AFB1(20) by inhibiting the biotransformation of AFB1 to aflatoxicol in liver(19). The other beneficial compounds of turmeric are tetrahydrocurcumin, niaцин, turmerone, curcule and cincinnamic acid(21), but are present in very low concentrations and contribute very little to the overall antioxidant activity. Previously, partial protection with 74 mg/kg TCMN against AFB1 has been reported(13). However, increasing the supplemental levels of TCMN to 222 and 444 mg/kg in the present study did not completely ameliorate the toxic effects of aflatoxin. The failure of increased levels of TCMN to further ameliorate the toxic effects of AFB1 is not unexpected since oxidative damage is not the only mode of action of AFB1. For example, AFB1 has also been shown to decrease the expression of hepatic genes involved in energy production and fatty acid metabolism, detoxification, coagulation and immune protection of broiler chickens(22). The poor performance of chickens fed the diet containing 444 mg/kg TCMN with AFB1 could be attributed to the pro-oxidant action of curcuminoids at higher concentrations. Some polyphenolic compounds have been reported to exhibit both antioxidant and pro-oxidant functions due to metabolic transformations in the presence of transition metals like Cu and Fe(12,23). However, the absence of performance depression in birds fed the diet containing 444 mg/kg TCMN alone suggests an interaction between the metabolites of curcuminoid pigments and AFB1, resulting in much poorer performance in chicks fed the combination of TCMN (444 mg/kg) and AFB1 when compared with lower levels (74 and 222 mg/kg) of TCMN supplementation. The beneficial effects observed in the present study are attributed to the TCMN content of turmeric powder. It should be, however, noted that different turmeric species are known to have different levels of curcuminoids (2–7%) (24), and hence requires analysis before using the commercial turmeric powder as a supplement. Also curcumin is highly sensitive to light, heat and alkaline pH (http://www.fao.org/inpho/content/compand/text/ch29/ch29.htm), and hence care need to be exercised while using curcumin in feed.
Liver antioxidant status

The antioxidant status in liver homogenates suggests that supplementation with TCMN stimulated the antioxidant system in the liver to counteract the oxidative damage caused by AFB1. Aflatoxin B1 is a potent carcinogen that forms adducts with DNA, induces cellular oxidative damage\(^{(31)}\) and causes lipid peroxidation in liver\(^{(32)}\). Supplementation of root extracts of *Picrorhiza kurroa* and seeds of *Silybum marianum* enhanced the activity of antioxidant enzymes and reduced peroxide levels in liver of rats fed AFB1\(^{(33)}\). The carboxyl functional group of curcuminoids from turmeric was shown to be responsible for its antimutagenic and anticarcinogenic action\(^{(34)}\). Moreover, curcumin has been shown to strongly inhibit superoxide anion generation\(^{(35)}\) and biotransformation of AFB1 to aflatoxicol in liver\(^{(36)}\). These findings support the hypothesised action of curcuminoids as antioxidants, and the results of the present study suggest that 222 mg/kg TCMN provided maximum protection against AFB1. Free radicals, apart from initiating lipid peroxidation, also release cytoplasmic Ca\(^{2+}\) that plays a crucial role in subsequent propagation of tissue injury, and hence protection against oxidative stress cannot be completely achieved by the action of radical scavenging antioxidants alone\(^{(36)}\).

Supplementation of TCMN at 444 mg/kg to the AFB1 diet, although it significantly increased total antioxidant concentration in the liver, did not increase the activity of superoxide dismutase or catalase and hence did not reduce the peroxide levels. The performance of these chickens was similar to those fed AFB1 alone. This finding is supported by the fact that certain naturally occurring polyphenolics like catechin, galangin and quercetin have been shown to inhibit lipid peroxidation, also release cytoplasmic Ca\(^{2+}\) that plays a crucial role in subsequent propagation of tissue injury, and hence protection against oxidative stress cannot be completely achieved by the action of radical scavenging antioxidants alone\(^{(36)}\).

From the present study, it is concluded that dietary supplementation of 222 mg/kg TCMN to a diet containing 1.0 mg/kg AFB1 provided the greatest amelioration and demonstrated highest antioxidant activity.

Acknowledgements

The Overseas Associateship granted by the Department of Biotechnology, Ministry of Science and Technology, Government of India to the first author for conducting research on mycotoxin and antioxidant status at the University of Missouri, Columbia, USA, is thankfully acknowledged. There is no conflict of interest among the authors and there is no specific funding for the present study. N. K. S. G. and D. R. L. planned and performed the present study, G. E. R. and A. J. B. helped in toxicological investigation and Y. C. C. did the total curcuminoid analysis.

References


