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Antioxidant efficacy of curcuminoids from turmeric (*Curcuma longa* L.) powder in broiler chickens fed diets containing aflatoxin B_1

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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 B_1 (AFB_1) 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 AFB_1; basal diet supplemented with 74 mg/kg TCMN and 1·0 mg/kg AFB_1; basal diet supplemented with 222 mg/kg TCMN and 1·0 mg/kg AFB_1; basal diet supplemented with 444 mg/kg TCMN and 1·0 mg/kg AFB_1. The addition of 74 and 222 mg/kg TCMN to the AFB_1 diet significantly ($P<0·05$) improved weight gain and feed efficiency. Increase ($P<0·05$) in relative liver weight in birds fed AFB_1 was significantly reduced ($P<0·05$) with the addition of 74, 222 and 444 mg/kg TCMN to the AFB_1 diet. The inclusion of 222 mg/kg TCMN ameliorated the adverse effects of AFB_1 on serum chemistry in terms of total protein, albumin and γ-glutamyl transferase activity. The decreased antioxidant functions due to AFB_1 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 AFB_1 diet demonstrated maximum antioxidant activity against AFB_1.

Aflatoxin B_1: 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 for aflatoxicosis in farm animals(2). Aflatoxin B_1 (AFB_1) is the most biologically active form of AF and causes poor performance, liver lesions and immunosuppression in poultry(3,4). Negative effects of AFB_1 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 AFB_1 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 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 AFB_1(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 35–36·1°C in the beginning and 30–31·1°C towards the end the 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

Abbreviations: AFB_1, aflatoxin B_1; ppb, parts per billion; TCMN, total curcuminoids.

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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]; 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_1were 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.

Table 1. Ingredient composition and calculated analysis of the basal diet

Ingredient	Composition (%)
Maize	53·38
Soyabean meal	34·61
Maize oil	5·89
Pork meal	3·54
Dicalcium phosphate	1·03
Limestone	0·75
Salt	0·41
DL-Methionine	0·19
Trace minerals mixture*	0·10
Se premix†	0·05
Vitamin mixture‡	0·05
Copper sulphate	0·004
Nutrient composition (calculated)	
Crude protein (%)	23·00
Metabolisable energy (MJ/kg)	13·39
Lys (%)	1·25
Met (%)	0·54
Met + Cys (%)	0·90
Ca (%)	1·00
Available P (%)	0·45

* Trace mineral mix provided (mg/kg of diet): Mn, 110 mg from $MnSO_4$; Fe, 60 mg from $FeSO_4 \cdot 7H_2O$; Zn, 110 mg from $ZnSO_4$; iodine, 2 mg from ethylenediamine dihydroiodide.

† Se premix provided 0·2 mg Se/kg of diet from $NaSeO_3$.

‡ Vitamin mix supplied (per kg of feed): vitamin A (retinyl acetate), 8800 IU (2·66 mg); cholecalciferol, 3855 ICU (96·37 μg); vitamin E (DL-α-tocopheryl acetate), 14 IU (14 μg); niacin, 55 mg; calcium pantothenate, 17 mg; riboflavin, 6·6 mg; pyridoxine, 2·2 mg, menadione sodium bisulphite, 1·7 mg; folic acid, 1·4 mg; thiamin mononitrate, 1·1 mg; biotin, 0·2 mg; cyanocobalamin, 11 μg.

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 (10 000 ***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* 1.11.1.1.6) 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, bisdemethoxycurcumin, and demethoxycurcumin and totalled to calculate the TCMN content[18]. Briefly, 10 g 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,

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250 × 4·6 mm HyperSil reverse-phase C_{18} 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)[19]. 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 of broiler chickens

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 AFB_1 had significantly lower feed intake and weight gain. Addition of TCMN (74 and 222 mg/kg) to the AFB_1 diet had no effect on feed intake, but significantly increased weight gain and improved feed conversion when compared with birds fed AFB_1 alone. Compared with controls, relative liver weight was increased significantly in birds fed AFB_1. The addition of all levels of TCMN significantly ameliorated the increase in relative weight of liver observed in birds fed AFB_1 alone, but relative liver weights were still heavier than those of control birds. The mortality included two birds in group fed AFB_1 alone and one bird in group fed AFB_1 with 444 mg/kg TCMN.

Serum biochemical parameters

Feeding diets containing 1·0 mg/kg AFB_1 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 AFB_1 diet significantly improved the serum values of total protein, albumen, globulin, γ-glutamyl transferase and aspartate aminotransferase compared with chickens fed AFB_1 alone or those fed AFB_1 plus 74 or 444 mg/kg TCMN. Birds fed diets containing TCMN with or without AFB_1 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 AFB_1 (Table 4). Supplementation of the AFB_1 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 AFB_1 (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 AFB_1, antioxidant protection was not evident in terms of level of peroxides and superoxide dismutase and catalase activities.

Discussion

Performance of broiler chickens

Performance of birds fed AFB_1 alone is in agreement with earlier reports of performance-depressing effects of AFB_1[4,11]. Supplementation of TCMN at 74 and 222 mg/kg to the AFB_1 diet partially improved the performance of chickens in

Table 2. Performance of broilers fed diets containing total curcuminoid (TCMN) and aflatoxin (1–21 d post-hatch)

Dietary treatment	Feed intake (g/bird)*	Body weight gain (g/bird)*	Feed:gain*	Relative liver weight (% body weight)†
Basal diet: control	789^{a}	655^{a}	1·201^{b}	2·91^{c}
Basal diet + 444 ppm TCMN	752^{ab}	650^{a}	1·221^{b}	2·80^{c}
Basal diet + 1·0 ppm AFB_1	716^{bc}	556^{d}	1·302^{a}	3·82^{a}
Basal diet + 1·0 AFB_1 + 74 ppm TCMN	733^{bc}	604^{bc}	1·201^{b}	3·41^{b}
Basal diet + 1·0 AFB_1 + 222 ppm TCMN	723^{bc}	639^{ab}	1·210^{b}	3·30^{b}
Basal diet + 1·0 AFB_1 + 444 ppm TCMN	689^{c}	581^{cd}	1·204^{b}	3·55^{b}
SEM	17·4	14·1	0·02	0·10

ppm, parts per million; AFB_1, aflatoxin B_1.
a,b,c Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* Means represent six cages per treatment and five birds per cage.
† Means represent twelve observations per treatment.

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

Dietary treatment	Glucose (mg/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	GGT (U/l)†	AST (U/l)‡	Uric acid (mg/l)	Ca (mg/dl)
Basal diet: control	2480[a]	26·1[a]	10·2[a]	16·0[a]	13·0[b]	221[b]	60·1[ab]	10·6[a]
Basal diet + 444 ppm TCMN	2010[bc]	25·0[a]	9·8[ab]	15·4[ab]	12·2[b]	229[b]	47·1[b]	10·1[ab]
Basal diet + 1·0 ppm AFB_1	2230[ab]	17·2[c]	6·2[d]	11·3[c]	15·9[a]	312[a]	65·2[a]	9·6[b]
Basal diet + 1·0 AFB_1 + 74 ppm TCMN	1910[c]	21·0[bc]	8·0[cd]	13·0[bc]	13·5[ab]	263[ab]	58·3[ab]	9·9[b]
Basal diet + 1·0 ppm AFB_1 + 222 ppm TCMN	1930[c]	22·2[ab]	9·2[abc]	13·1[bc]	11·9[b]	251[ab]	54·2[ab]	9·9[b]
Basal diet + 1·0 AFB_1 + 444 ppm TCMN	2090[bc]	19·0[c]	8·1[bcd]	12·4[c]	13·3[ab]	310[a]	59·3[ab]	9·8[b]
SEM	103·2	1·3	0·61	0·8	0·94	27·75	6·1	0·20

GGT, γ-glutamyl transferase; AST, aspartate amino transferase; ppm, parts per million; AFB_1, aflatoxin B_1.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* Means represent twelve observations per treatment.
† One unit of activity is the amount of enzyme that catalyses the liberation of 1 μM of *p*-nitroaniline per min at 25°C.
‡ One unit of activity is the amount of enzyme that converts 1·0 μM of α-ketoglutarate to L-glutamate in the presence of L-aspartic acid per min at pH 7·5 at 37°C.

the present study. Curcumin, the major pigment in TCMN of turmeric, is known to protect the liver against AFB_1[20] by inhibiting the biotransformation of AFB_1 to aflatoxicol in liver[9]. The other beneficial compounds of turmeric are tetrahydrocurcumin, niacin, turmerone, curlone and cinnamic 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 AFB_1 has been reported[11]. 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 AFB_1 is not unexpected since oxidative damage is not the only mode of action of AFB_1. For example, AFB_1 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 AFB_1 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 AFB_1, resulting in much poorer performance in chicks fed the combination of TCMN (444 mg/kg) and AFB_1 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/compend/text/ch29/ch29.htm), and hence care need to be exercised while using curcumin in feed pellets prepared at 75–80°C.

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 AFB_1 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 AFB_1, 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 aromaticum*) and African nutmeg (*Monodora myristica*) have also been shown to have protective properties against AFB_1 in both rats and chickens[28,29]. The reduction in serum glucose levels in birds supplemented with TCMN demonstrated a hypoglycaemic effect, and this finding is in agreement with

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

Dietary treatment	Total antioxidant (mM/ml)	Lipid peroxides (μM/ml)	Aqueous peroxide (μM/ml)	SOD (U/mg protein)†	Catalase (U/mg protein)‡
Basal diet: control	21·1[d]	0·20[cd]	0·13[b]	0·72[d]	20·6[ab]
Basal diet + 444 ppm TCMN	44·2[a]	0·16[d]	0·09[d]	0·81[cd]	21·7[a]
Basal diet + 1·0 ppm AFB_1	17·8[e]	0·36[a]	0·20[a]	0·84[bc]	18·4[c]
Basal diet + 1·0 AFB_1 + 74 ppm TCMN	25·2[c]	0·22[c]	0·12b[c]	0·96[ab]	19·6[bc]
Basal diet + 1·0 ppm AFB_1 + 222 ppm TCMN	33·1[b]	0·18[d]	0·10[cd]	1·01[a]	20·4[ab]
Basal diet + 1·0 AFB_1 + 444 ppm TCMN	45·4[a]	0·30[b]	0·14[b]	0·92[ab]	20·7[ab]
SEM	0·95	0·015	0·008	0·03	1·01

SOD, superoxide dismutase; ppm, parts per million; AFB_1, aflatoxin B_1.
[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* Means represent twelve observations per treatment.
† One unit of activity is the amount of enzyme that inhibits the rate of reaction by 100 %.
‡ One unit of activity is the amount of enzyme that degrades 1 μM of hydrogen peroxide per min at 25°C.

an earlier report on the hypoglycaemic effect of curcumin pigment in diabetic rats[30].

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 AFB_1. Aflatoxin B_1 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 AFB_1[33]. The carbonyl 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 AFB_1 to aflatoxicol in liver[9]. 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 AFB_1. 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 AFB_1 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 AFB_1 alone. This finding is supported by the fact that certain naturally occurring polyphenolics like catechin, galangin and quercetin have been shown to inhibit lipid oxidation, but also showed a pro-oxidant action during the lag phase of the oxidation due to transition metal (Cu/Fe)-induced generation of free radicals[23,37]. Other phenolics like eugenol (2-allyl-4-methoxyphenol) are modulated as a pro-oxidant or antioxidant under certain circumstances[12].

From the present study, it is concluded that dietary supplementation of 222 mg/kg TCMN to a diet containing 1·0 mg/kg AFB_1 provided the greatest amelioration and demonstrated highest antioxidant activity.

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