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Efficacy of DL- methionine in amelioration of aflatoxicosis in coloured broiler chicken

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ABSTRACT

The effect of supplementation of DL- methionine (0.025 and 0.05%) in diet containing 1 ppm total aflatoxin (AF: 76.45% AFB_1, 10.52% AFB_2, 9.89% AFG_1 and 3.14% AFG_2) in broiler chickens (day-old to 42 days of age) was investigated. Day-old broiler chicks (180) were divided into 6 treatment groups (T_1 – control; T_2 – T_1 + 1ppm AF, T_3 – T_1 + 0.025% Met, T_4 – T_1 + 0.05% Met, T_5 – T_2 + 0.025% Met, and T_6 – T_2 + 0.05% Met). Each diet was fed to 3 replicated groups of 10 birds each from day-old to 42 days of age. The results showed that the body weight gain (BWG) of aflatoxin fed group (T_2) was lower than that of control group (T_1). The weight gain in groups T_3 and T_4 did not differ from control, while in groups T_5 and T_6 gain was higher than that of T_2, but lower than that of control. The feed intake (FI) in T_5 and T_6 was higher than that of T_2, but lower than that of control, while FI did not differ in T3 and T4 from that of control. The FCR also increased to 2.41 in T_2 in comparison to control (2.15). Barring T2, the FCR in other groups were equal to that of control. Significantly reduced serum protein and cholesterol were reported in T_2 compared to T_1. The serum protein and cholesterol content in group T_5 and T_6 remained higher than that of T_2, but lower than that of control. ASAT and ALAT activities were increased by feeding aflatoxin (T_2) compared to T_1. Methionine supplementation to toxin contaminated diet (T_5 and T_6) reversed these parameters more or less comparable to that of control. Significant increase in the relative weights of liver and gizzard due to aflatoxin feeding (T_2) was reported compared to control. The relative weights of liver and gizzard in groups T_5 and T_6 were similar to that of control. The relative weight of bursa of Fabricius in T_2 was lower compared to control. The relative weights of bursa in groups T_5 and T_6 was similar to that of control. It was concluded that inclusion of slightly higher methionine (0.025 or 0.05%) in feed of coloured broiler chickens was beneficial to counteract the adverse effects of aflatoxin partially.

Key words: Aflatoxicosis, Broiler, Feed, Methionine

Poultry industry suffers greatly from the economic losses due to the adverse effects of mycotoxicosis attributed to reduced feed intake, poor feed conversion and nutrient utilization, growth and production performance, immuno-suppression and subsequently increased susceptibility to various infections (Xue *et al*. 2010). Aflatoxin is the most commonly occurring mycotoxin in India. Major forms of AF include B_1, B_2, G_1, and G_2, while AFB_1 is the most common and biologically active component (Busby and Wogan 1981). Several approaches were developed for decontamination of mycotoxins in foods (Shetty and Jespersen 2006, Diaz *et al*. 2004). Though many approaches are available for mycotoxin decontamination, most of them are not widely available due to high cost or practical difficulties involved in detoxification process (Shetty and Jespersen 2006). Now, the most promising and user-friendly approach is dietary manipulation, addition of adsorbents and detoxifying agent in feed. Selected adsorbents added to AF-contaminated feeds can sequester AF during the digestive process, allowing the mycotoxin to pass harmlessly through the animal (Davidson *et al*. 1987). However, not all of adsorbents are equally effective in protecting poultry against the toxic effects of AF and several adsorbents showed to impair nutrient utilization (Chung *et al*. 1990). Moreover, due to differences in binding capacity, it is obvious that some portion of the toxins gets absorbed and causes deleterious effect. Addition of sulphur amino acids to diets containing AF improved performance in chickens (Veltmann *et al.* 1981, 1983). Dietary methionine in excess amount (30–40% more) is reported to ameliorate the ill effects of aflatoxicosis in broiler chickens (Devegowda *et al.* 2000). However, such a level of methionine was very high and it may not be economical to add that amount. Moreover, methionine is considered to be a toxic amino acid. Therefore, the present

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study was conducted to determine the efficacy of additional methionine at lower level (excess by 5% and 10%) to ameliorate the toxic effects of aflatoxins.

MATERIALS AND METHODS

Aflatoxin production: Lyophilized preparation of *Aspergillus parasiticus* NRRL 2999 was obtained from U.S. Department of Agriculture, Peoria, Illinois (USA). The lyophilized preparation was revived on potato dextrose agar (PDA) medium and used for AF production. AF was produced from *Aspergillus parasiticus* NRRL 2999 by fermentation of cracked maize as per Shotwell *et al*. (1966). The fermented maize was steamed to kill the fungus spores, dried and ground to a fine powder. The aflatoxin concentration from maize powder was extracted as per Pons *et al*. (1966) and measured using thin layer chromatography (TLC). The total AF concentration in maize powder consisted of 76.45% AFB_1, 10.52% AFB_2, and 9.89% AFG_1 and 3.14% AFG_2. The maize powder containing known concentration of AF was incorporated into the basal diets of certain dietary treatments to get the desired amount of 1ppm total AF.

Birds and diets: Day-old broiler (IR-3) chicks (180) obtained from CARI hatchery were used in this study. These chicks were wing banded, weighed individually and divided into 6 treatment groups (T_1 to T_6), each replicated thrice and each replication consisted of 10 birds (each group containing 30 chicks). The chicks were housed in electrically heated compartments with continuous lighting and given broiler starter feed up to 21 days and broiler finisher feed from 22 to 42 days of age. The composition of basal diets is given in Table 1. There were 6 experimental diets viz. T_1, basal diet (0.5 and 0.44% Met in starting and finishing ration) + 0 ppb aflatoxin (control); T_2, basal diet + 1 ppm aflatoxin; T_3, basal diet + 0.025% Met; T_4, basal diet +0.05% Met; T_5, basal diet + 0.025% Met + 1ppm aflatoxin, and T_6, basal diet + 0.05% Met + 1ppm aflatoxin.

Table 1. Ingredient and chemical composition of basal diet

	Broiler starter	Broiler finisher
Feed ingredients		
Maize	56	60.65
Soybean meal	33	28.475
Sunflower cake	1.065	2
Rapeseed meal	4	4
Fish meal	3	3
Limestone	0.8	0/6
Di-calcium phosphate	1.4	1.2
Common salt	0.2	0.2
DL- methionine	0.08	0.04
Lysine	0.07	0.02
TM premix1	0.11	0.1
Vit premix1	0.15	0.15
B complex	0.015	0.015
Ch. chloride	0.05	0.05
Sodium bi- carbonate	0	0.05
Vitamin C	0.01	0.00
Coccidiostat	0.05	0.05
Total	100	100
Calculated composition		
Crude protein %	21.17	19.47
Metabolisable energy, Kcal/kg	2850	2888
Ca %	1.02	0.89
Available P %	0.44	0.41
Lysine	1.20	1.05
Methionine	0.50	0.44

Birds were inspected daily and their body weight and feed consumption were recorded weekly. Feed intake, body weight gain and feed conversion ratio (feed intake/ weight gain) were calculated. After completion of feeding trial at the age of 42 days, 9 birds from each treatment (3 from each replicate) were selected randomly for recording organ weights and to collect blood samples. Serum was separated and used for biochemical assays. Serum concentrations of total protein, total cholesterol, uric acid and the activities of ASAT and ALAT were determined spectrophotometrically using commercially available test kits. The data were analysed statistically using Duncan's multiple range test (SPSS 16.0).

RESULTS AND DISCUSSION

Body weight gain: Significant ($P<0.05$) differences in body weight gain (BWG) among various dietary treatments were recorded from third week of age onward (Table 2). At third week of age, the BWG was lower ($P\leq0.05$) in T_2 than in T_1, while BWG remained statistically similar in other treatments to that of control. At fourth, fifth and sixth weeks of age also the birds under T_2 had lower body weight than control. Moreover, the BWG in T_1, T_3 and T_4 were statistically similar. Dietary supplementation of methionine at both the levels (T_5 and T_6) to aflatoxin contaminated diet improved ($P\leq0.05$) the BWG, even then the values were lower ($P\leq0.05$) than that of control barring T_6 at sixth week of age wherein the BWG was statistically similar to that of control. During overall growth phase (0 to 6 weeks), the BWG decreased significantly on inclusion of 1 ppm of total aflatoxin in the diet. These results were in agreement with earlier workers who reported reduction in BWG at 0.2 to 0.5 ppm level of dietary aflatoxin (Azzam and Gopal 1997, Raju and Devegowda 2000, Sapocota *et al*. 2007, Silambarsan *et al*. 2013, Miazzo *et al*. 2000, Ledoux *et al*. 1999, Kubena *et al*. 1998, Santurio *et al*. 1999, and Rosa *et al*. 2001). In the present study, the results showed that supplementation of methionine to the basal diet did not produce any positive effect on weight gain of broilers, which validated its requirement of 0.50% as suggested by NRC (1994). Methionine supplementation in the aflatoxin contaminated diet resulted in significant improvement in the BWG. These findings are in agreement with earlier report (Sapocota *et al*. 2007) where partial improvement in BWG was reported when aflatoxin contaminated diet was supplemented with methionine. Devegowda *et al*. (2000) also reported that

Table 2. Effect of methionine on body weight gain (g/bird) for broiler chicks on a diet containing total aflatoxin between 1 to 42 days of age

Treatments	Weeks						
	1	2	3	4	5	6	0–6
T1	54.0±5.21	94.9±8.51	128.1±8.72^{bc}	218.9±6.57^{d}	246.1±2.89^{c}	278.4±9.87^{c}	1020.6±14.54^{d}
T2	57.5±1.58	88.4±4.01	94.7±3.50^{a}	110.6±5.26^{a}	150.5±2.51^{a}	205.9±14.67^{a}	707.9±16.90^{a}
T3	53.4±4.99	96.6±7.20	130.0±9.04^{bc}	221.5±7.25^{d}	248.4±2.85^{c}	279.0±9.33^{c}	1029.1±12.25^{d}
T4	53.9±4.32	94.1±8.55	134.7±12.74^{c}	212.3±5.19^{d}	249.3±3.33^{c}	273.3±10.28^{c}	1017.8±15.78^{d}
T5	62.8±2.61	89.3±3.24	104.7±2.60a^{b}	135.4±4.74^{b}	199.1±11.64^{b}	232.3±3.41^{ab}	823.9±3.61^{b}
T6	58.0±2.22	86.3±2.76	115.4±4.95^{abc}	170.0±5.63^{c}	209.9±3.58^{b}	261.1±14.49^{bc}	900.9±15.83^{c}

Means bearing different superscripts within a column differ significantly ($P \leq 0.05$).

Table 3. Effect of methionine on feed consumption (g/bird) for broiler chicks on a diet containing total aflatoxin between 1 to 42 days of age

Treatments	Weeks						
	1	2	3	4	5	6	0–6
T1	90.6±6.01	194.1±17.11	289.2±4.65^{b}	430.6±11.65^{b}	537.9±13.75^{b}	656.9±7.93^{c}	2199.6±12.44^{d}
T2	86.3±1.43	187.9±1.88	248.7±16.78^{a}	292.8±6.45^{a}	388.9±35.77^{a}	498.8±31.22^{a}	1699.2±27.37^{a}
T3	90.1±5.18	193.4±17.73	290.4±3.54^{b}	430.7±12.44^{b}	537.0±13.66^{b}	657.6±8.81^{c}	2199.5±12.07^{d}
T4	89.7±3.75	197.7±12.37	286.8±6.28^{b}	424.0±15.38^{b}	530.4±9.47^{b}	649.1±12.20^{c}	2178.0±27.95^{d}
T5	94.7±4.93	188.5±7.18	249.0±7.68^{a}	336.0±20.26^{a}	464.7±29.21^{b}	521.2±2.32^{a}	1854.2±37.05^{b}
T6	91.4±5.48	180.8±5.41	287.0±14.58^{b}	410.3±34.56^{b}	465.8±23.29^{b}	583.8±14.97^{b}	2019.4±25.86^{c}

Means bearing different superscripts within a column differ significantly ($P \geq 0.05$).

Table 4. Effect of methionine on FCR for broiler chicks on a diet containing total aflatoxin between 1 to 42 days of age

Treatments	Weeks						
	1	2	3	4	5	6	0–6
T1	1.686±0.06	2.046±0.07	2.283±0.17	1.973±0.10^{a}	2.183±0.03^{a}	2.366±0.06	2.153±0.02^{a}
T2	1.503±0.01	2.133±0.07	2.620±0.09	2.653±0.09^{b}	2.580±0.19^{b}	2.433±0.14	2.406±0.07^{b}
T3	1.698±0.07	1.997±0.06	2.255±0.16	1.951±0.11^{a}	2.161±0.05^{a}	2.360±0.05	2.137±0.01^{a}
T4	1.675±0.06	2.111±0.07	2.170±0.22	2.001±0.11^{a}	2.128±0.05^{a}	2.378±0.06	2.140±0.02^{a}
T5	1.506±0.02	2.109±0.01	2.378±0.11	2.487±0.17^{b}	2.332±0.03^{ab}	2.258±0.05	2.249±0.03^{a}
T6	1.575±0.07	2.096±0.06	2.486±0.09	2.405±0.13^{b}	2.219±0.11^{a}	2.243±0.07	2.242±0.04^{a}

Means bearing different superscripts within a column differ significantly ($P \leq 0.05$)

excess dietary methionine can ameliorate the adverse effects of aflatoxin in broiler chickens.

Feed consumption: Feed intake (FI) pattern differed significantly ($P<0.05$) among various dietary treatments from third week of age onward (Table 3). FI in T_2 was lower ($P \leq 0.05$) than that of T_1 from third week of age onward. The FI in T_3, T_4 and T_6 was statistically similar to that of control but was statistically lower in T_5 during third and fourth week of age. During overall growth phase (0–6 wk), the FI in aflatoxin fed group (T_2) was lower ($P \leq 0.05$) than control group (T_1). These results were in conformity with earlier reports where aflatoxin contaminated feed (0.3 ppm or higher) resulted in significant decrease in FI of broiler chickens (Kubena *et al*. 1990, Kubena *et al*. 1998, Ledoux *et al*. 1999, Santurio *et al*. 1999, Raju and Devegowda 2000). The overall FI in T_3 and T_4 was statistically similar to that of control indicating that supplementation of methionine to basal diet did not produce any positive effect on weight gain of broilers. Dietary addition of methionine at both levels (0.025 and 0.05%) to the aflatoxin contaminated diet (T_5 and T_6) significantly ($P \leq 0.05$) improved the FI, however the values were significantly ($P \leq 0.05$) lower than that of control. Significant difference ($P \leq 0.05$) in values of T_5 and T_6 was observed, being higher in T_6.

Feed conversion ratio: Feed conversion ratio (FCR) did not differ ($P \leq 0.05$) among various dietary treatments during first, second, third and sixth weeks of age (Table 4). During overall growth phase (0 to 6 wk), FCR of toxin fed group

Table 5. Effect of methionine on serum biochemical parameters in broiler chickens on diet containing 1ppm total aflatoxin at 1 to 42 days of age

Treatments	Total protein (*g/100 ml*)	Cholesterol (*mg/100 ml*)	Uric acid (*mg/100 ml*)	ASAT (*IU/L*)	ALAT (*IU/L*)
T1	$4.95{\pm}0.04^{c}$	$152.18{\pm}2.52^{c}$	$5.09{\pm}0.06^{c}$	$199.19{\pm}1.52^{ab}$	$7.60{\pm}0.09^{a}$
T2	$3.03{\pm}0.20^{a}$	$110.47{\pm}2.49^{a}$	$4.54{\pm}0.03^{a}$	$241.41{\pm}3.92^{c}$	$9.41{\pm}0.70^{b}$
T3	$4.91{\pm}0.06^{c}$	$153.57{\pm}2.33^{c}$	$4.98{\pm}0.11^{bc}$	$189.09{\pm}4.96^{a}$	$7.69{\pm}0.12^{a}$
T4	$4.88{\pm}0.05^{c}$	$148.60{\pm}1.05^{c}$	$4.85{\pm}0.05^{b}$	$190.32{\pm}2.59^{a}$	$7.94{\pm}0.09^{a}$
T5	$4.08{\pm}0.15^{b}$	$128.20{\pm}1.72^{b}$	$4.51{\pm}0.05^{a}$	$198.08{\pm}1.56^{ab}$	$8.66{\pm}0.33^{ab}$
T6	$3.81{\pm}0.03^{b}$	$126.01{\pm}1.86^{b}$	$4.47{\pm}0.03^{a}$	$202.17{\pm}2.30^{b}$	$7.78{\pm}0.15^{a}$

Means bearing different superscripts within a column differ significantly ($P \leq 0.05$).

(T_2) was poorer ($P \leq 0.05$) than of control group (T_1). Raju and Devegowda (2000) also reported poor feed efficiency in broilers fed diets containing 0.3 ppm aflatoxin. Several others have also reported a decrease in feed efficiency due to presence of aflatoxin in diet (Scheideler 1993, Kubena *et al*. 1998, Rosa *et al*. 2001). In present study, the inclusion of methionine in basal diet (T_3 and T_4) did not bring any positive effect on FCR. However, addition of DL-methionine at both the levels (0.025 and 0.05%) to the aflatoxin contaminated diet (T_5 and T_6) resulted in significant ($P \leq 0.05$) improvement in FCR, statistically comparable to control. These reports were in agreement with other workers (Sapocota *et al*. 2007, Naveenkumar *et al*. 2007), wherein methionine supplementation to the aflatoxin contaminated feed resulted in improvement of feed conversion efficiency in broiler chickens.

Biochemical parameters: The total serum protein in T_2 was lower ($P \leq 0.05$) than that of T_1 (Table 5), while it remained comparable to that of control in T_3 and T_4. The serum protein content of groups T_5 and T_6 were lower ($P \leq 0.05$) than that of control but higher ($P \leq 0.05$) than that of T_2. Methionine addition (0.025 and 0.05%) to aflatoxin contaminated diet partially reduced the adverse effects of aflatoxicosis on serum protein. Decrease in total serum protein due to aflatoxin contamination in diet was also reported by earlier researchers (Kubena *et al.* 1998, Ledoux *et al*. 1999, Raju and Devegowda 2000, Ahamad 2000). With regard to serum cholesterol content, it was lower ($P \leq 0.05$) in T_2 compared to T_1. Addition of methionine to the aflatoxin contaminated diet (T_5 and T_6) increased ($P \leq 0.05$) the cholesterol content, but the values remained lower ($P \leq 0.05$) than that of control. These results are in agreement with the other reports (Bailey *et al*. 1998, Kececi *et al*. 1998, Raju and Devegowda 2000, Ahamad 2000). The uric acid content in T_1 was higher ($P \leq 0.05$) than that of aflatoxin fed group (T_2). The uric acid content of groups T_5 and T_6 was statistically similar to that of T_2 but lower ($P \leq 0.05$) than that of control. Decrease in uric acid due to aflatoxin contamination in diet was reported earlier (Bailey *et al*. 1998, Kececi *et al*. 1998, Ahmad 2000). The aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities in T_1 were lower ($P \leq 0.05$) than that of T_2 and remained comparable to T_3, T_4, T_5 and T_6. Significant increase in the activities of ASAT was also reported by Basmacioglu *et al.* (2005) where 2 ppm aflatoxin was fed to broilers, whereas, Kubena *et al.* (1993) reported a decrease in ASAT activities at 3.5 ppm of aflatoxin in the diet of broilers. In present study, ALAT activities were increased ($P \leq 0.05$) by feeding 1 ppm aflatoxin. These results are in agreement with Santurio *et al*. (1999) where increase in ALAT activities was reported at 3 ppm level of aflatoxin. Methionine supplementations to toxin contaminated diet reversed these parameters significantly ($P \leq 0.05$) and were comparable to that of control. The information on effect of supplementation of methionine on ASAT and ALAT activities during aflatoxicosis in poultry is lacking in literature.

Table 6**.** Effect of methionine on relative weights (% of live weight) of organs in broiler chickens on diet containing 1ppm total aflatoxin at 1 to 42 days of age

Treatments	Liver	Gizzard	Heart	Bursa
T1	$2.67{\pm}0.08^{a}$	$2.05{\pm}0.02^{a}$	$0.54{\pm}0.01^{ab}$	0.26^{b}
T2	$3.34{\pm}0.07^{b}$	$2.36{\pm}0.06^{b}$	$0.57{\pm}0.00^{b}$	0.19^{a}
T3	$2.70{\pm}0.10^{a}$	$2.10{\pm}0.02^{a}$	$0.54{\pm}0.01^{ab}$	0.24^{b}
T4	$2.68{\pm}0.12^{a}$	$2.09{\pm}0.02^{a}$	$0.56{\pm}0.00^{ab}$	0.23^{b}
T5	$2.80{\pm}0.09^{a}$	$2.16{\pm}0.03^{a}$	$0.56{\pm}0.00^{ab}$	0.24^{b}
T6	$2.70{\pm}0.03^{a}$	$2.17{\pm}0.01^{a}$	$0.55{\pm}0.01^{ab}$	0.25^{b}

Means bearing different superscripts within a column differ significantly ($P \leq 0.05$).

Organ weights: The relative weights of liver and gizzard in control group was lower ($P \leq 0.05$) than that of aflatoxin fed group (T_2) (Table 6), while in other treatments (T_3 to T_6), the values were statistically similar to that of control. Significant increase in the relative weights of liver and gizzard due to aflatoxin feeding (0.3 to 5 ppm) was also reported earlier (Kubena *et al*. 1998, Raju and Devegowda 2000, Rosa *et al*. 2001, Miazzo *et al*. 2000, Sapocota *et al*. 2007). In the present study, incorporation of methionine at 0.025 and 0.05% levels in the aflatoxin containing diet (T_5

and T_6) reversed the relative weights of liver and gizzard as the values were statistically similar to that of control. These results were in agreement with Sapocota *et al*. (2007). In the present study, the relative weight of heart remained unaffected due to various dietary treatments, however, several researchers (Kubena *et al*. 1990, Bailey *et al*. 1998, Kubena *et al*. 1998, Rosa *et al*. 2001) reported an increase in the relative weight of heart in aflatoxin fed groups. In the present study, the relative weight of bursa of Fabricius in T_2 was lower ($P \leq 0.05$) than that of in T_1. The relative weight of bursa in T_3 to T_6 was statistically similar to that of T_1. Incorporation of methionine at 0.025 and 0.05% levels in the aflatoxin contaminated diet (T_5 and T_6) reversed ($P \leq 0.05$) the adverse effects of aflatoxicosis on relative weight of bursa of Fabricius. Kubena *et al*. (1990) also reported a decrease in relative weight of bursa when diet contained 3.5 ppm aflatoxin. However, Sapocota *et al*. (2007) observed no effect on the relative weight of bursa in broiler chickens fed diet with 0.3 ppm aflatoxin.

The results showed that incorporation of methionine at 0.025 and 0.05% levels to the basal diet (T_3 and T_4) did not produce any significant improvement on different parameters investigated. However, supplementation of methionine at 0.025 and 0.05% levels to the aflatoxin contaminated diet (T_5 and T_6) of broiler chickens provided partial protection against the ill effects of aflatoxicosis in terms of investigated parameters. Thakur *et al.* (2008) reported that the level of glutathione significantly decreased in aflatoxin fed group compared to that of control. Methionine acts as precursor of glutathione, which forms conjugated complexes with aflatoxin inside the animal and especially in the liver. These complexes are eliminated through faeces and urine.

It was concluded that inclusion of methionine at 0.025 and 0.05% levels in the 1 ppm total aflatoxin contaminated diet provided partial protection from the adverse effects of aflatoxicosis in broiler chickens.

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