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Influence of dietary supplementation of vitamin E in ameliorating adverse effects of ochratoxin on biochemical profile and immune response in broiler chickens

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ABSTRACT

In the present study, the effect of supplementation of vitamin E in ochratoxin A (OTA) contaminated diet in ameliorating adverse effects of ochratoxin on biochemical parameters and immune response in broiler chickens was investigated. Day old broiler chicks (240) were divided into 6 treatment groups (T1- control (basal diet), T2 - T1 + 200 ppb OTA, T3 -T1 + 100 mg vit. E, T4 -T1 + 200 mg vit. E, T5 -T2 + 100 mg vit. E and T6 -T2 + 200 mg vit. E). Each diet was fed to 5 replicated groups of 8 birds each from 0 to 42 days of age. The blood samples were collected on 42 days of age of broilers. The total serum protein and haemoglobin (Hb) concentrations in T2 (200 ppb OTA) and T5 (200 ppb OTA + 100 mg vit. E) were lower (P<0.05) than that of control (T1) diet. The serum protein and Hb contents in group T6 was higher (P<0.05) than that of T2 but statistically similar to that of control. The serum uric acid, creatinine, alkaline phosphatase (ALP), serum glutamic oxaloacetic transferase (SGOT) and serum glutamic pyruvic transferase (SGPT) value in T2 was higher (P<0.05) than that of T1. The serum uric acid, creatinine, ALP, SGOT and SGPT value in T6 (200 ppb OTA + 200 mg vit. E) was lower (P<0.05) than that of T2 and statistically similar to that of control (T1). The CMI and HA titre value in T2 was lower (P<0.05) than that of control (T1). The CMI and HA titre value of T3 and T4 was statistically similar to that of control. The CMI value of T5 and T6 was lower (P<0.05) than control, but higher (P<0.05) than T2. The CMI of group T6 was higher (P<0.05) than T5. The HA titre value of group T5 was higher (P<0.05) than T2 and lower than T1. The HA titre value in T6 was statistically similar to that of T1, indicating that inclusion of vit. E (200 mg/kg diet) ameliorated the adverse effect of ochratoxicosis in broiler chickens. Thus, it was concluded that ochratoxin contamination at 200 ppb level in broiler diet had adverse effect on blood biochemical profile and immune response, which were restored through supplementation of vitamin E at 200 mg/kg of feed in ochratoxin contaminated diet in broiler chickens.

Key words: Biochemical parameters, Broiler, Immune response, Ochratoxin, Vitamin E

Ochratoxin A (OTA) is a mycotoxin produced mainly by *Aspergillus ochraceus* and *Penicillium verrucosum*. The family of ochratoxins consists of 3 members, viz. ochratoxin A, B and C but ochratoxin A is the most toxic one (Chang *et al*. 1979). They are the second major group of mycotoxins characterized after the discovery of aflatoxins. OTA is an isocumarin derivative linked through the carboxy group to a L-β-phenylalanine (Engelhardt *et al*. 1999). Ochratoxin A is a natural contaminant of animal feedstuffs (Binder *et al.* 2007, Schiavone *et al*. 2008, Pozzo *et al*. 2010) and documented field outbreaks of ochratoxicosis, as well as experimental feeding trials with OTA contaminated feeds have indicated its detrimental effects on chicks (Santin *et al*. 2002, Stoev *et al*. 2002, Elaroussi *et al*. 2008, Hanif *et al*. 2008) and its potential risk for the poultry industry (Zaghini *et al*. 2007, Birü *et al*. 2002). In a survey conducted to investigate global occurrence of mycotoxins, the incidence of OTA in South Asia was found to be 55% on analysing the feed samples (Nahrer and Kovalsky 2014). Ochratoxin A causes significant losses and reduction in the profitability of poultry industry due to its effects on performance and health (Agawane and Lonkar 2004). It causes a reduction in productive performance (growth rate, feed consumption, poorer feed conversion) and increased mortality (Singh *et al*. 2015). Ochratoxin A is a mycotoxin known to be implicated in a diverse range of toxicological effects in a variety of animal species, its nephrotoxic, hepatotoxic, immunosuppressive, teratogenicity, neurotoxicity, mutagenicity. It causes kidney and liver tumors in poultry and possibly in humans (O'Brien and Dietrich 2005). Vitamin E plays an important role in the stimulation and enhancement of the chicken immune response. The objective of the present investigation was to study the efficacy of antioxidant (vit. E) in ameliorating the ill effects of ochratoxin on biochemical profile and immune response in broiler chickens.

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MATERIALS AND METHODS

Ochratoxin production: The lyophilised preparation of *Aspergillus westerdijkiae* NRRL 3147 was obtained from U.S. Department of Agriculture, Peoria, Illinois (USA). This lyophilised preparation was revived on potato dextrose agar 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 have a moisture level of 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 design (CRD). There were 6 dietary treatments. Each 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, T1 + 200 ppb OTA; T3, T1 + 100 mg vit. E; T4, T1 + 200 mg vit. E; T5, T2 + 100 mg vit. E and T6, T2 + 200 mg vit. E per kg diet.

Biological experiment and analysis: Day old broiler chicks (240) were wing banded, weighed individually and distributed randomly into 6 treatment groups. All the birds were reared under standard management conditions from 0–6 weeks of age and fed with broiler starter (1–21d) and finisher (22–42d) rations (Table 1). The basal diet was prepared as per BIS (2007). The protein content of the diet was estimated as per AOAC (1995) and calcium content as per Talapatra *et al*. (1940), while the concentrations of lysine, methionine, available P and metabolizable energy values were calculated. The cell mediated immune response to PHA-P antigen was evaluated by the method described by Corrier and DeLoach (1990). The microtitre haemagglutination procedure as described by Siegel and Gross (1980) was followed to measure total HA antibody titres in chickens. After 6 weeks, the blood samples from each treatment group were collected. The serum was separated and stored at –200°C and analysed for various biochemical parameters using commercial kit. The haemoglobin concentration in blood was estimated by Sahli's method. Haemoglobin is converted into acid haematin by addition of 0.1 N HCl. The resultant solution is then matched against a reference solution (Sahli's Haemoglobinometer). Reading on the graduated tube noted and this is expressed as haemoglobin level in g/dl. The heterophil/lymphocyte (H/L) ratio was calculated by dividing the number of heterophil by that of lymphocyte. The statistical analysis was done using SPSS 16.0 version.

Table 1. Ingredient and chemical composition of basal feed

Ingredients	Starter (%)	Finisher (%)
Maize	55.50	62.42
Deoiled rice bran	1.88	2.01
Soybean	31	20.5
Guar korma	4	4
Rape seed 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, 8,250 IU; Vit. D_3, 1,200 IU; Vit. K, 1 mg. ***B complex supplied per kg diet: Vit. B_1, 2 mg; Vit. B_2, 4 mg; Vit. B_{12}, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg.

RESULTS AND DISCUSSION

Effect on biochemical parameters: The data pertaining to various biochemical parameters (serum protein, is given in cholesterol, uric acid, creatinine, ALP, SGOT and SGPT) is given in Table 2.

Total serum protein and cholesterol: The total serum protein content of control group (T1) was higher ($P<0.05$) than that of ochratoxin alone fed group (T2). The serum protein content in groups T3, T4 and T6 was statistically similar to that of control. The serum protein content of group T5 was higher ($P<0.05$) than T2 but lower than T1. Contamination of ochratoxin at 200 ppb level in feed caused significant ($P<0.05$) reduction in serum protein content. The negative effects of ochratoxin on serum protein in the present study were in agreement with the previous investigations (Stoev *et al*. 2000, Santin *et al*. 2002, Hatab 2003, Elaroussi *et al*. 2008, El-Barkouky 2008, El-Barkouky and Abu-Taleb 2008, Singh *et al*. 2015). Reduction in serum protein is attributed to the decrease in protein absorption and/or utilisation or to the inhibition of protein synthesis by ochratoxin (Kubena *et al*. 1983, Kubena *et al*. 1988, Kubena *et al*. 1989). Similar decrease in serum protein was also reported when ochratoxin was administered to broiler chickens at 130–790 µg/kg (Stoev *et al*. 2000) and 567 µg/kg (Garcia *et al*. 2003). They suggested that

Table 2. Blood biochemical constituents of broilers fed different dietary treatments

Treatment	Total protein (g/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	ALP (KA units)	SGOT (IU/L)	SGPT (IU/L)
T_1	6.39±0.38^{cd}	185.12±4.14	5.32±0.33^{a}	0.31±0.02^{a}	155.20±3.89^{a}	171.59±3.98^{a}	32.30±3.98^{a}
T_2	4.54±0.50^{a}	182.88±4.12	7.41±0.39^{c}	0.37±0.03^{c}	191.85±5.81^{d}	208.58±5.28^{c}	42.22±2.26^{b}
T_3	6.71±0.64^{d}	185.68±5.67	5.40±0.28^{a}	0.33±0.02^{ab}	154.95±2.91^{a}	171.22±4.08^{a}	32.48±2.53^{a}
T_4	6.69±0.41^{d}	187.06±4.53	5.52±0.32^{a}	0.32±0.02^{a}	156.61±4.55^{ab}	172.07±4.77^{a}	32.85±1.73^{a}
T_5	5.31±0.44^{b}	183.14±5.22	6.33±0.41^{b}	0.36±0.02^{bc}	182.40±7.42^{c}	183.48±4.31^{b}	34.86±3.22^{a}
T_6	5.84±0.55^{bc}	185.89±3.78	6.73±0.49^{b}	0.30±0.03^{a}	162.65±2.78^{b}	171.34±5.70^{a}	32.62±3.11^{a}

low serum protein concentration might have been due to the decrease in albumin and globulin levels or to the degeneration of endoplasmic reticulum that led to pathological changes in the liver that in turn caused a reduction in hepatic protein synthesis, as ochratoxin is known to inhibit hepatic protein synthesis. Supplementation of 200 mg vit. E/kg to the 200 ppb ochratoxin contaminated feed ameliorated the ill effects of ochratoxicosis on serum protein in broiler chickens. With regard to serum cholesterol, the serum cholesterol content in various treatment groups varied from 182.9 to 186.7 mg/dl. The serum cholesterol content did not vary significantly (P<0.05) among various dietary treatments. However, Schaeffer *et al*. (1987) and Sreemannarayana *et al*. (1989) observed reduction in total serum cholesterol content due to ochratoxin contamination in feed of broiler chickens.

Serum uric acid and creatinine: The serum uric acid and creatinine content of control group (T1) was lower (P<0.05) than that of ochratoxin alone fed group (T2). The uric acid and creatinine content in groups T3 and T4 was statistically similar to that of control. The uric acid value in groups T5 and T6 was lower (P<0.05) than that of T2 but higher (P<0.05) than that of T1, indicating that the supplementation of vit. E at any level partially ameliorated the ill effects of ochratoxin on serum uric acid. The creatinine content of group T5 was statistically similar to T2 and higher (P<0.05) than that of T1. The creatinine content of group T6 was lower (P<0.05) than T2 and statistically similar to control (T1), indicating that addition of vit. E at 200 mg/kg level to the ochratoxin contaminated feed reversed the serum creatinine content equal to control. Ochratoxin contamination resulted in significant (P<0.05) increase in serum uric acid concentration. The significantly increased levels of uric acid in ochratoxin treated birds were in agreement with other reports (Stoev *et al*. 2000, Ayed *et al*. 1991, Garcia *et al*. 2003, Mohiuddin *et al*. 1993, Patil *et al*. 2005, Singh *et al.* 2015). Kubena *et al*. (1989) indicated that uric acid is the primary product of nitrogen catabolism in chickens and is excreted by the kidney. The elevation in serum uric acid level was accompanied by the increase in kidney weight in the ochratoxin fed birds, indicating impaired renal excretory functions. Supplementation of vit. E to the ochratoxin contaminated diet improved the serum uric acid content. Ochratoxin contamination of feed resulted in increased (P<0.05) creatinine content. Increased creatinine content due to ochratoxicosis in broilers was earlier reported in several investigations (Kumar *et al*. 2003, Koynarski *et al*. 2007, Sakhare *et al*. 2007, Hatab 2003, Elaroussi *et al*. 2008). Increase in creatinine concentration in the ochratoxin fed birds might be due to nephrotoxic action of ochratoxin, which caused renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and tubular damage (Agawane and Lonkar 2004). Inclusion of vit. E (200 mg/kg) to the ochratoxin contaminated diet ameliorated the ill effects of ochratoxicosis on creatinine content.

Alkaline phosphatase (*ALP*)*:* The ALP activities in ochratoxin alone fed group (T2) were higher (P<0.05) than that of control (T1). The ALP value in groups T3 and T4 was statistically similar to that of control. The ALP value in groups T5 and T6 was lower (P<0.05) than T2 but higher than T1. The ALP value in group T6 was lower (P<0.05) than that of T5, suggesting that the higher level of vit. E was more effective in ameliorating the ill effects of ochratoxin. In the present study, 200 ppb ochratoxin resulted in increased activities of ALP. Khan *et al*. (2014) also reported increased activities of ALP due to ochratoxicosis in broiler chickens. Addition of vit. E (200 mg/kg) to ochratoxin contaminated diet ameliorated the adverse effects of ochratoxin on ALP activities.

Serum glutamic oxaloacetic transferase (*SGOT*) *and Serum glutamic pyruvic transferase* (*SGPT*)*:* The SGOT and SGPT value in control group was lower (P<0.05) than that of ochratoxin alone fed group (T2). The SGOT and SGPT value in groups T3 and T4 was statistically similar to that of control. The SGOT value in group T5 was lower (P<0.05) than T2 but higher than T1. The SGOT value in group T6 was statistically similar to that of control (T1). The SGPT value in groups T5 and T6 was statistically similar to that of control (T1). In the present study, 200 ppb ochratoxin resulted in increased activities of SGOT. This result was in agreement with earlier reports (Santin *et al*. 2002, Kumar *et al*. 2003, Hatab 2003, Patil *et al*. 2005, Elaroussi *et al*. 2008). Under pathological conditions, increased level of SGOT in broiler serum was attributed to cellular damage and increased plasma membrane permeability (Raina *et al*. 1991), which was particularly true for ochratoxin containing diet. However, in the present study, addition of vit. E (200 mg/kg) to the ochratoxin contaminated feed increased the level of SGOT activities, which might be due to more protein synthesis as evidenced from more blood protein. With regard to SGPT, ochratoxin

Table 3. Blood haematological constituents of broilers fed different dietary treatments

Treatment	Haemoglobin (g/dl)	H/L ratio	CMI (mm)	HA titre
T_1	9.20±0.58^{c}	0.51±0.01	0.47±0.01^{d}	9.34±0.13^{c}
T_2	5.87±0.49^{a}	0.53±0.01	0.30±0.01^{a}	5.67±0.40^{a}
T_3	10.11±0.56^{c}	0.50±0.01	0.50±0.01^{dc}	9.69±0.45^{c}
T_4	9.37±0.40^{c}	0.51±0.01	0.48±0.01^{dc}	9.73±0.39^{c}
T_5	7.49±0.33^{b}	0.52±0.01	0.33±0.01^{b}	7.19±0.80^{b}
T_6	8.48±0.45^{c}	0.51±0.01	0.41±0.02^{c}	9.07±0.78^{c}

contamination resulted in increased activities of SGPT. This finding was in agreement with other reports in literature (Santin *et al*. 2006, Kumar *et al*. 2003, Hatab 2003, Patil *et al*. 2005, Elaroussi *et al*. 2008). Inclusion of vit. E (100 or 200 mg/kg) to the 200 ppb ochratoxin contaminated feed ameliorated the adverse effects of ochratoxicosis on SGPT activities in broiler chickens.

Effect on haematological parameters: The data pertaining to various haematological parameters (heamoglobin and heterophil-lymphocyte ratio) is given in Table 3. OTA contamination resulted in reduced Hb level in broiler chickens. The Hb value in groups T3 and T4 was statistically equal to that of control, however the Hb value for groups T5 and T6 was higher (P<0.05) than that of T2 but lower than that of control (T1). Ochratoxin contamination resulted in reduced Hb level in broiler chickens. Similar result was also reported by Agawane and Lonkar (2004), Sakhare *et al*. (2007), El-Barkouky (2008), El-Barkouky and Abu-Taleb (2008) who found that Hb concentration was significantly reduced due to ochratoxin concentration in feed ranging from 50 to 500 ppb level. Mohiuddin *et al*. (1992, 1993) reported that addition of ochratoxin at a level of 0.75, 1.5 or 3 µg/g feed for 4 weeks to the diet of 4 week old broiler chicks resulted in a significant decrease in Hb concentration in blood. Supplementation of vit. E at any levels to the basal diet did not produce any positive effect on haematology of broilers, however, supplementation of vit. E to the OTA contaminated diet improved the Hb level. The H/L ratio did not differ significantly (P<0.05) among various dietary treatments. Supplementation of vit. E at any levels to the basal diet or ochratoxin contaminated diet did not produce any effect on haematology.

Immune response: The data pertaining to CMI response to PHA-P measured as foot web index and humoral immune response measured as haemagglutination titre (HA) against SRBC's in broiler chickens fed various dietary treatments is given in (Table 3).

Effect on cell mediated immunity (*CMI*)*:* The CMI value of ochratoxin alone fed group (T2) was lower (P<0.05) than that of control group (T1). The CMI value of groups T3 and T4 was statistically similar to that of control. The CMI value of groups T5 and T6 was lower (P<0.05) than control, but higher (P<0.05) than ochratoxin alone fed group (T2). The CMI of group T6 was higher (P<0.05) than T5, indicating that 200 mg level was more efficacious than lower level of vit. E in ameliorating ochratoxicosis. Addition of ochratoxin to the diet of broiler chickens caused significant (P<0.05) reduction in CMI response to PHA-P. Singh *et al*. (1990) showed suppression of CMI response in chicken due to ochratoxicosis and described the significant reduction of T-lymphocyte count and phagocytic ability of spleenic macrophages after feeding 0.5 and 2.0 ppm ochratoxin. These findings were also supported by Verma *et al*. (2004) in broilers fed with 1–4 ppm ochratoxin for 47 days from one day of age. Reduction in CMI response was also observed by Wang *et al.* (2009), Dwivedi and Burns (1985) and Harvey *et al*. (1997). Supplementation of vit. E (100 and 200 mg/kg) to the 200 ppb OTA contaminated diet partially ameliorated the ill effect of ochratoxin on CMI response. Khatoon *et al*. (2013) also observed that vit. E (200 mg/kg diet) alone or in combination with silymarin (10g/kg) ameliorated the immunotoxic effects induced by 1.0 mg OTA/kg feed.

Effect on humoral immune response: The HA titre value in control group (T1) was higher (P<0.05) than that of ochratoxin alone fed group (T2). The HA titre value of groups T3 and T4 was statistically similar to that of control (T1). The HA titre value of group T5 was higher (P<0.05) than toxin fed group (T2) and lower than control. The HA titre value in group T6 was statistically similar to that of control, suggesting that inclusion of vit. E (200 mg/kg diet) ameliorated the adverse effect of ochratoxicosis in broiler chickens. Dietary ochratoxin reduced (P<0.05) the HA titre against sheep RBC's. Ochratoxin interferes with protein, DNA and RNA synthesis through competitive inhibition of phenylalanine-t-RNA synthetase by phenylalanine moiety of ochratoxin that leads to decrease plasma proteins and immunoglobulin (Hsieh 1987, Marguardt and Frohlich 1992). Also, leucocytopenia (lymphocytopenia and monocytopenia) that is noticed during ochratoxicosis can adversely affect immunoglobulin production (Campbell *et al.* 1983, Effat 1989).

It was concluded that ochratoxin contamination at 200 ppb level in broiler diet led to decreased protein, haemoglobin and creatinine, while increased uric acid, alkaline phosphatase, SGOT and SGPT levels in blood. Moreover, ochratoxin impaired both cell mediated and humoral immunity. Supplementation of vit. E at 200 mg/kg level to the ochratoxin contaminated diet ameliorated the ill effects of ochratoxicosis on biochemicals and immune response in broiler chickens.

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