



Associated efficiency of *Saccharomyces cerevisiae* and vitamin E in ameliorating adverse effects of ochratoxin A on biochemical profile and immune response in broiler chickens

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ABSTRACT

The associated efficacy of *Saccharomyces cerevisiae* and vitamin E, in ameliorating ochratoxicosis was investigated in broiler chickens. Day-old broiler chicks (320) were divided into 8 treatment groups, viz. T₁ (control; basal diet); T₂ (T₁ + 150 ppb OTA); T₃ (T₂ + 0.05% SC + 100 mg vitamin E-VE); T₄ (T₂ + 0.075% SC + 100 mg VE); T₅ (T₂ + 0.1% SC + 100 mg VE); T₆ (T₂ + 0.05% SC + 200 mg VE); T₇ (T₂ + 0.075% SC + 200 mg VE) and T₈ (T₂ + 0.1% SC + 200 mg VE/kg diet). Each diet was fed to 5 replicated groups of 8 birds from 0–42 days of age. The total serum protein, cholesterol and haemoglobin content of control group (T₁) was higher than that of ochratoxin fed group (T₂). The serum protein, cholesterol and haemoglobin value in groups T₅, T₇ and T₈ was higher than T₂ but statistically similar to that of control. The serum uric acid, creatinine, ALP, SGOT, SGPT and H/L ratio value in T₁ was lower than that of T₂. The uric acid, creatinine, ALP, SGOT, SGPT and H/L ratio value in T₅, T₇ and T₈ was lower than T₂ and statistically similar to that of control. The CMI and HA titre value of T₁ was higher than that of T₂. The CMI and HA titre value in T₅, T₇ and T₈ was higher than that of T₂ but statistically similar to that of control. It can be concluded that ochratoxin contamination at the rate of 150 ppb in the feed resulted in decreased total serum protein, cholesterol and haemoglobin content and increased serum uric acid, creatinine, ALP, SGOT, SGPT and H/L ratio value. Inclusion of *Saccharomyces cerevisiae* at 0.1% level along with 100 mg vitamin E or *Saccharomyces cerevisiae* at 0.075% level along with 200 mg vitamin E/kg diet to the ochratoxin (150 ppb) contaminated feed ameliorated the adverse effects of ochratoxicosis on biochemical profile and immune response in broiler chickens.

Key words: Biochemical profile, Broiler, Immune response, Ochratoxin, *Saccharomyces cerevisiae*, Vitamin E

Feed is the major input in poultry production. Poultry feed industry is facing some challenges and mycotoxins are one of them. Ochratoxin A (OTA), a mycotoxin, is receiving increasing attention worldwide because of the hazard it possesses to animal and human health. Ochratoxin A is a natural contaminant of animal feedstuffs (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. 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, poor feed conversion) and

increased mortality (Singh *et al.* 2015, Singh *et al.* 2016). Inclusion of 0.1% *S. cerevisiae* to the ochratoxin (200 ppb) contaminated diet ameliorated the ill effects of ochratoxicosis on production performance, relative weight of organs, serum protein, uric acid, creatinine, haemoglobin, ALP, SGPT, SGOT, H/L ratio and immune response in broiler chickens (Singh 2015, Singh *et al.* 2016). Various antioxidants are beneficial in reducing the toxicity of ochratoxins, involved in increasing oxidative stress (Sorrenti *et al.* 2013). Addition of vitamin E (200 mg/kg) to the ochratoxin contaminated diet improved the production performance, relative weight of liver and bursa, blood biochemical, haematological parameters and immune response of broiler chickens (Singh 2015). The objective of this investigation was to study the associated efficiency of *S. cerevisiae* and vitamin E to ameliorate the adverse effects of ochratoxicosis on biochemical profile and immune response in broiler chickens.

MATERIALS AND METHODS

Ochratoxin production: The lyophilised preparation of

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Aspergillus westerdijkiae NRRL 3147 was revived on potato dextrose agar medium. 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%. The flasks were plugged with non-absorbent cotton and sealed with aluminium foil and 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 eight dietary treatments, viz. T₁ (control; basal diet); T₂ (T₁ + 150 ppb OTA); T₃ (T₂ + 0.05% SC + 100 mg vitamin E-VE); T₄ (T₂ + 0.075% SC + 100 mg VE); T₅ (T₂ + 0.1% SC + 100 mg VE); T₆ (T₂ + 0.05% SC + 200 mg VE); T₇ (T₂ + 0.075% SC + 200 mg VE) and T₈ (T₂ + 0.1% SC + 200 mg VE/kg diet). 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 150 ppb OTA in basal diet.

Biological experiment and analysis: Day-old broiler chicks (320) were obtained from experimental hatchery, CARI, Izatnagar. The chicks were wing banded, weighed individually and distributed randomly into eight 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 22–42 days. The composition of broiler starter and finisher ration are given in Table 1. The starter diet contained 22.3% crude protein, 2,807 Kcal ME/kg, 1.28% lysine, 0.51% methionine, 1.09% calcium and 0.50% available P. 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 concentrations of lysine, methionine, available P and metabolizable energy values were calculated. After 6 weeks, the blood samples from each treatment group were collected. The serum was separated and stored at –20°C and analyzed for various biochemical parameters using commercial kit manufactured by Span Diagnostics Ltd, Sachin, Surat. 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 was then matched against a reference solution (Sahli's Haemoglobinometer). Reading on the graduated tube was noted and 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 cell mediated immune response to PHA-P antigen was evaluated by the method described by Corrier and DeLoach (1990). The microtitre haemagglutination procedure as

Table 1. Composition of broiler starter and finisher ration

Ingredient	Composition (%)	
	Starter diet	Finisher diet
Maize	55.5	62.42
Deoiled rice bran	1.88	2.01
Soybean meal	31.0	20.5
Guar coroma	4	4
Rapeseed meal	4	4
Fish meal	4.5	4
Limestone	0.7	0.5
Dicalcium 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

*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. D₃, 1200 IU; Vit. K, 1 mg/kg diet. ***B complex supplied Vit. B₁, 2 mg; Vit. B₂, 4 mg; Vit. B₁₂, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; choline, 500 mg/kg diet.

described by Siegel and Gross (1980) was followed to measure total HA antibody titres in chickens.

Statistical analysis: The collected data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS Version 16.0) available in the Central Avian Research Institute, Izatnagar, Bareilly, India. The recorded data were subjected to one-way analysis of variance (Snedecor and Cochran 1989) with comparison among means was made by Duncan's multiple range test with significance level of P<0.05.

RESULTS AND DISCUSSION

Total serum protein: The total serum protein content of control group (T₁) was higher (P<0.05) than that of ochratoxin alone fed group (T₂) (Table 2). The total serum protein value in T₃ and T₄ was statistically similar to that of T₂. The total serum protein value of T₆ was higher (P<0.05) than T₂ but lower (P<0.05) than that of T₁. The serum protein value in groups T₅, T₇ and T₈ was higher (P<0.05) than T₂ and statistically similar to that of control. The present study revealed that 150 ppb ochratoxin contamination in feed resulted in significant (P<0.05) reduction in serum protein content. Reduced serum protein content due to ochratoxin contamination in feed was in agreement with the earlier investigations (Stoev *et al.* 2002, Santin *et al.* 2002, Hatab 2003, Stoev *et al.* 2004, Koynarski *et al.* 2007, Elaroussi *et al.* 2008, El-Barkouky 2008, El-Barkouky and Abu-Taleb 2008, Singh *et al.* 2015, Singh 2015, Singh *et al.* 2016). Decreased serum protein is attributed to the decrease in protein absorption and/or utilization or to the inhibition of protein synthesis by ochratoxin (Kubena *et al.* 1983, Kubena *et al.* 1988, Kubena *et al.* 1989). Also, low serum protein

Table 2. Blood biochemical constituents of broilers fed different dietary treatment (Mean±SEM; n=5)

Treatment	Protein (g/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
T ₁	7.03±0.05 ^c	192.05±2.03 ^{cd}	5.87±0.10 ^a	0.34±0.01 ^{ab}
T ₂	4.58±0.16 ^a	150.74±2.10 ^a	7.78±0.14 ^c	0.42±0.00 ^c
T ₃	4.83±0.11 ^a	190.15±1.93 ^{cd}	7.77±0.12 ^c	0.41±0.00 ^c
T ₄	5.04±0.19 ^a	186.75±1.49 ^c	7.01±0.12 ^b	0.35±0.00 ^{ab}
T ₅	6.96±0.19 ^c	194.08±2.06 ^d	5.90±0.08 ^a	0.32±0.00 ^a
T ₆	5.59±0.16 ^b	166.45±3.88 ^b	6.99±0.08 ^b	0.39±0.00 ^c
T ₇	6.95±0.14 ^c	193.08±1.67 ^{cd}	5.84±0.08 ^a	0.36±0.00 ^b
T ₈	6.94±0.18 ^c	189.38±1.42 ^{cd}	5.92±0.07 ^a	0.34±0.00 ^{ab}

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

concentration might have been due to the decreased 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 OTA is known to inhibit hepatic protein synthesis (Stoev *et al.* 2000, Garcia *et al.* 2003). In our present study, inclusion of 0.1% *S. cerevisiae* + 100 mg vitamin E or 0.75% *S. cerevisiae* + 200 mg vitamin E/kg ochratoxin contaminated diet significantly (P<0.05) increased the serum protein content equal to that of control. Bailey *et al.* (1990), Bradley *et al.* (1994), Agawane and Lonkar (2004) also reported significant improvement in serum protein as a result of addition of probiotic containing *Saccharomyces* yeast culture at 10 mg/kg diet to 0.5 ppm OTA intoxicated broiler chickens. Significant improvement in serum protein content due to addition of *S. cerevisiae* in OTA contaminated feed was also reported by El-Barkouky (2008) and Khalil (2008). Singh (2015) also reported that inclusion of 0.1% *S. cerevisiae* or 200 mg vitamin E/kg ochratoxin contaminated diet significantly increased the serum protein content equal to that of control.

Total serum cholesterol: The serum cholesterol content in T₁ was higher (P<0.05) than that of T₂ (Table 2). The cholesterol content of T₆ was higher (P<0.05) than T₂ but lower than that of control. The serum cholesterol content of groups T₃, T₄, T₅, T₇ and T₈ was higher (P<0.05) than T₂ and statistically similar to that of control. These results indicated that inclusion of 0.05% *S. cerevisiae* + 100 mg vitamin E or 0.075% *S. cerevisiae* + 200 mg vitamin E/kg ochratoxin contaminated feed removed the harmful effects of ochratoxicosis on serum cholesterol. The results of the present investigation showed that 150 ppb ochratoxin contamination in feed resulted in decreased serum cholesterol, which might be due to decreased availability of acetyl-CoA because of impaired carbohydrate metabolism. This result was in agreement with earlier reports by Schaeffer *et al.* (1987), Sreemannarayana *et al.* (1989), Sawale *et al.* (2009). The present study revealed that addition of 0.05% *S. cerevisiae* along with 100 mg vitamin E or 0.075% *S. cerevisiae* along with 200 mg vitamin E/kg ochratoxin contaminated feed reversed the

serum cholesterol concentration equal to control.

Serum uric acid: The serum uric acid content in T₁ was lower (P<0.05) than that of T₂ (Table 2). The uric acid value in groups T₃, T₄ and T₆ was higher (P<0.05) than that of control. The uric acid value in T₅, T₇ and T₈ was lower (P<0.05) than T₂ and statistically similar to that of control. This result revealed that inclusion of *S. cerevisiae* (0.1%) + vitamin E (100 mg/kg) or *S. cerevisiae* (0.075%) + vitamin E (200 mg/kg) to the ochratoxin contaminated feed reversed the serum uric acid concentration equal to control. In our study, 150 ppb ochratoxin contamination in feed resulted in significant (P<0.05) increase in serum uric acid content. Significantly increased concentration of uric acid in ochratoxin treated birds was in agreement with earlier reports (Stoev *et al.* 2002, Dalia 2003, Garcia *et al.* 2003, Patil *et al.* 2005, Koynarski *et al.* 2007, Singh 2015, 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 OTA fed birds, indicating impaired renal excretory functions. Our study revealed that addition of *S. cerevisiae* (0.1%) along with vitamin E (100 mg/kg) or *S. cerevisiae* (0.075%) along with vitamin E (200 mg/kg) to the OTA contaminated feed reversed the uric acid content equal to control. Singh (2015) also reported that addition of 0.1% *S. cerevisiae* or 200 mg vitamin E/kg diet partially ameliorated the ill effects of ochratoxicosis on uric acid contamination in broiler chickens. Increased uric acid level in serum might be due to reduced protein synthesis and breakdown of amino acids.

Serum creatinine: The creatinine content in T₁ was lower (P<0.05) than that of T₂ (Table 2). The creatinine content in T₃ and T₆ was statistically similar to T₂ and higher (P<0.05) than that of T₁. The creatinine content of groups T₄, T₅, T₇ and T₈ was lower (P<0.05) than T₂ and similar to that of T₁. The present study revealed that ochratoxin (150 ppb) contamination of feed resulted in increased (P<0.05) creatinine content. Increased creatinine content due to ochratoxin contamination in feed was earlier reported in several investigations (Stoev *et al.* 2002, Kumar *et al.* 2003, Hatab 2003, Koynarski *et al.* 2007, Sakhare *et al.* 2007, Elaroussi *et al.* 2008, Singh 2015). Increase in creatinine content in the ochratoxin fed birds might be due to nephrotoxic action of OTA, which caused renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and tubular damage (Agawane and Lonkar 2004). Agawane and Lonkar (2004) also reported significant improvement in creatinine concentration of broilers as a result of addition of probiotic containing yeast culture *Saccharomyces boulardii* at 10 mg/kg in diet of broilers intoxicated with ochratoxin. Also, Singh (2015) reported that inclusion of *S. cerevisiae* (0.1%) or vitamin E (200 mg/kg) to the 200 ppb ochratoxin contaminated diet ameliorated the ill effects of ochratoxicosis on creatinine content in broiler chickens.

Alkaline phosphatase (ALP): The ALP activities in

control group was lower ($P<0.05$) than that of ochratoxin alone fed group (T_2) (Table 3). The ALP activities in T_3 , T_4 and T_6 was higher ($P<0.05$) than that of control. The ALP value in T_5 , T_7 and T_8 was lower ($P<0.05$) than T_2 and similar to that of control, indicating that addition of *S. cerevisiae* + vitamin E in T_5 , T_7 and T_8 reversed the ALP value equal to that of control. The present study indicated that 150 ppb ochratoxin contamination in feed resulted in increased activities of ALP. Increased activities of ALP due to ochratoxicosis were earlier reported in several investigations in chickens (Koynarski *et al.* 2007, Denli *et al.* 2008, Hanif *et al.* 2008, Sawale *et al.* 2009, Khan *et al.* 2014, Singh 2015). Our study revealed that addition of 0.1% *S. cerevisiae* along with 100 mg vitamin E or 0.075% *S. cerevisiae* along with 200 mg vitamin E/kg to the 150 ppb ochratoxin contaminated diet ameliorated the ill effects of ochratoxicosis on ALP activities in broiler chickens. Singh (2015) also reported that inclusion of *S. cerevisiae* (0.1%) or vitamin E (200 mg/kg) alleviated the harmful effects of ochratoxicosis on ALP activities in broiler chickens.

Serum glutamic oxaloacetic transferase (SGOT): The SGOT value in T_1 was lower ($P<0.05$) than that of T_2 (Table 3). The SGOT value in groups T_3 , T_4 and T_6 was higher ($P<0.05$) than the control. The SGOT value in groups T_5 , T_7 and T_8 was lower ($P<0.05$) than T_2 and similar to that of control. In the present study, ochratoxin (150 ppb) contaminated in feed 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, Singh 2015). Raina *et al.* (1991) reported that increased level of SGOT in broiler was attributed to cellular damage and increased plasma membrane permeability. Our study revealed that inclusion of 0.1% *S. cerevisiae* along with 100 mg vitamin E or 0.075% *S. cerevisiae* along with 200 mg vitamin E/kg to the ochratoxin contaminated feed alleviated the ill effects of ochratoxicosis on SGOT activities in broiler chickens. Also, Raju and Devegowda (2000) reported that inclusion of esterified glucomannan (EG) at 1 g/kg level in feed resulted in decreased activities of SGOT that were elevated by mycotoxins. Singh (2015) also reported that inclusion of 0.1% *S. cerevisiae* or vitamin E (200 mg) to the 200 ppb

ochratoxin containing feed alleviated the ill effects of ochratoxicosis on SGOT activities in broiler chickens.

Serum glutamic pyruvic transferase (SGPT): The SGPT value in T_1 was lower ($P<0.05$) than that of T_2 (Table 3). The SGPT value in groups T_3 , T_4 and T_6 was higher ($P<0.05$) than that of control. The SGPT value in groups T_5 , T_7 and T_8 was lower ($P<0.05$) than the toxin fed group and statistically similar to that of control. The present study revealed that ochratoxin (150 ppb) contamination resulted in increased activities of SGPT. This result was in agreement with other reports in literature (Kumar *et al.* 2003, Hatab 2003, Patil *et al.* 2005, Santin *et al.* 2006, Elaroussi *et al.* 2008, Singh 2015). In our present study, inclusion of *S. cerevisiae* (0.1%) + vitamin E (100 mg/kg) or *S. cerevisiae* (0.075%) + vitamin E (200 mg/kg) to the 150 ppb ochratoxin contaminated feed alleviated the adverse effects of ochratoxicosis on SGPT activities in broiler chickens. Agawane and Lonkar (2004) also reported that addition of probiotic containing yeast culture *S. boulardii* at 10 mg/kg feed to 0.5 ppm OTA containing feed showed beneficial effects in decreasing SGPT in broilers. They indicated that increased serum concentration of SGPT in broilers fed OTA containing diet may be due to the damage of hepatocytes releasing the enzyme after the damage. Singh (2015) also reported that addition of *S. cerevisiae* (0.1%) or vitamin E (200 mg/kg) to the 200 ppb OTA containing feed ameliorated the adverse effects of ochratoxicosis on SGPT activities in broiler chickens.

Haemoglobin (Hb): The Hb content in control group was higher ($P<0.05$) than that of ochratoxin fed group (Table 4). The Hb content in T_3 , T_4 and T_6 was lower ($P<0.05$) than that of control. The Hb value in groups T_5 , T_7 and T_8 was higher ($P<0.05$) than that of toxin fed group (T_2) and similar to that of control. In the present study, ochratoxin contamination (150 ppb) in the feed of broiler chickens decreased Hb level. These results were in agreement with those reported by Agawane and Lonkar (2004), Sakhare *et al.* (2007), El-Barkouky (2008), El-Barkouky and Abu-Taleb (2008) who reported that Hb concentration was significantly ($P<0.05$) reduced due to ochratoxin contamination in feed ranging from 50–500 ppb level. Our study revealed that addition of 0.1% *S. cerevisiae* + 100 mg vitamin E or 0.075 *S. cerevisiae* + 200 mg vitamin E/kg to the ochratoxin contaminated feed reversed the Hb level which was statistically equal to that of control. Bradley *et al.* (1994) and Agawane and Lonkar (2004) reported significant improvement in Hb value as a result of addition of probiotic containing yeast culture to ochratoxin intoxicated birds. El-Barkouky (2008) also reported that supplementation of broiler diet with *S. cerevisiae* (200 mg/kg feed) significantly counteracted the deleterious effects of ochratoxin on Hb and PCV of broilers subjected to ochratoxin intoxication. Singh (2015) also reported that inclusion of 0.1% *S. cerevisiae* or 200 mg vitamin E/kg ochratoxin (200 ppb) contaminated feed alleviated the deleterious effects of ochratoxin on Hb concentration in broiler chickens.

Table 3. Serum enzymes of broilers fed different dietary treatment (Mean $\pm$ SEM; n=5)

Treatment	ALP (KA units)	SGOT (IU/L)	SGPT (IU/L)
T_1	146.38 $\pm$ 1.23 ^a	180.52 $\pm$ 1.94 ^{ab}	36.23 $\pm$ 1.25 ^a
T_2	190.29 $\pm$ 1.61 ^d	220.54 $\pm$ 2.73 ^d	48.36 $\pm$ 0.81 ^b
T_3	188.33 $\pm$ 1.47 ^{cd}	218.89 $\pm$ 1.35 ^d	50.29 $\pm$ 0.72 ^{bc}
T_4	180.37 $\pm$ 0.93 ^b	209.44 $\pm$ 1.64 ^c	47.84 $\pm$ 0.84 ^b
T_5	148.50 $\pm$ 0.80 ^a	178.64 $\pm$ 1.24 ^a	35.20 $\pm$ 1.12 ^a
T_6	185.61 $\pm$ 2.05 ^c	215.76 $\pm$ 2.20 ^d	51.44 $\pm$ 0.66 ^c
T_7	149.28 $\pm$ 0.53 ^a	180.73 $\pm$ 0.97 ^{ab}	34.94 $\pm$ 1.06 ^a
T_8	150.18 $\pm$ 0.82 ^a	184.33 $\pm$ 1.58 ^b	36.11 $\pm$ 1.07 ^a

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

Table 4. Blood haematological constituents and immune response of broilers fed different dietary treatment (Mean±SEM; n=5)

Treatment	Haematological constituent		Immune response	
	Haemoglobin (gm/dl)	H/L ratio	CMI	HA Titre
T ₁	10.71±0.24 ^c	0.53±0.00 ^a	0.48±0.00 ^d	10.37±0.03 ^d
T ₂	6.30±0.10 ^a	0.55±0.00 ^c	0.31±0.00 ^a	6.40±0.02 ^a
T ₃	6.17±0.08 ^a	0.54±0.00 ^{bc}	0.32±0.00 ^a	6.40±0.02 ^a
T ₄	6.87±0.09 ^b	0.54±0.00 ^{bc}	0.34±0.00 ^b	7.04±0.06 ^b
T ₅	10.71±0.23 ^c	0.53±0.00 ^a	0.50±0.00 ^d	10.38±0.02 ^d
T ₆	6.94±0.22 ^b	0.53±0.00 ^a	0.36±0.00 ^c	6.98±0.06 ^b
T ₇	10.60±0.21 ^c	0.53±0.00 ^a	0.50±0.00 ^d	10.22±0.05 ^{cd}
T ₈	10.53±0.17 ^c	0.53±0.00 ^a	0.51±0.00 ^d	10.13±0.17 ^{cd}

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

Heterophil/Lymphocytes ratio: The H/L ratio in control group was lower ($P<0.05$) than that of ochratoxin fed group (Table 4). The H/L ratio in T₃ and T₄ was statistically similar to the T₂ and higher ($P<0.05$) than that of control. The H/L ratio in T₅, T₆, T₇ and T₈ was lower ($P<0.05$) than T₂ and statistically similar to the control, indicating that addition of *S. cerevisiae* along with vitamin E at any level in T₅ to T₈ reversed the H/L ratio equal to that of control. The present study revealed that 150 ppb ochratoxin contamination in feed resulted in increased H/L ratio in broiler chickens. This result was in agreement with Singh (2015) who reported increased H/L ratio due to 200 ppb OTA contamination in the diet of broiler chickens. In our study, addition of 0.1% *S. cerevisiae* + 100 mg vitamin E or 0.075% *S. cerevisiae* + 200 mg vitamin E/kg OTA contaminated feed reversed the H/L ratio value equal to control. Singh (2015) also reported that inclusion of 0.1% *S. cerevisiae* to the 200 ppb OTA contaminated feed reversed the H/L ratio equal to control.

Effect on cell mediated immunity (CMI): The CMI value of control group was higher ($P<0.05$) than that of ochratoxin alone fed group (Table 3). The CMI value in T₃, T₄ and T₆ was lower ($P<0.05$) than that of control. The CMI value in T₅, T₇ and T₈ was higher ($P<0.05$) than that of T₂ and statistically similar to that of control. This result indicated that inclusion of *S. cerevisiae* (0.1%) + vitamin E (100 mg) or *S. cerevisiae* (0.075%) + vitamin E (200 mg/kg) to the 150 ppb ochratoxin contaminated feed ameliorated the ill effects of ochratoxicosis on CMI response in broiler chickens. Our study revealed that ochratoxin (150 ppb) concentration in the diet of broiler chickens resulted in reduction ($P<0.05$) in CMI response to PHA-P. Elaroussi *et al.* (2006) also reported that feeding ochratoxin @ 400 to 800 µg/kg feed to day-old broiler chicks until 5th week of age significantly reduced the CMI response as determined by wattle response to PHA-P antigen. Singh *et al.* (1990) showed suppression of CMI response in chickens due to ochratoxicosis and described the significant reduction of T-lymphocyte count and phagocytic ability of splenic macrophages after feeding 0.5 and 2.0 ppm ochratoxin.

Verma *et al.* (2004) reported similar findings in broilers fed with 1–4 ppm ochratoxin for 47 days from day one of age. In the present study, addition of *S. cerevisiae* (0.1%) along with vitamin E (100 mg/kg) or *S. cerevisiae* (0.075%) along with vitamin E (200 mg/kg) to the ochratoxin contaminated feed ameliorated the ill effects of ochratoxin on CMI response. Khalil (2008) also observed that dietary inclusion of *S. cerevisiae* at 2 g/kg ameliorated the negative effects of ochratoxin in growing quails. Singh (2015) also reported that inclusion of 0.1% *S. cerevisiae* or 200 mg vitamin E/kg ochratoxin contaminated diet partially ameliorated the ill effects of ochratoxin on CMI response. Also, Khatoon *et al.* (2013) observed that vitamin E (200 mg/kg diet) alone or in combination with silymarin (10 g/kg) ameliorated the immunotoxic effects induced by 1.0 mg OTA/kg feed.

Humoral immune response: The HA titre value in T₁ was higher ($P<0.05$) than that of T₂ (Table 4). The HA titre value in groups T₃, T₄ and T₆ was lower ($P<0.05$) than that of control. The HA titre value in T₅, T₇ and T₈ was higher ($P<0.05$) than T₂ and statistically similar to that of control, indicating that inclusion of 0.1% *S. cerevisiae* + 100 mg vitamin E or 0.075% *S. cerevisiae* + 200 mg vitamin E to the ochratoxin contaminated diet alleviated the adverse effects of ochratoxicosis on humoral immune response. In the present study, dietary ochratoxin at 150 ppb level reduced ($P<0.05$) the HA titre against SRBC'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 decreased 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). Singh (2015) reported that dietary ochratoxin at 200 ppb level reduced ($P<0.05$) the HA titre against SRBC's. Our study revealed that incorporation of 0.1% *S. cerevisiae* + 100 mg vitamin E or 0.075% *S. cerevisiae* + 200 mg vitamin E to the 150 ppb ochratoxin contaminated feed ameliorated the ill effects of ochratoxicosis on humoral immune response. El-Barkouky (2008) also found that inclusion of 0.1% *S. cerevisiae* improved humoral immune response negatively affected by 50 and 100 ppb ochratoxin. Singh (2015) also reported that inclusion of 0.1% *S. cerevisiae* or 200 mg vitamin E/kg ochratoxin (200 ppb) contaminated feed ameliorated the ill effect of ochratoxicosis on humoral immune response of broiler chickens.

It can be concluded that ochratoxin contamination at the rate of 150 ppb in the feed resulted in decreased total serum protein, cholesterol and haemoglobin content, and increased serum uric acid, creatinine, ALP, SGOT, SGPT and H/L ratio value. Inclusion of *Saccharomyces cerevisiae* at 0.1% level along with 100 mg vitamin E or *Saccharomyces cerevisiae* at 0.075% level along with 200 mg vitamin E/kg diet to the ochratoxin (150 ppb) contaminated feed ameliorated the adverse effects of ochratoxicosis on

biochemical profile and immune response in broiler chickens.

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