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Efficacy of Choline in Ameliorating Aflatoxicosis in Broiler Chickens

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A B S T R A C T

The present study was undertaken to evaluate the efficacy of choline in ameliorating aflatoxicosis in broiler chickens. Day-old broiler chicks (n=240) were divided into 6 treatment groups (T_1-control, basal diet; T_2-T_1+250 ppb AFB_1; T_3-T_1+ 200 ppm Choline; T_4-T_1+ 400 ppm Choline;T_5-T_2+ 200 ppm Choline and T_6- T_2+ 400 ppm Choline). Each diet was fed to 4 replicated groups of 10 birds each from day-old to 42 days of age. During overall growth period (0-6 weeks), the BWG of control group (T_1) was higher ($P<0.05$) than that of toxin fed group (T_2).The BWG in T_5 and T_6 was higher ($P<0.05$) than that of toxin fed group (T_2) but lower ($P<0.05$) than that of control, indicating that addition of choline to the aflatoxin contaminated diet partially ameliorated the adverse effects of aflatoxicosis on body weight gain. The feed intake did not vary significantly among various treatment groups. The FCR in control group (T_1) was lower ($P<0.05$) than that of T_2. The FCR in other treatment groups (T_3 to T_6) was statistically similar to that of control. The relative weight of liver in control group (T_1) was lower ($P<0.05$) than that of aflatoxin fed group (T_2). The relative weight of liver in groups T_5 and T_6 was significantly ($P<0.05$) lower than that of toxin fed group (T_2), but significantly ($P<0.05$) higher than that of control (T_1). The relative weight of bursa of Fabricius in control group (T_1) was higher ($P<0.05$) than that of T_2.The relative weight of bursa in group T_6 was significantly ($P<0.05$) higher than that of T_5, but lower than that of control (T_1). The total serum protein, cholesterolanduric acid content of control group (T_1) was higher ($P<0.05$) than that of T_2.The total serum protein, cholesterol and uric acid content of groups $T_5$and $T_6$was higher ($P<0.05$) than that of T_2 but lower than that of T_1.The SGPT and SGOT activities in aflatoxin alone fed group (T_2) were higher ($P<0.05$) than that of control (T_1).The SGPT and SGOT value in groups T_5 and T_6 was lower ($P<0.05$) than that of T_2 but higher than that of T_1.It was concluded that aflatoxicosis caused by 250 ppb level of dietary aflatoxin resulted in depression of growth, feed intake, feed conversion efficiency; enlargement of liver, regression of bursa, decreased total protein, cholesterol, uric acid, and increased level of SGPT and SGOT activity. Inclusion of choline to the 250 ppb aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxicosis on production performance, organ weights and blood biochemistry in broiler chickens.

Introduction

Contamination of poultry feeds with aflatoxin is one of the major problems associated with the feeding of poultry. Aflatoxin occurs over a wide variety of substrates of practical importance to poultry feeding (maize, rice, wheat, groundnut cake, cottonseed meal, sunflower cake, soybean meal and compounded feeds). Aflatoxins are a group of secondary metabolites produced by a certain species of fungus of the genus *Aspergillus* (especially *A. flavus* and *A. parasiticus*). These fungiare capable of growing and contaminating the grains and cereals at any time before and after the harvest, during storage, transportation and processing of feed ingredients and the formulated feeds after processing. The spores of the fungi remain dormant but when the level of moisture is more than 12 per cent with a temperature of 25-35°C, with humidity of 80 percent and adequate aeration initiate their growth. Aflatoxin contamination of feedstuffs has been reported to be of a wide range from 1 to900µg/kg in commonly used ingredients as well as mixed feed samples in developing countries (Mohanamba *et al.,* 2007). Aflatoxicosis in poultry causes lowered performance in terms of reduced weight gain, feed intake and feed efficiency (Silambarasan *et al.,* 2013; Singh *et al.,* 2015[a]; Singh *et al.,* 2015[b]; Singh *et al.,* 2016), reduced nutrient utilisation (Silambarasan *et al.,* 2013), increased mortality (Khatke *et al.,* 2012[b]; Sharma *et al.,* 2014), anemia (Singh *et al.,* 2015[a]; Singh *et al.,* 2016), hepatotoxicosis and haemorrhage (Singh *et al.,* 2015[a]; Singh *et al.,* 2016), altered biochemistry (Singh and Mandal 2013; Singh *et al.,* 2013) and reduced immunity resulting in susceptibility to environmental and infectious agents (Khatke *et al.,* 2012[a]) leading to severe economic loss, pathological changes in the liver, kidney and bile duct (Silambarsan *et al.,* 2016; Khatke *et al.,* 2012[a]). Poultry industry suffers greater economic losses due to the greater susceptibility of the species in comparison with other animals to the toxin apart from continuing intermittent occurrences in feeds (Thapa, 2008). As mycotoxins are one of the major factors suppressing poultry productivity and also product quality, control of their impact is critical (Oguz, 2011). Choline plays an essential role in fat metabolism in the liver. It prevents abnormal accumulation of fat (fatty livers) by promoting its transport as lecithin or by increasing the utilization of fatty acids in the liver itself (Xue *et al.,* 2010). Choline is thus referred to as a "lipotropic" factor due to its function of acting on fat metabolism by hastening removal or decreasing deposition of fat in liver. In broiler liver, fat content was reduced by adding choline at 760 mg per kg (345 mg per lb) of diet for birds fed different energy sources (Rao *et al.,* 2001). Spires *et al.,* (1982) found that supplemental choline could replace up to two-thirds of the supplemental methionine required in broiler diets from 0 to 47 days in diets containing 0.30% methionine and 0.43% cystine in the starter phase, and 0.25% and 0.42% methionine and cystine, respectively, in the finisher phase. Growth retardation and perosis result from choline deficiency in young poultry. Perosis is the primary clinical sign of a choline deficiency in chicks and turkey poults, whereas quail develop enlarged hocks and bowed legs (NRC, 1994). The objective of the present investigation was to test the efficacy of choline in ameliorating adverse effects of aflatoxicosis in broiler chickens.

Materials and Methods

Production and analysis of aflatoxin

Aflatoxin was produced using the fungal strain *Aspergillus flavus* NRRL 6513 that was obtained from U.S. Department of Agriculture, USA. To get the fresh spores, the

culture was regularly subcultured on potato dextrose agar (PDA) medium slants and stored at5°C. Aflatoxin was produced on maize substrate. Fermentations were carried out in batches as per Shot well *et al.,* (1966). 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 a UV spectrophotometer.

Experimental design

Experimental design was completely randomized design (CRD). There were six dietary treatments. Each dietary treatment had 4 replicates and each replicate had 10 chicks. The experiment was conducted in broiler chickens from day-old to 6 weeks of age. The various dietary treatments were prepared by mixing mouldy maize to get the desired concentration of250 ppb AFB_1 (Table 1).

Biological experiment, feed formulation and analysis

Day-old broiler chicks (n=240) were obtained from experimental hatchery, CARI, Izatnagar. The chicks were wing banded, weighed individually and distributed randomly into six groups. All birds were reared under standard management conditions from 0-6 weeks. All birds were fed with broiler starter ration from 1-21 days and broiler finisher ration from 22 to 42 days. The composition of broiler starter and finisher ration was as below: The starter diet with maize 55.5, Deoiled rice bran 1.88, soybean meal 31.0, guar corma 4, rapeseed meal 4, fish meal 4.5, limestone 0.7, dicalcium phosphate 1.6, common salt 0.2, DL-methionine 0.07, lysine 0.125, TM premix 0.11, vitamin premix 0.15, B complex 0.015, choline chloride 0.05 and coccidiostat 0.05%; and finisher diet with maize 62.42, Deoiled rice bran 2.01, soybean meal 20.5, guar corma 4, rapeseed meal 4, fish meal 4, limestone 0.5, dicalcium phosphate 1.6, common salt 0.25, DL-methionine 0.03, lysine 0.07, TM premix 0.10, vitamin premix 0.15, B complex 0.015, choline chloride 0.05 and coccidiostat 0.05% were formulated. TM premix supplied Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4 mg/kg diet. Vitamin premix supplied Vit A, 8250 IU; Vit. D_3, 1200 IU; Vit. K, 1mg per kg diet.B complex supplied Vit. B_1, 2mg; Vit. B_2, 4mg; Vit. B_{12}, 10mcg; niacin, 60mg; pantothenic acid, 10mg; choline, 500mg per kg diet. The starter diet contained 22.3% crude protein, 2,807 Kcal ME/kg, lysine 1.28%, methionine 0.51%, calcium 1.09% and available P 0.50%.The corresponding values in finisher diet were 20.06%, 2,876 Kcal/kg, 1.04%, 0.43%, 1.09% and 0.42%.The protein as per AOAC (1995) and calcium contents as per Talapatra *et al.,* (1940) were estimated, while the concentration of lysine, methionine, available P and metabolizable energy value were calculated. Fortnightly individual body weight and feed consumption of each group were recorded and the FCR (feed: gain) was calculated. At the end of sixth week of experimental trial, ten birds per dietary treatment were sacrificed randomly in order to record relative (% of body weight) weights of liver, heart, spleen and bursa of Fabricius. The blood samples from each treatment group were collected. The serum was separated and stored at -20^0C and analyzed for various biochemical parameters using commercial kit manufactured by Span Diagnostics Ltd, SACHIN, Surat.

Statistical analysis

The collected data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS Version 16.0). The recorded data were subjected to one-way analysis of variance with comparison among means was made by Duncan‘s multiple range test with significance level of $P<0.05$.

Results and Discussion

The data pertaining to production performance {body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR)} at different growth phases are presented in Table 2.

Body weight gain (BWG)

During starting growth phase (0-3 weeks), the BWG of broilers in control group (T_1) was higher ($P<0.05$) than that of aflatoxin fed group (T_2). The BWG in T_3 and T_4 was statistically similar to that of control (T_1). The BWG in T_5 was statistically similar to that of toxin fed group (T_2). However, the BWG in T_6 was statistically similar to that of control. During finisher phase (4-6 weeks), the BWG among various dietary treatments did not differ significantly. During overall growth period (0-6 weeks), the BWG of control group (T_1) was higher ($P<0.05$) than that of toxin fed group (T_2). The BWG of T_3 and T_4 was statistically similar to that of control (T_1).The BWG in T_5 and T_6 was higher ($P<0.05$) than that of toxin fed group (T_2) but lower ($P<0.05$) than that of control, indicating that addition of choline at any level to the aflatoxin contaminated diet partially ameliorated the adverse effects of aflatoxicosis on body weight gain of broiler chickens. The present investigation indicated that the addition of 250 ppb aflatoxin to the basal diet of broiler chickens resulted in significant decrease in body weight gain. The present study indicated that inclusion of 250 ppb of aflatoxin in the diet of broiler chickens resulted in significant reduction in BWG during 0-6 wk of growth trial. Significant reduction in BWG of broilers at 300 ppb level of dietary aflatoxin was also reported by previous researchers (Silambarsan *et al.*, 2013; Abaji 2012; Raju and Devegowda 2000). In the present study, incorporation of choline to the 250 ppb aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxicosis on body weight gain of broiler chickens.

Feed intake

During starter phase (0-3wk), finisher phase (4-6 wk) and overall growth period (0-6 wk), the feed intake (FI) in T_1 did not differ ($P<0.05$) from other treatment groups. However, the FI during starter phase, finisher phase and overall growth period varied between 750.64 to 797.29, 1864.77 to 1984.63 and 2615.41 to 2772.39, respectively. In all the three growth phases, the FI was numerically lowest in toxin fed groups. The results revealed that aflatoxin contamination in feed at 250 ppb level resulted in decreased feed intake in broiler chickens. Decreased feed intake was also reported by Beura *et al.*, (1993), who also reported reduced feed consumption in pure bred and commercial broiler chicken at 300 and 800 ppb, respectively. Significantly reduced feed consumption at 300 ppb aflatoxin was also reported by Silambarasan *et al.,* (2013); Abaji (2012) and Raju and Devegowda (2000). Several researchers (Kubena *et al.,* 1990; Kubena *et al.,* 1998; Ledoux *et al.,* 1999; Verma *et al.,* 2004; Santurio *et al.,* 1999) also reported decreased feed consumption due to aflatoxin contamination ranging from 1 to 5 ppm. In the present study, inclusion of choline to the 250 ppb aflatoxin contaminated feed ameliorated the adverse effects of aflatoxicosis on feed consumption of broiler chickens, however, the feed consumption was numerically lower than that of control.

Feed conversion ratio (FCR)

During starter phase (0-3wk), finisher phase (4-6 wk) and overall growth period (0-6 wk), the feed conversion ratio (FCR) of control group (T_1) was lower ($P<0.05$) than that of aflatoxin fed group (T_2). The FCR of groups

T_3, T_4, T_5 and T_6 did not vary significantly from that of control. Deterioration of feed efficiency is a common feature of aflatoxicosis in poultry. In the present study, aflatoxin contamination in feed resulted in poor feed efficiency in broilers during 0-6 weeks of age. Silambarasan *et al.,* (2013); Abaji (2012) and Raju and Devegowda (2000) also reported significantly poor feed efficiency in broiler chickens at 0.3 ppm level of dietary aflatoxin. Similarly, other researchers have also reported a dose dependent significant reduction in feed efficiency due to presence of aflatoxin in diet (Verma *et al.,* 2004; Reddy *et al.,* 1982; Rosa *et al.,* 2001). In the present study, inclusion of choline to the 250 ppb aflatoxin contaminated feed ameliorated the adverse effects of aflatoxicosis on feed efficiency of broiler chickens.

Organ weight

The average value of relative organ weights (liver, heart, spleen and bursa of Fabricius) expressed as percentage of live weight were statistically analyzed and presented in Table 3.

Liver

The relative weight of liver (percent of live body weight) in T_1 was lower ($P<0.05$) than that of aflatoxin fed groups (T_2).The relative weight of liver in treatment groups T_3 and T_4 was statistically similar to that of control (T_1). The relative weight of liver in groups T_5 and T_6 was significantly ($P<0.05$) lower than that of toxin fed group (T_2), but significantly ($P<0.05$) higher than that of control (T_1).The relative weight of liver in groups T_5 was statistically ($P<0.05$) higher than that of T_6. In the present study, aflatoxin contamination in the diet of broiler chickens resulted in increased ($P<0.05$) relative weight of liver. Significant increase in the relative weight of liver due to aflatoxin feeding (0.3 to 5 ppm) was also reported earlier (Silambarsan 2011; Giambrone *et al.,* 1985[a]; Giambrone *et al.,* 1985[b]; Kubena *et al.,* 1998; Raju and Devegowda 2000; Rosa *et al.,* 2001; Miazzo *et al.,* 2000; Sapcota *et al.,* 2007). In the present study, supplementation of choline to the aflatoxin contaminated diet (T_4 and T_5) partially ameliorated the adverse effects of aflatoxicosis on relative weight of liver. The higher level of choline was more efficaceous in ameliorating the adverse effects of aflatoxicosis on relative liver weight in broiler chickens.

Heart

The relative weight of heart among various dietary treatments did not differ significantly i.e. addition of aflatoxin to feed did not produce any significant effect on relative heart weight. Contrary to this, significant increase in the relative heart weight due to dietary addition of aflatoxin ranging from 3 to 5 ppm was reported by several researchers (kubena *et al.,* 1990; Kubena *et al.,* 1998; Bailey *et al.,* 1998; Leudoux *et al.,* 1999; Rosa *et al.,* 2001). In the present study, no effect of dietary aflatoxin on relative weight of heart could be due to low level of aflatoxin in feed. Also, supplementation of choline did not show any significant effect on relative weight of heart.

Spleen

There was no significant difference in relative weight of spleen among various dietary treatments i.e. addition of aflatoxin to feed did not produce any significant effect on relative spleen weight. Contrary to this, significant increase in relative spleen weight due to dietary aflatoxin content ranging from 3.5 to 5 ppm has also been reported by earlier researchers (Kubena *et al.,* 1990; Bailey *et al.,* 1998; Kubena *et al.,* 1998 and Rosa *et al.,*

2001).In the present study, no significant effect of aflatoxin addition on the relative weight of spleen could be due to low level of aflatoxin in the feed. Also, supplementation of choline did not show any significant effect on relative weight of spleen.

Bursa of fabricius

The relative weight of bursa of Fabricius in T_1 was higher ($P<0.05$) than that of aflatoxin fed group (T_2). The relative weight of bursa in groups T_3 and T_4 was statistically similar to that of control. The relative weight of bursa of group T_5 was statistically similar to that of aflatoxin fed group T_2, indicating that addition of choline at 200 ppm level to the 250 ppb aflatoxin contaminated feed did not ameliorate the adverse effect of aflatoxicosis on immunity of birds. The relative weight of bursa in group T_6 was significantly ($P<0.05$) higher than that of T_5, but lower than that of control (T_1).

In the present study, aflatoxin contamination in feed resulted in significant ($P<0.05$) reduction in relative weight of bursa. Silambarsan (2011) also reported a significant decrease in the relative weight of bursa at 300 ppb level of dietary aflatoxin. Significant reduction in the relative weight of bursa was also reported in chicks receiving 2 ppm of aflatoxin (Verma *et al.,* 2004). A severe and significant regression of bursa in broilers was observed by Thaxton *et al.,* (1974) at 0.75 ppm and higher level of aflatoxin. Similar results have also been reported by Chattopadhyay *et al.,* (1985); Gopi (2006); Beura (1988) who also observed a significant reduction in bursal weight due to dietary aflatoxin. In the present study, supplementation of choline at 400 ppm level to the 250 ppb aflatoxin contaminated feed partially ameliorated the ill effects of aflatoxicosis on bursa of Fabricius in birds.

Effect on biochemical parameter

The data of various biochemical parameters (total serum protein, cholesterol, uric acid, SGPT and SGOT) was statistically analyzed and the mean values are presented in Table 4.

Total serum protein

The total serum protein content of control group (T_1) was higher ($P<0.05$) than that of aflatoxin fed group (T_2). The serum protein content in groups $T_3$and T_4 was statistically similar to that of control. The serum protein content of group$T_5$and $T_6$was higher ($P<0.05$) than that of T_2 but lower than that of T_1. The results of the present investigation showed that administration of aflatoxin at 250 ppb level of contamination in feed caused significant ($P<0.05$) reduction in serum protein content. A significant decrease in serum protein due to feeding aflatoxin contaminated diet has also been reported by earlier workers (Kubena *et al.,* 1998, Ledoux *et al.,* 1999; Raju and Devegowda 2000; Gopi 2006; Silambarasan 2011). The decrease in total serum protein by aflatoxin feeding has been reported due to reduced content of albumin and β globulin (Pier 1992). Reduced value of serum albumin and globulin has also been reported by Huff *et al.,* (1992). Other researchers reported that decrease in serum protein by aflatoxin feeding was attributed to failure in digestion and absorption of protein in gastro-intestinal tract (Voight *et al.,* 1980) and inhibition of protein synthesis due to aflatoxin contamination in diet (Sarasin and Moule 1973). Groopman *et al.,* (1996) also reported that the decline in serum protein may be due to decline in protein synthesis by forming adduct with DNA, RNA and protein and inhibit RNA synthesis and DNA-dependent RNA polymerase activity as well as causing degranulation of endoplasmic reticulum. In the present study, incorporation of choline to the 250 ppb aflatoxin

contaminated feed partially ameliorated the adverse effects of aflatoxicosis on total serum protein content in broiler chickens.

Cholesterol

With regard to serum cholesterol, the cholesterol content of control group (T_2) was lower ($P<0.05$) than that of control (T_1). The cholesterol content in groups $T_3$and T_4 was statistically similar to that of control. The cholesterol content of group$T_5$and $T_6$was higher ($P<0.05$) than that of T_2 but lower than that of T_1. The results revealed that aflatoxin contamination of feed at 250 ppb level resulted in reduced ($P<0.05$) serum cholesterol content of broiler chickens. These results are in agreement with those reported by earlier workers (Bailey *et al.,* 1998; Kececi *et al.,* 1998; Raju and Devegowda, 2000; Ahamad, 2000). In the present study, inclusion of choline to the 250 ppb aflatoxin contaminated diet partially alleviated the adverse effects of aflatoxicosis on serum cholesterol in birds.

Serum uric acid

The serum uric acid content of control group (T_1) was lower ($P<0.05$) than that of aflatoxin fed group (T_2). The uric acid content in groups T_3 and T_4 was statistically similar to that of control. The uric acid value in groups T_5 and T_6 was higher ($P<0.05$) than that of T_2 but lower ($P<0.05$) than that of T_1.The results revealed that addition of 250 ppb aflatoxin to the diet reduced ($P<0.05$) the serum uric acid content in broiler chickens. Oguz *et al.,* (2000) reported that serum uric acid was decreased when 50 ppb aflatoxin containing diet was fed to broiler chickens. Denli*et al.,* (2009) also observed that 1 ppm aflatoxin containing diet resulted in decrease in serum uric acid concentration. Safameher (2008) also reported that significant reduction in serum uric acid with 0.5 ppm of aflatoxin containing diet. A significant decrease in the uric acid concentration was also reported by several other researchers (Bailey *et al.,* 1998; Kececi *et al.,* 1998). In the present study, addition of choline to the 250 ppb aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxicosis on uric acid in broiler chickens.

Serum Glutamic Pyruvic Transferase (SGPT)

The SGPT activities in aflatoxin alone fed group (T_2) were higher ($P<0.05$) than that of control (T_1). The SGPT value in groups T_3 and T_4 was statistically similar to that of control. The SGPT value in groups T_5 and T_6 was lower ($P<0.05$) than that of T_2 but higher than that of T_1. In the present study, 250 ppb level of dietary aflatoxin resulted in increased activities of SGPT. Denli *et al.,* (2009) and Eraslan*et al.,* (2006) also reported an increase in the activity of SGPT with 1 ppm of aflatoxin contaminated diet. Increased level of SGPT activity due to aflatoxin was also reported by several researchers (Shi *et al.,* 2009; Kermanshahi *et al.,* 2009). In the present study, addition of choline to the 250 ppb aflatoxin contaminated diet partially alleviated the ill effects of aflatoxicosis on SGPT activity in broiler chickens.

Serum Glutamic OxaloaceticTransferase (SGOT)

The SGOT activities in aflatoxin alone fed group (T_2) were higher ($P<0.05$) than that of control (T_1). The SGOT value in groups T_3 and T_4 was statistically similar to that of control. The SGOT value in groups T_5 and T_6 was lower ($P<0.05$) than that of T_2 but higher than that of T_1. The results revealed that aflatoxin contamination at 250 ppb level in the feed resulted in increased ($P<0.05$) SGOT activity. Denli *et al.,* (2009) and Eraslan *et al.,* (2006) also reported an increase in the SGOT

activity with 1ppm of aflatoxin contaminated diet. Safameher (2008) also observed elevated SGOT activity in chickens with 0.5 ppm of aflatoxin contaminated diet. Increased activities of SGOT due to dietary aflatoxin were also reported by Shi *et al.,* (2009); Raju and Devegowda (2000). The present study revealed that inclusion of choline to the 250 ppb aflatoxin contaminated diet partially ameliorated the ill effects of aflatoxicosis on SGOT activity in birds.

Table.1 Experimental group and treatments

Group	Dietary treatment
T_1	Basal diet (Control)
T_2	T_1+ 250 ppb AFB_1
T_3	T_1+ 200 ppm Choline
T_4	T_1+ 400 ppm Choline
T_5	T_2+ 200 ppm Choline
T_6	T_2+ 400 ppm Choline

Table.2 Effect of aflatoxin and choline supplementation on body weight gain, feed consumption and FCR of broiler chickens between 1 to 42 days of age

Treatments	0-3 wk	4-6 wk	0-6 wk
Body weight gain (g/bird)			
T_1	479.13 ± 7.62^{b}	971.12 ± 8.47^{a}	1450.26 ± 10.47^{c}
T_2	409.68 ± 18.78^{a}	797.41 ± 16.37^{a}	1203.16 ± 29.84^{a}
T_3	471.91 ± 7.95^{b}	799.04 ± 175.29^{a}	1451.55 ± 22.73^{c}
T_4	460.60 ± 19.60^{b}	993.44 ± 22.33^{a}	1454.04 ± 40.73^{c}
T_5	444.54 ± 14.56^{ab}	885.39 ± 15.18^{a}	1329.93 ± 25.91^{b}
T_6	463.18 ± 6.75^{b}	930.36 ± 25.46^{a}	1393.60 ± 28.17^{bc}
Feed intake (g/bird)			
T_1	778.52 ± 22.03^{a}	1984.63 ± 38.17^{a}	2763.15 ± 51.79^{a}
T_2	750.64 ± 19.41^{a}	1864.77 ± 22.73^{a}	2615.41 ± 26.46^{a}
T_3	793.32 ± 13.34^{a}	1979.07 ± 24.13^{a}	2772.39 ± 33.59^{a}
T_4	768.59 ± 6.62^{a}	1967.74 ± 30.21^{a}	2736.33 ± 27.98^{a}
T_5	797.29 ± 11.26^{a}	1930.98 ± 63.12^{a}	2728.27 ± 72.57^{a}
T_6	789.07 ± 14.47^{a}	1936.08 ± 63.50^{a}	2725.16 ± 62.16^{a}
Feed conversion ratio (FCR)			
T_1	1.623 ± 0.02^{a}	2.043 ± 0.03^{a}	1.904 ± 0.02^{a}
T_2	1.846 ± 0.09^{b}	2.341 ± 0.05^{b}	2.179 ± 0.06^{b}
T_3	1.683 ± 0.04^{ab}	2.023 ± 0.05^{a}	1.912 ± 0.04^{a}
T_4	1.681 ± 0.07^{ab}	1.982 ± 0.03^{a}	1.886 ± 0.04^{a}
T_5	1.797 ± 0.04^{ab}	2.182 ± 0.07^{ab}	2.051 ± 0.04^{ab}
T_6	1.703 ± 0.03^{ab}	2.088 ± 0.09^{a}	1.958 ± 0.06^{a}

Values bearing different superscripts in a column differ significantly ($P<0.05$)

Table.3 Effect of aflatoxin and choline supplementation on relative organ weights (% of live weight) of broiler chickens fed at 1 to 42 days of age

Treatments	Liver	Heart	Spleen	Bursa
T_1	2.36±0.03^a	0.60±0.00^a	0.31±0.02^a	0.28±0.01^c
T_2	3.54±0.02^d	0.61±0.00^a	0.31±0.01^a	0.15±0.00^a
T_3	2.43±0.17^a	0.60±0.00^a	0.30±0.01^a	0.31±0.01^c
T_4	2.38±0.03^a	0.60±0.01^a	0.31±0.01^a	0.29±0.01^c
T_5	3.16±0.03^c	0.60±0.01^a	0.32±0.01^a	0.17±0.00^a
T_6	2.80±0.11^b	0.60±0.00^a	0.30±0.01^a	0.20±0.00^b

Values bearing different superscripts in a column differ significantly (P<0.05)

Table.4 Effect of aflatoxin and choline on blood biochemical parameters of broiler chickens

Treatments	Total protein (g/dl)	Cholesterol (mg/dl)	Uric Acid (mg/dl)	SGPT (IU/L)	SGOT (IU/L)
T_1	6.74±0.08^c	209.67±1.74^c	8.15±0.11^c	39.41±1.04^a	139.47±2.47^a
T_2	4.56±0.09^a	156.54±2.18^a	5.88±0.09^a	52.39±0.79^c	249.94±4.08^c
T_3	6.77±0.07^c	210.67±1.43^c	8.12±0.12^c	39.35±1.03^a	140.32±1.86^a
T_4	6.78±0.10^c	211.15±1.89^c	8.06±0.07^c	39.40±0.95^a	138.60±2.34^a
T_5	5.71±0.17^b	186.76±6.17^b	6.35±0.15^b	48.75±1.00^b	195.08±3.12^b
T_6	5.70±0.19^b	186.59±8.52^b	6.44±0.14^b	48.50±1.30^b	190.27±4.48^b

Values bearing different superscripts in a column differ significantly (P<0.05)

It was concluded that aflatoxicosis caused by 250 ppb level of dietary aflatoxin resulted in depression of growth, feed intake, feed conversion efficiency; enlargement of liver, regression of bursa, decreased total protein, cholesterol, uric acid, and increased level of SGPT and SGOT activity. Inclusion of choline to the 250 ppb aflatoxin contaminated feed partially ameliorated the adverse effects of aflatoxicosis on production performance, organ weights and blood biochemistry in broiler chickens.

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