Indian Journal of Animal Sciences **90** (1): 61–66, January 2020/Article
https://doi.org/10.56093/ijans.v90i1.98221

Influence of *Saccharomyces cerevisiae* to ameliorate adverse effects of ochratoxin on biochemical profile and immune response in broiler chickens

MOHIT SINGH[1], RAM SINGH[2] and A B MANDAL[3]

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

Received: 13 November 2018; Accepted: 25 April 2019

ABSTRACT

To establish the ochratoxicosis ameliorating efficacy of dietary *Saccharomyces cerevisiae* (SC), day-old broiler chicks (240) were divided into 6 treatment groups (T_1, control (basal diet); T_2, T_1 + 200 ppb OTA; T_3, T_1 + 0.05% SC; T_4, T_1 + 0.1% SC; T_5, T_2 + 0.05% SC and T_6, T_2 + 0.1% SC). Each diet was fed to 5 replicated groups of 8 birds each from 1 to 42 days of age. The total serum protein and haemoglobin (Hb) in T_2 and T_5 were lower than that of control (T_1). The serum protein and Hb content in group T_6 was higher than that of T_2 and statistically similar to that of control. The serum uric acid, creatinine, ALP, SGOT, SGPT and H/L ratio in ochratoxin fed group (T_2) was higher than that of T_1. The serum uric acid, creatinine, ALP, SGOT, SGPT and H/L ratio in T_6 was lower than that of T_2 and statistically similar to that of T_1. The CMI and HA titre value in ochratoxin fed group (T_2) was lower than that of T_1. The CMI and HA titre value in T_5 and T_6 was lower than T_1 but higher than T_2. It was concluded that ochratoxin contamination at 200 ppb level in broiler diet altered the biochemical parameters and immune response. Inclusion of *Saccharomyces cerevisiae* at 0.1% level to the ochratoxin contaminated diet ameliorated the adverse effects of ochratoxicosis on biochemical parameters and immune response of broiler chickens.

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

Ochratoxin A (OTA) is a mycotoxin that is receiving increasing attention worldwide because of the hazard it poses to animal and human health. This mycotoxin was isolated as metabolite of the fungus *Aspergillus ochraceus* from which the toxin acquired its name. However, ochratoxin production is not unique to *Aspergillus ochraceus* as additional several *Aspergillus species* and several *Penicillium species* produce ochratoxins. Ochratoxicosis causes a reduction in production performance, viz. reduced growth rate, decreased feed consumption, poorer feed conversion (Singh *et al.* 2016b, Singh *et al.* 2018) and increased mortality (Singh *et al.* 2015, Singh *et al.* 2016b, Singh *et al.* 2018). Ochratoxin impaired both cell mediated and humoral immunity (Singh *et al.* 2016a). Besides the production of OTA by storage fungi in several different food and feed, the presence of OTA residues in animal products is another concern. Ochratoxin A has also been detected in meat, egg and milk (Jorgensen 1998, Skaug 1999). Moreover, OTA has been frequently found in human plasma, milk and urine indicating a diffuse and continuous human exposure to this mycotoxin in many countries (Rosner *et al.* 2000, Skaug *et al.* 2001). 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, Singh *et al.* 2018). 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). *S. cerevisiae* has shown considerable binding ability with several commonly occurring mycotoxins (Devegowda *et al.* 1998, Singh *et al.* 2018), and is also found effective as a low-inclusion binder to bind mycotoxins present in contaminated feed (Mahesh and Devegowda 1996, Singh *et al.* 2018). The objective of this investigation was to study the effect of dietary *S. cerevisiae* to ameliorate ochratoxicosis in broiler chickens.

Present address: [1]Mycotoxin Laboratory (singh.mohit204@gmail.com), [2]Principal Scientist (carirsingh@yahoo.co.in), [3]Director (abmcari@rediffmail.com), Avian Nutrition and Feed Technology Division.

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 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 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 (1990).

Experimental design: Experimental design was completely randomized design (CRD). There were 6 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 T_1, Control (basal diet free from ochratoxin); T_2, T_1 + 200 ppb OTA; T_3, T_1+ 0.05% SC; T_4, T_1+ 0.1% SC; T_5, T_2 + 0.05% SC and T_6, T_2+ 0.1% SC.

Biological experiment and analysis: Day-old broiler chicks (240) were obtained from experimental hatchery, CARI, Izatnagar. The chicks were wing banded, weighed individually and distributed randomly into 6 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 ingredient and chemical composition of broiler starter and finisher ration are presented in Table 1.

Table 1. Ingredient and chemical composition of basal feed

Ingredient	Starter (g/kg)	Finisher (g/kg)
Maize	555.00	624.20
Deoiled rice bran	18.80	20.10
Soybean	280.00	205.00
Guar korma	40.00	40.00
Rape seed meal	40.00	40.00
Fish meal	40.00	40.00
Limestone	5.00	5.00
Di–calcium phosphate	16.00	16.00
Common salt	2.00	2.50
DL– methionine	0.70	0.30
Lysine	1.25	0.70
TM Premix*	1.10	1.00
Vitamin Premix**	1.50	1.50
B complex***	0.15	0.15
Choline chloride	0.50	0.50
Coccidiostat	0.50	0.50
Chemical composition of basal diet		
Crude protein (g/kg)	223	200.6
ME (MJ/Kg)	11.75	12.04
Calcium (g/kg	10.9	10.9
Available phosphorus (g/kg)	5.0	4.2
Lysine (g/kg)	12.8	10.4
Methionine (g/kg)	5.1	4.3

*TM premix supplied mg/kg diet: $MgSO_4.5H_2O$, 300; $MnSO_4.H_2O$, 55; KI, 0.4; $FeSO_4.7H_2O$, 56; $ZnSO_4$, $7H_2O$, 30; $CuSO_4.5H_2O$, 4.**Vitamin premix supplied per kg diet: vitamin A (retinol), 8250 IU; vitamin D3 (cholecalciferol), 1200 IU; vitamin K (menadione), 1 mg.***B complex supplied per kg diet: vitamin B_1 (thiamine), 2 mg; Vit. B_2, 4 mg; vitamin B_2 (riboflavin), 10 mcg; niacin (nicotinic acid), 60 mg; pantothenic acid, 10 mg; choline, 500 mg.

The protein as per AOAC (1990) 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. 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 –20°C and analyzed for various biochemical parameters using commercial kit manufactured by Span Diagnostics Ltd, 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 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.

Statistical analysis: The collected data was subjected to statistical analysis using Software 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

Total serum protein: The total serum protein in ochratoxin fed group (T_2) was lower ($P<0.05$) than that of control (T_1). The serum protein content in groups T_3 and T_4 was statistically similar to that of T_1 (Table 2). The serum protein in T_5 was statistically similar to that of ochratoxin fed group (T_2) and lower ($P<0.05$) than that of T_1, indicating that supplementation of *S. cerevisiae* at 0.05% level did not improve serum protein content. The serum protein content in group T_6 was higher ($P<0.05$) than that of T_2 and statistically similar to that of control, indicating that addition of *S. cerevisiae* at 0.1% level improved the serum protein content equal to that of control. In the present study, 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 (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 low serum

Table 2. Certain blood biochemical constituents of broilers fed various dietary treatments (Mean±SEM; n=5)

Treatment	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^{c}	185.12±4.14	5.32±0.33^{a}	0.31±0.02^{a}	155.20±3.89^{ab}	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^{d}	42.22±2.26^{b}
T_3	6.49±0.49^{c}	185.99±3.97	5.35±0.29^{a}	0.31±0.01^{a}	154.34±3.36^{a}	171.10±4.14^{a}	32.14±2.58^{a}
T_4	6.58±0.42^{c}	186.60±5.00	5.39±0.29^{a}	0.32±0.02^{ab}	155.41±4.39^{a}	172.10±4.46^{a}	32.54±2.46^{a}
T_5	4.72±0.42^{a}	182.87±4.72	6.29±0.40^{b}	0.35±0.03^{bc}	181.72±7.25^{c}	189.67±3.91^{c}	42.64±2.13^{b}
T_6	5.56±0.35^{b}	185.00±4.83	6.70±0.38^{b}	0.31±0.02^{a}	161.66±2.68^{b}	181.41±7.91^{b}	35.10±3.70^{a}

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

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. In the present study, inclusion of *S. cerevisiae* (0.1%) to the ochratoxin contaminated diet increased (P<0.05) the serum protein content equal to that of control. This result was in agreement with Bailey *et al.* (1990), Bradley *et al.* (1994) and Agawane and Lonkar (2004) who 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 ochratoxin intoxicated broiler chickens. Significant improvement in serum protein content due to addition of *S. cerevisiae* in ochratoxin contaminated feed was also reported by El-Barkouky (2008) and Khalil (2008).

Total serum cholesterol: The serum cholesterol in various dietary treatment groups varied between 182.87 and 186.60 mg/dl. The cholesterol concentration in all the treatment groups was statistically similar to that of control (Table 2). However, Schaeffer *et al.* (1987) and Sreemannarayana *et al.* (1989) observed reduction in serum cholesterol content due to ochratoxin contamination in feed of broiler chickens.

Serum uric acid: The serum uric acid content in T_2 was higher (P<0.05) than that of T_1. The uric acid concentration in groups T_3 and T_4 was statistically similar to that of control (Table 2). The uric acid content of groups T_5 and T_6 was lower (P<0.05) than T_2 but higher (P<0.05) than control (T_1), indicating that addition of *S. cerevisiae* partially ameliorated the ill effects of ochratoxicosis on serum uric acid content. In the present study, 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. In the present study, addition of *S. cerevisiae* to ochratoxin contaminated feed partially reversed the serum uric acid concentration.

Serum creatinine: The creatinine content in T_2 was higher (P<0.05) than that of T_1. The creatinine concentration in groups T_3 and T_4 was statistically similar to that of T_1 (Table 2). The creatinine content in group T_5 was statistically similar to that of T_2. The creatinine content of group T_6 was lower (P<0.05) than that of T_2 and statistically similar to that of T_1, indicating that inclusion of *S. cerevisiae* at 0.1% level to the ochratoxin contaminated diet ameliorated the adverse effects of ochratoxicosis on creatinine concentration. In the present study, 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). In the present study, supplementation of 0.1% *S. cerevisiae* to the OTA contaminated feed reversed the creatinine content equal to that of control. 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.

Alkaline phosphatase (*ALP*): The ALP activities in T_1 was lower (P<0.05) than that of T_2. The ALP activities in groups T_3 and T_4 was statistically similar to that of control

Table 3. Certain blood haematological constituents and immune response of broilers fed different dietary treatment (Mean±SEM; n=5)

Treatment	Haemoglobin (g/dl)	H/L ratio	CMI (mm)	HA titre Log $_2$
T_1	9.20±0.58^{c}	0.51±0.01^{ab}	0.47±0.01^{d}	9.34±0.13^{d}
T_2	5.87±0.49^{a}	0.53±0.01^{c}	0.30±0.01^{a}	5.67±0.40^{a}
T_3	9.24±0.40^{c}	0.51±0.01^{ab}	0.47±0.01^{d}	9.42±0.31^{d}
T_4	9.27±0.73^{c}	0.49±0.00^{a}	0.48±0.01^{d}	9.43±0.54^{d}
T_5	7.32±0.40^{b}	0.51±0.01^{ab}	0.34±0.01^{b}	6.40±0.41^{b}
T_6	8.45±0.84^{c}	0.51±0.01^{ab}	0.41±0.01^{c}	8.30±1.61^{c}

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

(Table 2). The ALP activities in group T_5 was lower (P<0.05) than that of T_2, but higher (P<0.05) than that of T_1. The ALP value in T_6 was lower (P<0.05) than that of T_2 and statistically similar to that of control (T_1), indicating that the addition of 0.1% *S. cerevisiae* to the ochratoxin contaminated diet ameliorated the adverse effects of ochratoxicosis on ALP activities. In the present study, ochratoxin resulted in increased activities of ALP. Khan *et al.* (2014) also reported increased activities of ALP due to ochratoxicosis in broiler chickens. In the present study, addition of *S. cerevisiae* at 0.1% level to ochratoxin contaminated diet ameliorated the adverse effects of ochratoxicosis on ALP activities.

Serum glutamic oxaloacetic transferase (*SGOT*)*:* The SGOT activities in T_2 was higher (P<0.05) than that of T_1. The SGOT level in groups T_3 and T_4 was statistically similar to that of control (T_1) (Table 2). The SGOT level in groups T_5 and T_6 was lower (P<0.05) than T_2 but significantly higher than control, indicating that the supplementation of *S. cerevisiae* at any level to the ochratoxin contaminated diet partially ameliorated the adverse effects of ochratoxicosis on SGOT activities. The SGOT level in T_6 was lower (P<0.05) than that of T_5, suggesting that 0.1% level of *S. cerevisiae* was more effective than 0.05% level in ameliorating ochratoxicosis. In the present study, 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). Raina *et al.* (1991) reported that increased level of SGOT in broiler serum was attributed to cellular damage and increased plasma membrane permeability. In present study, inclusion of 0.1% *S. cerevisiae* to ochratoxin contaminated diet reversed the SGOT value equal to normal. Raju and Devegowda (2000) also reported that inclusion of esterified glucomannan (EG) @ 1 g/kg level in feed resulted in decreased activities of SGOT that were elevated by mycotoxins.

Serum glutamic pyruvic transferase (*SGPT*)*:* The SGPT level in T_2 was higher (P<0.05) than that of T_1. The SGPT level in groups T_3, T_4 and T_6 was statistically equal to that of control (Table 2). The SGPT level in group T_5 was higher (P<0.05) than that of control. The SGPT level in T_6 was lower (P<0.05) than that of T_2 and statistically similar to that of control, indicating that addition of 0.1% *S. cerevisiae* to the ochratoxin contaminated feed ameliorated the adverse effects of ochratoxicosis on SGPT activities. In the present study, ochratoxin contamination resulted in increased activities of SGPT. This finding was in agreement with other reports in literature (Kumar *et al.* 2003, Hatab 2003, Patil *et al.* 2005, Elaroussi *et al.* 2008). In the present study, addition of *S. cerevisiae* (0.1%) to ochratoxin contaminated feed ameliorated the ill effects of ochratoxicosis on SGPT activities. Agawane and Lonkar (2004) also reported that addition of probiotic containing yeast culture *S. boulardii* @ 10 mg/kg feed to 0.5 ppm OTA contaminated feed showed beneficial effects in decreasing SGPT activities in broiler chickens. They indicated that increased serum concentration of SGPT in broilers fed ochratoxin containing diet may be due to the damage of hepatocytes releasing the enzyme after the damage.

Effect on haematological parameters: The haemoglobin content in T_1 was higher (P<0.05) than that of T_2. The Hb content is T_3, T_4 and T_6 was statistically similar to that of control (Table 3). The Hb value of T_5 was higher (P<0.05) than T_2 but lower than T_1, indicating that inclusion of 0.05% *S. cerevisiae* may not be sufficient to reverse the haemoglobin concentration however, addition of 0.1% dietary *S. cerevisiae* reversed the Hb concentration equal to control (Table 3). In the present study, ochratoxin contamination resulted in reduced Hb level in broiler chickens. Similar result was also reported by Agawane and Lonkar (2004), Sakare *et al.* (2007), El-Barkouky (2008) and 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. Also, 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. In the present study, inclusion of 0.1% *S. cerevisiae* to the ochratoxin contaminated diet reversed the Hb level which was statistically equal to that of control. These findings were in agreement with Bradley *et al.* (1994) and Agawne and Lonkar (2004) who reported significant improvement in Hb value as a result of addition of probiotic containing yeast culture to ochratoxin intoxicated broilers. 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 values of broilers subjected to ochratoxin intoxication. H/L ratio in control group was 0.51 which increased (P<0.05) to 0.53 in ochratoxin fed group (T_2). The H/L ratio in T_3, T_4, T_5 and T_6 was statistically similar to that of control, indicating that supplementation of *S. cerevisiae* at both levels improved (P<0.05) the H/L ratio in ochratoxin intoxicated broiler chicks.

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 presented in Table 3. One of the most profound effects of ochratoxicosis is its ability to impair immune system functions.

Effect on cell mediated immunity (*CMI*): The CMI of group T_1 was higher (P<0.05) than that of T_2. The results revealed that contamination of dietary ochratoxin in feed reduced (P<0.05) the CMI response as compared to that of control. The CMI in T_3 and T_4 was statistically similar to that of control, indicating that the addition of *S. cerevisiae* to the basal feed did not produce any effect on CMI response. The CMI in group T_5 and T_6 was lower (P<0.05) than that of T_1 but higher than that of T_2, however, the CMI in group T_6 was higher (P<0.05) than T_5. These findings indicated that supplementation of *S. cerevisiae* at both levels

(0.05 and 0.1%) partially ameliorated the ill effect of ochratoxin on CMI response; however, the higher level (0.1%) was more efficacious in ameliorating the adverse effect of ochratoxin. The present study indicated that ochratoxin inclusion in the diet of broiler chickens caused significant (P<0.05) reduction in CMI response to PHA-P. This result was in agreement with those of Singh *et al.* (2015), Singh *et al.* (2016b), Singh *et al.* (2018). Also, 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). In the present study, inclusion of *S. cerevisiae* to the ochratoxin contaminated feed partially ameliorated the ill effects of ochratoxin on CMI response. However, 0.1% inclusion of *S. cerevisiae* was more efficacious in ameliorating the adverse effect of ochratoxin. Khalil (2008) also observed that dietary inclusion of *S. cerevisiae* at 2 g/kg ameliorated all the negative effects of ochratoxin in growing quails.

Humoral immune response: The HA titre in T_2 was lower (P<0.05) than that of T_1. The HA titre of T_3 and T_4 was statistically similar to that of control. The HA titre in T_5 and T_6 was lower (P<0.05) than T_1 but higher than T_2 indicating that addition of *S. cerevisiae* at both levels partially ameliorated the adverse effect of ochratoxicosis on humoral immune response. The HA titre of group T_6 was higher than that of T_5, indicating that 0.1% level of *S. cerevisiae* was more efficacious than 0.05% level in ameliorating ill effect of ochratoxicosis. In the present study, 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). In the present study, incorporation of *S. cerevisiae* (0.05 and 0.1%) to the ochratoxin contaminated feed partially ameliorated the ill effect of ochratoxicosis on humoral immune response of broiler chickens which resulted in welfare of birds. El-Barkouky (2008) also found that inclusion of *S. cerevisiae* at 0.1% improved humoral immune response negatively affected by 50 and 100 ppb OTA.

It can be 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. Inclusion of *Saccharomyces cerevisiae* at 0.1% level to the ochratoxin contaminated diet ameliorated the ill effects of ochratoxicosis on biochemical parameters and immune response of broiler chickens.

REFERENCES

Agawane S B and Lonkar P S. 2004**.** Effect of probiotic containing *Saccharomyces boulardii* on experimental ochratoxicosis in broilers: hematobiochemical studies. *Journal of Veterinary Science* **5**: 359–67.

AOAC. 1990. *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington DC, USA.

Ayed I A M, Defalla R, Yagi A I and Adams S E I. 1991**.** Effect of ochratoxin A on Lohman-type chicks. *Veterinary and Human Toxicology* **33**: 557–60.

Bailey C A, Gibson R M, Kubena L F, Huff W E and Harvey R B. 1990. Impact of L-Phynelalanine supplementation on the performance of three-week-old broilers fed diets containing ochratoxin A. 2-Effects on hematological and clinical chemistry. *Poultry Science* **69**: 420–25.

Bradley G I, Savage T F and Timm K I. 1994. The effect of supplementing diet with *Saccharomyces cervecei* var. *boulardii* on male poults performance and ileal morphology. *Poultry Science* **73**: 1766–70.

Campbell M L, May J D, Huff W E and Doerr J A. 1983. Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. *Poultry Science* **62**: 2138–44.

Corrier D E and Deloach J R. 1990. Evaluation of cell mediated cutaneous basophil, hypersensitivity in young chickens by an interdigital skin test. *Poultry Science* **69**: 403–08.

Devegowda G, Raju M V L N, Afzali N and Swamy H V L N. 1998. Mycotoxin picture worldwide: Novel solutions for their counteraction. *Feed Compounder* **18**: 22–27.

Effat M M. 1989. 'Ochratoxin A and its effect on chicken immune response'. MSc Thesis, Faculty of Veterinary Medicine. Cairo University, Egypt. p.105

Elaroussi M A, Mohamed F R, Elgendy M S, El-Barkouky E M, Abdou A M and Hatab M H. 2008. Ochratoxicosis in broiler chickens: Functional and histological changes in target organs. *International Journal of Poultry Science* **75**: 414–22.

El-Barkouky E M and Abu-Taleb A M. 2008. The role of vitamin C in improving the performance of male broiler chickens feed ration contaminated with ochratoxin. *Egyptian Journal of Applied Science* **23**: 1–12.

El-Barkouky E M. 2008. The role of yeast in improving the performance of male broiler chicken feed ration contaminated with ochratoxin. *Egyptian Journal of Applied Science* **23**: 13–24.

Garcia A R, Avila E, Rosiles R and Petrone V M. 2003. Evaluation of two mycotoxin binders to reduce toxicity of broiler diets containing ochratoxin A and T–2 toxin contaminated grain. *Avian Diseases* **47**: 691–99.

Hatab M H. 2003**.** Determination of the toxic effects of Ochratoxin A on broiler performance and immune system response by the use of nuclear techniques. MSc Thesis, Faculty of Agriculture, Cairo University, Egypt. p. 336.

Hsieh D P H. 1987. Mode of action of mycotoxins. (Ed.) Krogh P. *Mycotoxins in food*. Academic Press, London pp. 149–76.

Jorgensen K. 1998. Survey of pork, poultry, coffee, beer and pulses for ochratoxin A. *Food Additives and Contaminants* **15**(5): 550–54.

Khalil M R. 2008. Effects of ochratoxin on immune response in quails and traits for control. Ph.D. Thesis, Faculty of Veterinary

Medicine, Benha University, Egypt. p. 182.

Khan S A, Venancio E J, Hirooka E Y, Rigobello F, Ishikawa A, Nagashima L A, Oba Alexandre and Itano E N. 2014. Avian ochratoxicosis: A review. *African Journal of Microbiology Research* **8**: 3216–19.

Koynarski V, Stoev S, Grozeva N, Mirtcheva T, Daskalo H, Mitev J and Mantle P. 2007. Experimental coccidiosis provoked by *Eimeria acervulina* in chicks simultaneously fed on ochratoxin A contaminated diet. *Research in Veterinary Science* **82**: 225–31.

Kubena L F, Harvey R B, Huff W E, Corrier D E, Philips T D and Creger C R. 1988. Influence of ochratoxin A and deoxynivalenol on growing broiler chicks. *Poultry Science* **67**: 253–60.

Kubena L F, Harvey R B, Huff W E, Corrier D E, Philips T D and Rottinghaus G E. 1989. Influence of ochratoxin A and T-2 toxin singly and in combination on broiler chickens. *Poultry Science* **68**: 867–72.

Kubena L F, Philips T D, Creger C R, Witzel D A and Heidelbaugh N D. 1983. Toxicity of ochratoxin A and tannic acid to growing chicks. *Poultry Science* **62**: 1786–92.

Kumar A, Jindal N, Shukla C L, Pal Y, Ledoux D R and Rottinghaus G E. 2003. Effect of ochratoxin A on *Escherichia coli*-challenged broiler chicks. *Avian Diseases* **47**: 415- 24.

Mahesh B K and Devegowda G. 1996. Ability of aflatoxin binders to bind aflatoxin in contaminated poultry feeds. An *in vitro* study. *Proceedings of the 20th World's Poultry Congress* **4**: 296–303.

Marquardt R R and Frohlich A A. 1992. A review of recent advances in understanding Ochratoxicosis. *Journal of Animal Science* **70**: 3968–88.

Mohiuddin S M, Reddy M V and Ahmed S R. 1992. Studies on ochratoxicosis in broiler chicks. *Indian Veterinary Journal* **69**: 1011–14.

Mohiuddin S M, Warasi S M A and Reddy M V. 1993. Hematological and biochemical changes in experimental ochratoxicosis in broiler chickens. *Indian Veterinary Journal* **70**: 613–17.

O'Brien E and Dietrich D R. 2005. Ochratoxin A: The continuing enigma. *Critical Reviews in Toxicology* **35**: 33–60.

Patil R D, Degloorkar N M, Moregaonkar S D and Kulkarni G B. 2005. Ameliorative efficacy of Bantox® in induced ochratoxicosis in broilers: A haemato-biochemical study. *Indian Journal of Veterinary Pathology* **29**: 23–30.

Raina J S, Roy K S and Singh B. 1991. Biochemical and histochemical studies in experimental mycotoxicosis in chicks. *Indian Journal of Animal Sciences* **62**: 1276–81.

Raju M V L N and Devegowda G. 2000. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and hematology in broilers exposed to individual and combined mycotoxicosis aflatoxin, ochratoxin and T-2 toxin. *British Poultry Science* **41**: 640–50.

Rosner H, Rohrmann B and Peiker G. 2000. Ochratoxin A in human serum. *Archiv Fur Lebensmittelhygiene* **51**: 104–107.

Sakhare P S, Harne S D, Kalorey D R, Warke S R, Bhandarkar A G and Kurkure N V. 2007. Effect of Toxiroak® polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. *Veterinarski Arhiv* **77**: 129–46.

Santin E, Maiorka A, Krabbe E L, Paulillo A C and Alessi A C. 2002. Effect of hydrated sodium calcium aluminosilicates on the prevention of the toxic effects of ochratoxin. *Journal of Applied Poultry Research* **11**: 22–28.

Schaeffer J L, Tyczkowski J K and Hamilton P B. 1987. Alterations in carotenoid metabolism during ochratoxicosis in young broiler chickens. *Poultry Science* **66**: 318–24.

Siegel P B and Gross W B. 1980. Production and persistence of antibodies to sheep erythrocytes. 1. Directional selection. *Poultry Science* **59**: 1–5.

Singh G S P, Chauhan H V S, Jha G J and Singh K K. 1990. Immunosuppression due to chronic ochratoxicosis in broiler chicks. *Journal of Comparative Pathology* **103**: 399–410.

Singh M, Singh R and Mandal AB. 2016b. Use of *Saccharomyces cerevisiae* to suppress the effects of ochratoxicosis in broiler chickens. *Indian Journal of Animal Sciences* **86**(7): 790–94.

Singh M, Singh R, Mandal AB and Sharma M 2016a. Influence of dietary supplementation of vitamin E in ameliorating adverse effects of ochratoxin on biochemical profile and immune response in broiler chickens. *Indian Journal of Animal Sciences* **86**(12): 1447–52.

Singh R, Mandal A B, Sharma M and Biswas A. 2015. Effect of varying levels of dietary ochratoxin A on the performance of broiler chickens. *Indian Journal of Animal Sciences* **85**: 296–300.

Singh R, Tyagi P K, Divya and Sharma M. 2013. Ochratoxigenic potential of *Aspergillus westerdijkiae* NRRL 3174 under laboratory conditions. *Indian Journal of Poultry Science* **48**: 247–49.

Singh S, Singh R, Mandal A B and Singh M. 2018. Associated efficiency of *Saccharomyces cerevisiae* and vitamin E in ameliorating adverse effects of ochratoxin A on production performance in broiler chickens. *Indian Journal of Animal Sciences* **88**(8): 938–43.

Skaug M A, Helland I, Solvoll K and Saugstad O D. 2001. Presence of ochratoxin A in human milk in relation to dietary intake. *Food Additives and Contaminants* **18**(4): 321–27.

Skaug M A. 1999. Analysis of Norwegian milk and infant formulas for ochratoxin A. *Food Additives and Contaminants* **16**: 75–78.

Sreemannarayana O, Marquardt R R, Frohlich A A, Abramson D and Phillips T D. 1989. Organ weights, liver constituents and serum components in growing chicks fed ochratoxin A. *Archives of Environmental Contamination and Toxicology* **18**: 404–10.

Stoev S D, Anguelov G, Ivanov I and Pavlov D. 2000. Influence of ochratoxin A and an extract of artichoke on the vaccinal immunity and health in broiler chicks. *Experimental and Toxicologic Pathology* **52**: 43–55.

Talapatra S K, Ray S C and Sen K C. 1940. Estimation of phosphorus, choline, calcium, magnesium, sodium and potassium in feeding stuffs. *Journal of Veterinary Science and Animal Husbandary* **10**: 243–45.

Verma J, Johri T S, Swain B K and Ameena S. 2004. Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. *British Poultry Science* **45**: 512–18.

Wang G H, Xue C Y, Chen F, Ma Y L, Zhang X B, Bi Y Z and Cao Y C. 2009. Effects of combinations of ochratoxin A and T-2 toxin on immune function of yellow-feathered broiler chickens. *Poultry Science* **88**: 504–10.