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Efficacy of zinc in amelioration of aflatoxicosis in broiler chickens

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ABSTRACT

In the present study the effect of zinc supplementation in ameliorating aflatoxicosis in broiler chickens was studied. Day-old broiler chicks (240) were divided into 6 treatment groups containing 5 replicates of 8 birds each (T_1–control; T_2- T_1+ 250 ppb AFB_1; T_3- T_1+ 20 mg Zn/kg; T_4- T_1+ 40 mg Zn/kg; T_5– T_2 + 20 mg Zn/kg; T_6- T_2 + 40 mg Zn/kg diet) and the experiment was conducted from day 1 to 42 days of age. During overall growth period (0–6 weeks), the weight gain of broilers in control (T_1) was 1,341.90 g as against 1,123.43 g in aflatoxin (AF) fed group (T_2) which was significantly lower. The body weight gain (BWG) in T_3, T_4 and T_6 was statistically similar to that of control, however the BWG in T_5 was higher than T_2 but could not match with that of control (T_1). Supplementation of 40 mg Zn/kg in AF contaminated diet significantly improved the BWG. The overall feed intake (FI) in groups (T_3, T_4, T_5 and T_6) was statistically similar to that of control group, however, the FI in group T_2 was significantly reduced compared to control. The overall FCR in AF fed group (T_2) was higher than that of control. The FCR in all other treatment groups was statistically similar to that of control. AF increased the relative weights of liver and spleen while decreased in weight of bursa. These effects of AF were ameliorated by supplementation of 40 mg Zn/kg feed. It was concluded that aflatoxin contamination (250 ppb) in broiler diet impaired the performance in terms of body weight gain, feed intake and feed conversion efficiency and relative organ weights. Supplementation of 40 mg Zn/kg to the aflatoxin contaminated diet ameliorated the ill effects of aflatoxicosis on performance of the birds.

Key words: Aflatoxicosis, Broiler chicken, Zinc

Presence of mycotoxins in feed is one of the major constraints in maintain feed quality because the mycotoxins are widely present in feedstuffs around the world and may affect production even in very low concentration. The most widespread and in most studied group of mycotoxins, aflatoxins are of great concern in warm and humid climatic conditions like India (Singh *et al*. 2010). Aflatoxin in poultry causes reductions in growth performance (Silambarasan *et al*. 2013, Patil *et al.* 2013), suppressed immunity (Okan *et al*. 2004), carcinogenesis, hepatotoxicity ((Busby and Wogan 1984), anaemia (Oguz *et al.* 2000) and increased mortality (Miazzo *et al.* 2000). Aflatoxins also cause oxidative stress (Shen *et al*. 1994, Souza *et al*. 1999) by free radical-mediated lipid peroxidation and cause cellular degeneration (Meki *et al*. 2001, Shi *et al*. 2006). Changes in the composition of poultry diets (dietary modifications) alleviate the adverse effects of aflatoxin in the poultry. Dietary fortification with certain vitamins (Hamilton *et al.* 1972), protein (Smith *et al.* 1971), fat (Hamilton *et al.* 1972), fatty acids (Lanza *et al.* 1981) and trace mineral (Zn, Cr, Se) (Hagazy and Adachi, 2000) reduce the effect of aflatoxin on the performance of poultry. Zinc participates in antioxidant defence mechanism as cofactor of super-oxide dismutase. The objective of this study was to evaluate the efficacy of zinc in counteracting the deleterious effects of aflatoxin B_1 (AFB_1) in broiler chickens.

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MATERIALS AND METHODS

Production and analysis of aflatoxin: Aflatoxin was produced using the fungal strain *Aspergillus flavus* NRRL 6513. To get the fresh spores, the culture was regularly sub-cultured on potato dextrose agar (PDA) medium slants and stored at 5°C. Aflatoxin was produced on maize substrate (Shotwell *et al*. 1996). The AFB_1 concentration in fermented maize was 990 mg/kg of maize. The extraction and estimation of aflatoxin was done as per Pons *et al*. (1966). Aqueous acetone was used for extraction of the toxin. Aflatoxin contents were finally quantified using spectrophotometry.

Experimental design: Experimental design was completely randomised design (CRD). There were 6 dietary treatments. Each dietary treatment consisted of 5 replicates and each replicate had 8 chicks. The experiment was conducted in broiler chickens from day-old to 6 weeks of age. The basal diet was mixed with the required quantity of mouldy maize to get the desired concentration of 250 ppb AFB_1 (Table 1).

Biological experiment and analysis: Day-old broiler

Table 1. Experimental groups and treatments

Group	Dietary treatment
T_1	Control (basal diet)
T_2	Basal diet + AF @ 250 ppb
T_3	Basal diet + 20 mg Zn/kg diet
T_4	Basal diet + 40 mg Zn/kg diet
T_5	Basal diet + AF @ 250 ppb + 20 mg Zn/kg diet
T_6	Basal diet + AF @ 250 ppb + 40 mg Zn/kg diet

Table 2. Ingredients and chemical composition of basal feed

Ingredient	Starter (%)	Finisher (%)
Maize	55.525	61.765
Soybean	41.00	35.00
Limestone	1.00	1.10
Di-calcium phosphate	1.75	1.50
Common salt	0.30	0.30
DL-Met	0.11	0.02
TM premix	0.10	0.10
Vitamin premix	0.15	0.15
B complex	0.015	0.015
Choline chloride	0.05	0.05
Chemical composition of basal feed		
Crude protein (%)	21.50	19.50
ME (kcal/kg)	2859.82	2919.78
Calcium (%)	1.04	0.99
Available phosphorus (%)	0.45	0.40
Lysine (%)	1.29	1.14
Met (%)	0.52	0.43

chicks (240) were obtained from experimental hatchery, CARI, Izatnagar. The chicks were wing banded, weighed individually and distributed randomly into 6 treatment groups. All the birds were reared under standard managemental conditions from 0–6 weeks. All birds were fed with broiler starter ration from 1–21 days and broiler finisher ration from 22 – 42 days. Weekly individual body weight and feed consumption of each group were recorded. The composition of broiler starter and finisher ration are presented in Table 2.

The protein as per AOAC (1990) and calcium as per Talapatra *et al.* (1940) contents were estimated, while the concentration of lysine, methionine, available P and metabolizable energy value were calculated. Data were analyzed following completely randomized design (Snedecor and Cochan 1980). The statistical analysis was done using SPSS 16.0 version.

RESULTS AND DISCUSSION

The data pertaining to body weight gain, feed intake and feed conversion ratio is given in Table 3.

Body weight gain (BWG): Significant ($P<0.05$) differences in body weight gain (BWG) among various dietary treatments were recorded from second week of age onward. At the second, third and fifth weeks of age, the BWG of groups T_2 and T_5 was significantly ($P<0.05$) lower than that of control but the BWG in other groups was statistically similar to that of control. During fourth week of age the BWG in groups T_3 and T_4 was similar to that of control (T_1), whereas the BWG in groups T_5 and T_6 was lower ($P<0.05$) than that of control but higher ($P<0.05$) than that of aflatoxin fed group (T_2). At sixth week of age, the BWG of all treatment groups did not show any statistical difference compared to the control except T_2 which showed the significant ($P<0.05$) lower BWG as compare to that of control. During starter phase of growth (0–3 weeks) the weight gain of birds in group T_2 was significantly reduced compared to control, whereas BWG in other groups T_3 to T_6 was statistically similar to that of control. During 4–6 weeks and 0–6 weeks (overall growth period), the BWG of groups T_2 was lower ($P<0.05$) than that of control but the BWG in other groups was statistically similar to that of control barring T_5, where BWG was lower ($P<0.05$) than control but higher ($P<0.05$) than toxin fed group (T_2).

Table 3. Body weight gain, feed intake and FCR in different growth phases of different dietary treatments

Treatments	0–3 wk	4–6 wk	0–6 wk
Body weight gain (g/bird)			
T_1	$440.83±7.80^b$	$909.06±7.96^c$	$1341.90±8.58^c$
T_2	$381.65±17.88^a$	$741.77±15.90^a$	$1123.43±29.00^a$
T_3	$436.49±49.00^b$	$914.45±16.15^c$	$1350.95±20.46^c$
T_4	$428.96±20.69^b$	$920.39±24.03^c$	$1349.36±43.68^c$
T_5	$414.76±14.85^{ab}$	$821.56±15.32^b$	$1236.32±26.16^b$
T_6	$434.37±7.90^b$	$870.58±25.52^{bc}$	$1304.95±28.11^{bc}$
Feed intake (g/bird)			
T_1	739.25±15.97	$1885.11±25.55^b$	$2624.36±35.50^b$
T_2	721.26±18.26	$1750.64±22.64^a$	$2471.91±29.74^a$
T_3	755.08±6.63	$1895.35±16.18^b$	$2650.43±20.02^b$
T_4	723.84±9.07	$1893.36±25.86^b$	$2617.20±30.73^b$
T_5	739.71±11.57	$1794.02±54.23^{ab}$	$2533.73±64.37^{ab}$
T_6	752.36±11.82	$1826.31±57.18^{ab}$	$2578.67±49.30^{ab}$
Feed conversion ratio (FCR)			
T_1	$1.67±0.01^a$	$2.09±0.02^a$	$1.95±0.01^a$
T_2	$1.90±0.08^b$	$2.36±0.04^c$	$2.20±0.05^b$
T_3	$1.73±0.03^a$	$2.07±0.05^a$	$1.96±0.04^a$
T_4	$1.69±0.06^a$	$2.06±0.04^a$	$1.94±0.05^a$
T_5	$1.78±0.04^{ab}$	$2.08±0.07^{ab}$	$2.05±0.04^a$
T_6	$1.73±0.02^a$	$2.10±0.09^a$	$1.98±0.06^a$

^{abc} Values in a column with different superscript differ significantly ($P<0.05$).

Contamination of 250 ppb of aflatoxin in the diet of broilers resulted in significant reduction in BWG. Significant reduction in BWG of broiler at 300 ppb level of dietary aflatoxin was reported by previous researchers (Raju and Dewegowda 2000, Sapocota *et al*. 2007, Abaji 2012, Silambarasan *et al*. 2013). Earlier studies also indicated that dietary aflatoxin at 0.5 ppm or more in commercial broiler diet adversely affected growth in a dose-dependent fashion (Johri *et al*. 1988, Beura *et al*. 1993, Verma 1994, Rosa *et al.* 2001). The present study revealed that supplementation of 20 mg Zn/kg diet failed to ameliorate the adverse effect of aflatoxicosis on BWG caused by 250 ppb aflatoxin B_1, however supplementation of 40 mg Zn/kg diet to the aflatoxin contaminated diet significantly (P<0.05) increased the overall BWG and the gain was statistically similar to that of control. Similar finding was reported by Hagazy and Adachi (2000) where supplementation of 60 mg Zn/kg diet resulted in significant improvement in weight gain of chicks exposed to aflatoxin.

Feed intake (FI): Significant (P<0.05) differences in feed consumption (FC) pattern among various dietary treatments were recorded from fifth week of age onward. At fifth week of age, the FC of groups T_2 was lower (P<0.05) than that of control but the FC in other groups was statistically similar to that of control. During sixth weeks of age, the FC in T_3 group was significantly (P<0.05) higher than that of toxin alone fed group (T_2). The FC in all other groups was statistically similar. During starter phase (0–3 weeks) of the trial, the FI in various treatment groups did not differ significantly. During finisher (0–4 weeks) and overall growth phase (0–6 weeks), the FC in T_2 and T_5 was significantly (P<0.05) reduced compared to that of control. The FC in other treatment groups was statistically similar to that of control (T_1).

The present study revealed that aflatoxin contamination in diet resulted in significant reduction in feed consumption. Similar observation was reported by Beura *et al*. (1993), who reported reduced feed consumption in purebred and commercial broiler chickens at 0.3 and 0.8 ppm level of aflatoxin, respectively. Significantly reduced feed consumption at 0.3 ppm aflatoxin was also reported by Silambarasan *et al*. (2013), Abaji (2012) and Raju and Devegowda (2000). In present study, zinc supplementation (40 mg/kg) to aflatoxin contaminated diet resulted in significant improvement in feed consumption of broilers equal to that of control. Hagazy and Adachi (2000) also reported significant improvement in feed consumption of broilers due to zinc supplementation in aflatoxin contaminated diet.

Feed conversion ratio (FCR): At the first, third and sixth week of age, there was no differences in FCR among various dietary treatments. At second week of age, the FCR in T_2 and T_5 was higher (P<0.05) than that of control, however, the FCR in other groups was statistically similar to that of control. During fourth week of age, the FCR in T_2 was significantly (P<0.05) higher compared to control. The FCR in T_3 and T_4 was statistically similar to that of control whereas, in groups T_5 and T_6 FCR was lower (P<0.05) than toxin fed group but higher (P<0.05) than that of control group (T_1). At sixth week of growth period, the FCR of T_2 was significantly higher compared to control, however the FCR of other groups was statistically similar to that of control (T_1). During starter phase (0–3 weeks), finisher phase (4–6 weeks) and overall growth period (0–6 week), the FCR of T_2 was statistically higher than that of control group (T_1), however the FCR of other groups was statistically similar to that of control (T_1) indicating that supplementation of zinc at both levels (20 and 40 mg/kg) to the AF contaminated diet curb the ill effect of AF on FCR in broiler chickens.

In the present study, aflatoxin contamination in feed resulted in poor feed efficiency in broilers. Silambarasan *et al*. (2013), Abaji (2012) and Raju and Devegowda (2000) also reported significantly poor feed efficiency in broiler chickens with 0.3 ppm level of dietary aflatoxin. Scheideler (1993) and Rosa *et al*. (2001) also reported impaired feed efficiency due to 2.5 ppm and 5.0 ppm aflatoxin feeding, respectively. In the present study, addition of zinc (20 and 40 mg/kg diet) resulted in significant improvement in feed efficiency during aflatoxicosis. This finding is in agreement with Hegazy and Adachi (2000) where zinc fortification (60 mg/kg) in diet resulted in significant improvement in feed efficiency of chicks exposed to aflatoxicosis.

Liveability: The liveability (Table 4) percentage did not vary significantly among various dietary treatment groups. At sixth week of age, the liveability percentage in control group was 92.50 which numerically reduced to 85.00 and 82.00 in T_2 and T_5, respectively. The liveability percentage in groups T_3, T_4 and T_6 was higher compared to toxin alone fed group. In the present study, 250 ppb aflatoxin level in the diet resulted in higher mortality compared to control. Inclusion of aflatoxin at 250 ppb did not produce heavy mortality, which might be due to the low level of aflatoxin inclusion in the diet. Silambarasan (2011) and Abaji (2012) also reported an increase in mortality due to 300 ppb level of aflatoxin in the diet of broilers. Occurrence of mortality due to aflatoxin contamination in the diet was been reported by Denli *et al.* (2009), Reddy *et al.* (1982) and Gopi (2006). In the present study, zinc supplementation (40 mg/kg) to the aflatoxin contaminated feed resulted in improved liveability percentage in broiler chickens.

Organ weights: The relative weight of liver in the control group was significantly (P<0.05) lower than that of T_2. The relative weight of liver in T_3, T_4 and T_6 was statistically similar to that of control however, the relative weight of liver in T_5 was lower (P<0.05) than that of T_2 but higher

Table 4. Liveability percentage as influenced by different dietary treatments

Treatments	First wk	Second wk	Third wk	Forth wk	Fifth wk	Sixth wk
T_1	100.0±0.0	97.50±2.50	95.00±3.06	95.00±3.06	95.00±3.06	92.50±3.06
T_2	100.0±0.0	92.50±3.06	90.00±2.50	87.50±0.0	87.50±0.0	85.00±2.50
T_3	100.0±0.0	97.50±2.50	95.00±3.06	95.00±3.06	90.00±2.50	90.00±2.50
T_4	100.0±0.0	97.50±2.50	95.00±3.06	95.00±3.06	92.50±3.06	92.50±3.06
T_5	100.0±0.0	95.00±3.06	95.00±3.06	92.50±3.06	90.00±2.50	82.00±2.50
T_6	100.0±0.0	97.50±2.50	97.50±2.50	95.00±3.06	92.50±3.06	90.00±2.50

Table 5. Relative weights of organs (% of live weight) fed different dietary treatments

Treatments	Liver	Spleen	Bursa	Thymus
T_1	2.37±0.04[a]	0.24±0.01[a]	0.19±0.02[b]	0.31±0.02
T_2	3.03±0.09[c]	0.35±0.01[b]	0.10±0.01[a]	0.34±0.02
T_3	2.40±0.08[ab]	0.26±0.02[a]	0.17±0.01[b]	0.28±0.03
T_4	2.37±0.07[a]	0.25±0.02[a]	0.19±0.04[b]	0.29±0.02
T_5	2.49±0.06[b]	0.27±0.02[a]	0.15±0.01[b]	0.32±0.03
T_6	2.50±0.05[ab]	0.27±0.02[a]	0.17±0.02[b]	0.31±0.03

[abc] Values in a column with different superscript differ significantly (P<0.05).

(P<0.05) than that of T_1. In the present study, contamination of aflatoxin at 250 ppb level in the diet of broiler chickens resulted in significant (P<0.05) increase in the relative weight of liver (Table 5). Similar observations were also reported by Silambarasan (2011), Abaji (2012), Smith and Hamilton (1970) and Verma (1994). Raju and Devegowda (2000) also reported a significant increase in the relative weight of liver due to 300 ppb of aflatoxin contamination in the diet of broilers. In the present study, supplementation of zinc at 40 mg/kg diet ameliorated the adverse effects of aflatoxin on relative weight of liver.

The relative weight of spleen in control group was lower (P<0.05) than that of T_2. The relative weight of spleen in all other groups was significantly (P<0.05) lower than that of T_2 and statistically similar to that of control. In the present study, a significant increase in the relative spleen weight was recorded at 250 ppb level of dietary aflatoxin. Abaji (2012) also reported a significant increase in the relative weight of spleen at 300 ppb level of dietary aflatoxin. Significant increase in relative spleen weight due to dietary aflatoxin content ranging from 3.5 to 5 ppm was also reported by earlier researchers (Kubena *et al.* 1990, Kubena *et al.* 1993, Bailey *et al.* 1998, Kubena *et al.* 1998, Rosa *et al.* 2001). In the present study, supplementation of zinc at both levels (20 and 40 mg/kg) to the aflatoxin contaminated diet reversed the effect of aflatoxin on relative weight of spleen.

The relative weight of bursa of Fabricius in control group (T_1) was higher (P<0.05) than that of aflatoxin alone fed group (T_2). The relative weight of bursa of Fabricius in other treatment groups was statistically similar to that of control. In the present study, a significant (P<0.05) decrease in the weight of bursa was reported at 250 ppb level of dietary aflatoxin. These results corroborated well with earlier reports of Silambarasan (2011) and Abaji (2012). In their study, a significant decrease in the relative bursa weight was observed at 300 ppb level of aflatoxin in the diet. In the present study, supplementation of zinc to the aflatoxin contaminated diet ameliorated the ill effect of aflatoxin on relative weight of bursa of Fabricius. There was no significant difference in relative weight of thymus among various dietary treatments. Nonsignificant difference in relative weight of thymus was also reported by Ortatatli *et al*. (2005) when diet containing 100 ppb was fed to broiler chickens.

It was concluded that 250 ppb aflatoxin in broiler diet impaired the performance in terms of body weight gain, feed intake, feed conversion efficiency and relative organ weights. Supplementation of zinc at 40 mg/kg to the aflatoxin contaminated diet ameliorated the ill effects of aflatoxicosis on performance of the broiler chickens.

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