



Influence of supplementation of vitamin E on amelioration of ochratoxicosis in broiler chickens

MOHIT SINGH¹, RAM SINGH² and A B MANDAL³

ICAR-Central Avian Research Institute, Izatnagar Uttar Pradesh 243 122 India

Received: 3 November 2018; Accepted: 26 March 2019

ABSTRACT

In the present study, the effect of vitamin E supplementation in ochratoxin A (OTA) contaminated diet in amelioration of ochratoxicosis in broiler chickens was investigated. Day-old broiler chicks (n=240) were divided into six treatment groups (T₁-control (basal diet); T₂-T₁ + 200 ppb OTA; T₃-T₁ + 100 mg vitamin E; T₄-T₁ + 200 mg vitamin E; T₅-T₂ + 100 mg vitamin E and T₆-T₂ + 200 mg vitamin E). Each diet was fed to 5 replicated groups of 8 birds each from 0 to 42 days of age. During overall growth period (0–6 week of age), the body weight gain (BWG) in ochratoxin contaminated diet (T₂) fed group was lower than that of control. The BWG in group T₅ was statistically similar to that of T₂ fed diet and lower than that of control (T₁). However, BWG in group T₆ (200 mg vitamin E/kg) was higher than T₂ and statistically similar to that of control group. Ochratoxin contamination in diet caused significant reduction in feed consumption, feed efficiency and livability percentage in broiler chickens. Addition of vitamin E (200 mg/kg) to the ochratoxin contaminated diet ameliorated the adverse effects on feed intake, FCR and livability percentage. Supplementation of vitamin E (200 mg/kg) to the ochratoxin contaminated diet also resulted in significant improvement in the relative weight of liver and bursa. It was concluded that experimentally induced ochratoxicosis with 200 ppb ochratoxin resulted in reduced production performance, enlargement of liver and regression of bursa. Addition of vitamin E (200 mg/kg) to the ochratoxin contaminated diet improved the production performance and relative weight of liver and bursa during 0–6 weeks of age of broiler chickens in experimentally induced ochratoxicosis caused by 200 ppb of dietary ochratoxin.

Key words: Body weight gain, Broiler, FCR, Ochratoxin feed intake, Vitamin E

Moulds adversely influence poultry performance by altering the nutrient composition of feed ingredients, decreasing the efficiency of nutrient utilization and by producing toxic secondary metabolites called mycotoxins. The ubiquitous nature of fungi makes mycotoxins unavoidable contaminants of agricultural based foods and feeds (Bryden 2007). Ochratoxin A is a mycotoxin known to have a diverse range of toxicological effects like nephrotoxic, hepatotoxic, immunosuppressive, teratogenicity, neurotoxicity, mutagenicity (Singh *et al.* 2019a; Singh *et al.* 2019b). It causes kidney and liver tumors in poultry and possibly in humans (O'Brien and Dietrich 2005). Thus, ochratoxin A is receiving attention worldwide. In a survey conducted to investigate global occurrence of mycotoxins, the incidence of contamination in feed of OTA in South Asia was found to be 55% (Nahrer and Kovalsky 2014). It causes a reduction in productive performance (growth rate, feed consumption, poorer feed conversion), increased mortality, and reduction in profitability due to its

effects on performance and health (Singh and Mandal 2018a; Singh and Mandal 2018b; Singh *et al.* 2016a; Singh *et al.* 2016b; Agawane and Lonkar 2004; Singh *et al.* 2015; Singh *et al.* 2018). For preventive management of mycotoxins in the field and during storage, new approaches have been employed including physical, chemical and nutritive treatments that can be advised to detoxify mycotoxins in contaminated feeds and feedstuffs (Varga and Toth 2005) along with amelioration of its toxicity in animal body system. Various antioxidants are beneficial in reducing the toxicity of ochratoxins, involved in increasing oxidative stress (Sorrenti *et al.* 2013). Vitamin C and E, being antioxidants, play an important role in the stimulation and enhancement of the chicken immune response. Thus, the objective of the present investigation was to study the efficacy of antioxidant (vitamin E) in ameliorating the ill effects of ochratoxin in broiler chickens.

MATERIALS AND METHODS

Ochratoxin production: The lyophilised preparation of *Aspergillus westerdijkiae* NRRL 3174 was obtained from US Department of Agriculture, Peoria, Illinois (USA). This lyophilised preparation was revived on potato dextrose agar

Present address: ¹Mycotoxin laboratory (singh.mohit204@gmail.com), ²Principal Scientist (carirsingh@yahoo.co.in), ICAR-CIRB, Hisar. ³Director (abmcari@rediffmail.com), Avian Nutrition and Feed Technology Division.

medium and used for experimentation. Ochratoxin was produced as per the method described by Singh *et al.* (2013). Cracked maize (50 g) was taken in 250 ml conical flasks. The moisture content of substrate was adjusted to 35%. Thus, flasks were plugged with non-absorbent cotton and sealed with aluminium foil. The flasks were autoclaved for 20 min at 121°C and inoculated with 1-week old mycelium of *Aspergillus westerdijkiae* NRRL 3174. The inoculated flasks were incubated in a BOD incubator for 14 days. After removal from the incubator, the flasks were dried at 70°C and the ochratoxin assays were performed as per AOAC (1995).

Experimental design: Experimental design was completely randomized (CRD). There were six dietary treatments. Each dietary treatment had 5 replicates and each replicate had 8 chicks. The experiment was conducted in broiler chickens from day-old to 6 weeks of age. The various dietary treatments were prepared by mixing the required quantity of mouldy maize to get the desired concentration of 200 ppb OTA in basal diet. The dietary treatments were T₁: Control (basal diet free from ochratoxin); T₂: basal diet + 200 ppb OTA, T₃: basal diet + 100 mg vitamin E; T₄: basal diet + 200 mg vitamin E; T₅: basal diet + 200 ppb OTA + 100 mg vitamin E and, T₆: basal diet + 200 ppb OTA + 200 mg vitamin E.

Biological experiment 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 6 treatment groups. All birds were reared under standard management conditions from 0 to 6 weeks of age. All birds were fed broiler starter ration from 1 to 21 days and broiler finisher ration from 22 to 42 days. Phase-wise body weight and feed consumption of each group were recorded and FCR was calculated. The ingredient and chemical composition of broiler starter and finisher ration are presented in Table 1.

The protein as per AOAC (1995) and calcium contents as per Talapatra *et al.* (1940) were estimated, while the concentrations of lysine, methionine, available P and metabolizable energy values were calculated. At the end of the experiment, 10 birds/dietary treatment were sacrificed randomly and their organs were collected, weighed and expressed as percentage of live weight. 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 was statistically analysed and the average values are presented in Table 2.

Body weight gain (BWG): During starter phase of growth period (0–3 wk), the BWG of birds in groups T₂ and T₅ was lower ($P < 0.05$) than that of control, however, the BWG in groups T₃, T₄ and T₆ was statistically similar to that of control. During 3–6 and 0–6 wk of growth periods, the average BWG in ochratoxin fed group (T₂) was lower ($P < 0.05$) than that of control. The BWG in groups T₃ and T₄ was statistically equal to that of control, indicating that

Table 1. Ingredient and chemical composition of basal feed

Ingredient	Starter (%)	Finisher (%)
Maize	55.50	62.42
De-oiled rice bran	1.88	2.01
Soybean	31	20.5
Guar korma	4	4
Rapeseed meal	4	4
Fish meal	4.5	4
Limestone	0.7	0.5
Di-calcium phosphate	1.6	1.6
Common salt	0.2	0.25
DL-methionine	0.07	0.03
Lysine	0.125	0.07
TM Premix*	0.11	0.10
Vitamin Premix **	0.15	0.15
B complex***	0.015	0.015
Choline chloride	0.05	0.05
Coccidiostat	0.05	0.05
Chemical composition of basal diet		
Crude protein (%)	22.3	20.06
ME (Kcal/kg)	2807	2876
Calcium (%)	1.09	1.09
Available phosphorus (%)	0.50	0.42
Lysine (%)	1.28	1.04
Methionine (%)	0.51	0.43

*TM premix supplied mg/kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. **Vitamin premix supplied per kg diet: Vit. A, 8250 IU; Vit. D₃, 1200 IU; Vit. K, 1 mg. ***B complex supplied per kg diet: Vit. B₁, 2 mg; Vit. B₂, 4 mg; Vit. B₁₂, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg.

Table 2. Body weight gain, feed intake and FCR in different growth phases as influenced by various dietary treatments

Treatment	0–3 wk	3–6 wk	0–6 wk
<i>Body weight gain (g/bird)</i>			
T ₁	460.76±7.69 ^b	941.00±8.56 ^{cd}	1401.77±8.57 ^c
T ₂	396.44±17.82 ^a	776.75±15.90 ^a	1173.19±28.97 ^a
T ₃	456.89±11.68 ^c	917.24±23.25 ^b	1374.13±32.45 ^b
T ₄	466.66±14.26 ^c	919.91±20.27 ^b	1386.58±30.73 ^b
T ₅	418.14±6.32 ^{ab}	823.93±30.70 ^a	1242.08±26.07 ^a
T ₆	448.62±8.01 ^{bc}	912.49±28.20 ^b	1361.12±31.91 ^b
<i>Feed intake (g/bird)</i>			
T ₁	771.50±16.28	1961.54±26.35 ^{ab}	2733.05±36.77 ^b
T ₂	747.13±18.05	1840.31±23.36 ^a	2587.44±29.66 ^a
T ₃	764.54±10.66	1948.40±27.20 ^b	2712.94±37.72 ^b
T ₄	782.46±11.65	1966.44±20.32 ^b	2748.90±30.83 ^b
T ₅	766.94±11.95	1893.59±29.15 ^{ab}	2660.53±33.37 ^{ab}
T ₆	753.16±16.45	1944.65±26.68 ^b	2697.8±30.68 ^b
<i>Feed conversion ratio (FCR)</i>			
T ₁	1.673±0.01 ^a	2.084±0.02 ^a	1.948±0.01 ^a
T ₂	1.897±0.08 ^b	2.372±0.04 ^b	2.210±0.05 ^b
T ₃	1.676±0.03 ^a	2.126±0.02 ^a	1.976±0.02 ^a
T ₄	1.680±0.03 ^a	2.139±0.02 ^a	1.984±0.02 ^a
T ₅	1.834±0.01 ^b	2.413±0.10 ^b	2.147±0.06 ^b
T ₆	1.678±0.02 ^a	2.136±0.04 ^a	1.984±0.03 ^a

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

addition of vitamin E to the basal diet did not produce any effect on BWG of birds. The BWG in group T₅ was statistically similar to that of toxin fed group (T₂) and lower ($P < 0.05$) than that of control (T₁). However, the BWG in group T₆ was higher ($P < 0.05$) than T₂ and statistically similar to that of control, indicating that addition of 200 mg vitamin E per kg to the ochratoxin contaminated diet ameliorated the adverse effects of ochratoxin on weight gain of broiler chickens. The present study revealed that contamination of 200 ppb ochratoxin in the diet of broiler chickens resulted in significant reduction in BWG of birds. Significant reduction in BWG of broiler chickens was reported in earlier investigations with dietary ochratoxin levels of 50–100 ppb (Stoev *et al.* 2004, El-Barkouky 2008, El-Barkouky and Abu-Taleb 2008); 150 ppb (Singh *et al.* 2018); 200 ppb (Singh *et al.* 2016a; Sakhare *et al.* 2007; Singh *et al.* 2015 and El-Barkouky *et al.* 2010) and 567 ppb (Garcia *et al.* 2003). Several other workers also reported significant reduction in BWG of broilers due to ochratoxin contamination in feed (Singh *et al.* 2015; Raju and Devegowda 2000; Santin *et al.* 2006; Hatab 2003; Kumar *et al.* 2003; Elaroussi *et al.* 2006; Verma *et al.* 2004 and Hanif *et al.* 2008). In the present study, supplementation of vitamin E at 200 mg/kg level ameliorated the adverse effects of ochratoxicosis caused by 200 ppb ochratoxin in feed. Scanty observations are available on effect of OTA in birds but OTA is involved in the development of different types of cancers in rats, mice and humans (Sorrenti *et al.* 2013). A growing number of *in vitro* and *in vivo* studies conducted revealed role for oxidative stress in OTA toxicity and carcinogenicity. The use of α -tocopherol could offer new strategies to reduce OTA cytotoxicity, supporting its defensive role in the cell membrane and its multiple functions in cellular metabolism (Baldi *et al.* 2004; Fusi *et al.* 2010).

Feed Intake (FI): During starter phase (0–3 wk) there was no significant difference in feed intake due to various dietary treatments. However, during 3–6 wk of age, the FI in control group (T₁) was higher ($P < 0.05$) than that of ochratoxin fed group (T₂). The FI values in other treatment groups (T₃ to T₆) were statistically similar to that of control. The FI in group T₆ was statistically similar to that of control (T₁), indicating that addition of vitamin E (200 mg/kg) to the ochratoxin contaminated feed ameliorated the ill effects of ochratoxicosis on FI in broilers. During overall growth phase (0–6 wk), the FI in T₂ group was lower ($P < 0.05$) than that of control. The FI in T₃ and T₄ was statistically similar to that of control, suggesting that addition of vitamin E to the basal diet did not produce any effect on FI of birds. The FI in group T₅ was statistically similar to T₁ and T₂. The FI of group T₆ was higher ($P < 0.05$) than that of toxin fed group (T₂) and statistically similar to that of control, suggesting that inclusion of vitamin E (200 mg/kg) to the 200 ppb ochratoxin contaminated feed ameliorated the adverse effects of ochratoxicosis on feed consumption of broiler chickens. In the present study, ochratoxin contamination in diet caused significant reduction in feed

consumption of broiler chickens. Similarly, significantly reduced FI in broilers fed ochratoxin contaminated feed at a concentration ranging from 50 to 200 ppb was observed by earlier workers (Singh *et al.* 2016a; Singh *et al.* 2015; Garcia *et al.* 2003; Kumar *et al.* 2003; Verma *et al.* 2004; El-Barkouky 2008; El-Barkouky and Abu-Taleb 2008; Singh *et al.* 2018 and El-Barkouky *et al.* 2010). In the present study, addition of vitamin E (200 mg/kg) to 200 ppb ochratoxin contaminated diet ameliorated the ill effects of ochratoxicosis on feed intake in broiler chickens.

Feed Conversion Ratio (FCR): The FCR during starter phase (0–3 wk) in control (T₁) was lower ($P < 0.05$) than that of ochratoxin fed group (T₂). The FCR of group T₅ was statistically similar to T₂ and higher ($P < 0.05$) than that of control. The FCR of other groups T₃, T₄ and T₆ was statistically similar to that of control. During 3–6 weeks of growth period, the FCR of group T₅ was statistically similar to that of ochratoxin fed group (T₂). However, the FCR of groups T₃, T₄ and T₆ was statistically similar to that of control (T₁). During overall growth period (0–6 wk), the FCR of groups T₃ and T₄ was statistically similar to that of control, suggesting that inclusion of vitamin E to the basal diet did not produce any effect on FCR of birds. The overall FCR in group T₅ was statistically similar to that of ochratoxin fed group (T₂). The overall FCR of group T₆ was statistically similar to that of control, indicating that supplementation of vitamin E (200 mg/kg) to the ochratoxin contaminated feed curbed the ill effects of ochratoxin on feed efficiency of broiler chickens. The present study revealed that ochratoxin contamination (200 ppb) in feed decreased ($P < 0.05$) the efficiency of feed utilization in broiler chickens. These results were in agreement with earlier reports (Santin *et al.* 2006; Kojnarski *et al.* 2007; Hanif *et al.* 2008) wherein dietary inclusion of ochratoxin adversely affected FCR in broiler chickens. Poor feed efficiency in broilers fed ochratoxin (50–200 ppb) contaminated diet was also observed by other workers (Sakhare *et al.* 2007; El-Barkouky 2008; El-Barkouky and Abu-Taleb 2008; El-Barkouky *et al.* 2010; Singh *et al.* 2015; Singh *et al.* 2016a; and Singh *et al.* 2018). In the present study, supplementation of vitamin E at 200 mg/kg level to ochratoxin contaminated feed resulted in significant improvement in feed efficiency of broiler chickens. The improvement regarding effect of vitamin E on feed efficiency during ochratoxicosis is lacking in literature.

Livability: The results of week-wise livability percentage in broiler chickens kept on various dietary treatments are presented in Table 3. At first week of age, no mortality was recorded in any of the treatments. During second week of age, there was no difference ($P < 0.05$) in livability percentage among various dietary treatments. During third week of age, the livability percentage in group T₂ was lower ($P < 0.05$) than that of control, however the livability percentage in other treatment groups was statistically similar to that of control. During fourth and fifth weeks, there was no difference ($P < 0.05$) in livability percentage among different dietary treatments. At sixth week of age, the

Table 3. Livability percentage as influenced by various dietary treatments

Treatment	I wk	II wk	III wk	IV wk	V wk	VI wk
T ₁	100.00±0.00	100.00±0.00	100.00±0.00 ^b	98.00±4.47	94.00±5.47	94.00±5.47 ^b
T ₂	100.00±0.00	96.00±5.47	94.00±5.47 ^a	92.00±4.47	88.00±4.47	84.00±8.94 ^a
T ₃	100.00±0.00	100.00±0.00	100.00±0.00 ^b	98.00±4.47	96.00±5.47	94.00±5.47 ^b
T ₄	100.00±0.00	100.00±0.00	98.00±4.47 ^{ab}	96.00±5.47	92.00±8.36	92.00±8.36 ^b
T ₅	100.00±0.00	98.00±4.47	96.00±5.47 ^{ab}	92.00±8.36	90.00±7.07	90.00±7.07 ^b
T ₆	100.00±0.00	100.00±0.00	98.00±4.47 ^{ab}	96.00±5.47	92.00±4.47	90.00±7.07 ^b

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

livability percentage in control group (T₁) was 94 which significantly (P<0.05) decreased to 84 due to ochratoxin feeding in group T₂. The livability percentage in groups T₃ and T₄ was statistically equal to that of control. The livability percentage of groups T₅ and T₆ was higher (P<0.05) than the ochratoxin fed group (T₂) and statistically similar to that of control group, indicating that supplementation of vitamin E to the ochratoxin contaminated feed ameliorated the adverse effects of ochratoxicosis on mortality of broiler chickens. In the present study, ochratoxin contamination of broiler diet caused higher mortality compared to control. These findings were in agreement with earlier reports (Singh *et al.* 2016a; Singh *et al.* 2018; El-Barkouky and Abu-Taleb 2008; El-Barkouky *et al.* 2010; Kumar *et al.* 2003; Hatab 2003; Elaroussi *et al.* 2006). In the present study, supplementation of vitamin E at 100 or 200 mg/kg diet improved the livability percentage in broiler chickens fed ochratoxin contaminated diet (200 ppb).

Organ weights

The data pertaining to relative weight of liver, thymus and bursa of fabricius (percent of live body weight) was statistically analysed and the average values are presented in Table 4.

Liver: The relative weight of liver in control group (T₁) was lower (P<0.05) than that of OTA fed group (T₂). The relative weight of liver in other treatment groups was statistically similar to that of control. The relative weight of liver in group T₆ was lower (P<0.05) than that of T₅, indicating that the higher level of vitamin E supplementation was more effective in ameliorating the ill effects of ochratoxicosis on relative weight of liver. The present study revealed that ochratoxin contamination in the diet of broiler

chickens resulted in increased (P<0.05) relative weight of liver. Similar results were also reported by other workers (Singh *et al.* 2017; Singh *et al.* 2015; Singh *et al.* 2016a; Gibson *et al.* 1989; Huff *et al.* 1992; Elkady 1993; Stoev *et al.* 2004; Hatab 2003; Elaroussi *et al.* 2008; Sakhare *et al.* 2007; El-Barkouky *et al.* 2010; Hanif *et al.* 2008). In the present study, supplementation of vitamin E (200 mg/kg) level ameliorated the adverse effects of ochratoxin on relative weight of liver. The information regarding effect of vitamin E supplementation on liver during ochratoxicosis is scanty in literature.

Thymus: The relative weight of thymus was statistically similar among various dietary treatments. In the present study, supplementation of vitamin E had no effect on the relative weight of thymus in broiler chickens. Similarly, no difference in the relative weight of thymus due to ochratoxin in feed was earlier reported by Singh *et al.* (2016a); Huff and Doerr (1981) and Hanif *et al.* (2008). However, Dwivedi and Burns (1984) in chickens, and Dwivedi and Burns (1985) in turkeys reported dose-related significant reductions in the relative weight of thymus. Also, a significant decrease in the thymus weight was observed when broiler chicks were fed OTA (4 µg/g feed) contaminated diet (Elkady 1993). Singh *et al.* (2017) also reported significant reduction in relative weight of thymus due to ochratoxicosis caused by 150 ppb of aflatoxin in broiler chickens.

Bursa of Fabricius: The relative weight of bursa of fabricius in control group (T₁) was higher (P<0.05) than that of ochratoxin fed group (T₂). The relative weight of bursa in group T₃ and T₄ was statistically similar to that of control. The relative weight of bursa in group T₅ was statistically similar to that of T₂ and lower (P<0.05) than that of control. However, the relative weight of bursa in T₆ was statistically similar to that of control, suggesting that vitamin E supplementation at 200 mg/kg feed level ameliorated the ill effects of ochratoxin on relative weight of bursa. In the present study, ochratoxin contamination (200 ppb) in feed resulted in significant (P<0.05) reduction in relative weight of bursa. Significant reduction in relative weight of bursa due to ochratoxin contamination was earlier reported by Singh *et al.* (2017); Singh *et al.* (2015); Elkady (1993); Sakhare *et al.* (2007) and Gupta *et al.* (2008). In this regard, these results also agree with those reported by Campbell *et al.* (1983), Hatab (2003), and Elaroussi *et al.* (2008). In the present study, supplementation of vitamin E

Table 4. Relative weight (% of live weight) of organs fed different dietary treatments

Treatment	Liver	Thymus	Bursa
T ₁	2.43±0.12 ^a	0.31±0.03	0.20±0.01 ^{bc}
T ₂	3.35±0.08 ^b	0.36±0.02	0.11±0.01 ^a
T ₃	2.31±0.10 ^a	0.32±0.04	0.20±0.02 ^b
T ₄	2.43±0.10 ^{ab}	0.35±0.04	0.20±0.03 ^b
T ₅	2.60±0.08 ^b	0.36±0.01	0.12±0.01 ^a
T ₆	2.41±0.09 ^a	0.34±0.04	0.18±0.02 ^b

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

(200 mg/kg) to the 200 ppb ochratoxin contaminated diet ameliorated the adverse effects of ochratoxin on relative weight of bursa.

It was concluded that experimentally induced ochratoxicosis with 200 ppb ochratoxin resulted in reduced production performance, enlargement of liver and regression of bursa in broiler chickens during 0–6 weeks of age, and addition of vitamin E (200 mg/kg) in diet ameliorated the ill effects of ochratoxicosis as evidenced by improved production performance, relative weight of liver and bursa.

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