ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 8 (8): 798-803, 2009
ISSN 1682-8356

Experimental Mycotoxicosis in Layer Induced by Ochratoxin A and its Amelioration with Herbomineral Toxin Binder 'Toxiroak'

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Abstract: A study was conducted in 80 white leghorn 26 week old laying hen to evaluate toxic effects of ochratoxin A and preventive efficacy of herbomineral toxin binder product (Toxiroak®). Birds were randomly divided into four groups of 20 each. Group I served as control and given no treatment, Group II comprised healthy birds fed standard basal diet and administered Toxiroak@1.25 kg/tonne of feed for 60 days, birds of group III received ochratoxin A@1 ppm while those of group IV were given ochratoxin A@1 ppm and herbomineral toxin binder product Toxiroak@1.25 kg/tonne of feed for 60 days. Ochratoxin A adversely affects body weight gain, feed consumption, laying performance of hens besides haematobiochemical disturbances & severe immunosupression. However, supplementation of herbomineral toxin binder feed supplement has provided a moderate amelioration in mycotoxicosis.

Key words: Layer, performance, ochratoxicosis, herbo-mineral, toxin-binder

INTRODUCTION

Ochratoxins (OTA) are mycotoxins isolated from *Aspergillus ochraceus* but can also be produced by a series of *Aspergillus* and *Penicillium* species (Gibson *et al*., 1990). Of this group of isocumarins, only ochratoxin A has been naturally isolated from cereals and is the most toxic mycotoxin for birds. The natural occurrence of OTA in food and feedstuffs of plant and animal origin is common. Due to its long half-life OTA accumulates in the food chain and threatens human and animal health because of its extreme toxicity, widespread occurrence and the variety of commodities that it can contaminate (Scott, 1978). OTA has been implicated in a diverse range of toxicological effects, including renal toxicity, mutagenicity, teratogenicity, neurotoxicity and immunotoxicity in both animals and man (O'Brien and Dietrich, 2005). OTA causes significant loss to poultry industry, intoxication of birds by ochratoxin results in reduced weight gain, impaired feed efficiency, reduced egg production and quality (Page *et al*., 1980). Furthermore, it produces a reduction in total blood proteins (Huff *et al*., 1988; Stoev *et al*., 2000), suppression of immune function (Chang *et al*., 1979; Singh *et al*., 1990; Stoev *et al*., 2000; Santin *et al*., 2002; Politis *et al*., 2005) and impairment of blood coagulation (Raju and Devegowda, 2000). OTA induces degenerative changes and an increase in the weight of kidney and liver, as well as a decrease in weights of the lymphoid organs (Stoev *et al*., 2000). Another implication of ochratoxicosis is leucocytopenia as a result of immunosupression (Chang *et al*., 1979; Stoev *et al*., 2000; Elaroussi *et al*., 2006), impaired complement activity (Campbell *et al*., 1983), reduction in immunoglobulins (Dwivedi and Burns, 1984) and finally it causes atrophy of the lymphoid organs along with depletion of lymphocytes (Politis *et al*., 2005; Stoev *et al*., 2004 and Kumar *et al*., 2004). In the last few years, several studies have suggested that aluminosilicates, certain minerals, chemical adsorbants, herbs etc. can reduce the effects of mycotoxins in several animal species, due to their ability to bind to or to adsorb mycotoxins. Deleterious effects of Aflatoxins could be overcome, or at least diminished, by adsorbants in rats (Abdel-Wahhab *et al*., 2002), chemical adsorbants (Kubena *et al*., 1993), Levamisole hydrochloride (Kalorey, 1993), glucamannan (Raju and Devegowda, 2000) have been attempted with varying degrees of success to reduce toxicity. Stoev *et al*. (2002) reported that 5% aqueous extract of *Artichoke* and *Curcuma longa* powder@0.5 g/kg feed reduces the toxic effect of ochratoxin A and Aflatoxin B1, in chicks. Sakhare *et al*. (2007) also reported on efficacy of polyherbal feed supplement during induced mixed mycotoxicosis in broilers. In the present study protective role of polyherbal and mineral feed supplement Toxiroak® is studied during induced aflatoxicosis in broilers. *In vitro* antitoxic and antifungal activity of the same was confirmed earlier (Kalorey *et al*., 2000).

MATERIALS AND METHODS

The current research study is a part of master's thesis programme of the first author in the department of Veterinary Pathology, College of Veterinary and Animal Husbandry, IGKV Raipur, Anjora, Durg, India. A study was conducted on 80 white leghorn 26 week old laying hen, randomly divided into four groups, each comprising of 20 birds. Group I served as control and given no treatment, Group II comprised healthy birds fed standard

basal diet and administered herbomineral toxin binder product Toxiroak@1.25 kg/tonne of feed for 60 days, birds of group III received Ochratoxin A@1 ppm while those of group IV were given Ochratoxin A@1 ppm and Toxiroak@1.25 kg/tonne of feed for 60 days. Toxiroak® is a polyherbal preparation prepared from extracts of *Allium sativum, Azadirachta indica, Solanum nigram, Emblica officinalis, Curcuma longa* and hydrated alluminosilicates (HSCAS), was procured from Ayurvet Ltd., Baddi (H.P.), India, for present study.

Production of ochratoxin A: OTA was produced on rice as per the procedure described above, using a known ochratoxin A-producing strain of *Aspergillus ochraceus* (NRRL 3174) available at the Department of Microbiology, Nagpur Veterinary College, Nagpur. OTA standard (3 ug/ml) was used for quantification of OTA, according to Tapia (1985). Laying birds of all the four groups were offered standard layer mash as per the NRC, 1998 requirements and water, *adlibitum*. In order to achieve required toxin level in calculated feed, a quantity of fungus was mixed in feed of group III and IV, respectively.

Experimental observations: All the birds from group I to IV were weighed at fortnightly intervals during complete experimental study. Also, feed offered to birds and feed that was left uneaten, was recorded weekly in order to calculate cumulative Feed Conversion Ratio (FCR). Laying performance of birds was assessed by recording number of eggs laid in each group per day and calculated at weekly intervals. Haematological observations (Hb, PCV, TEC, TLC) were recorded on day 0, 30th and 60th of experiment randomly selecting five birds per group. Blood samples were collected from jugular vein in heparinized vials (Heparin @ 10 I.U./ml of blood). For biochemical estimations, blood was collected in non-heparinized vials for serum separation on day 0, 30th and 60th. Serum samples were analyzed for total serum protein, albumin, cholesterol, liver marker enzymes: Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) using semi automated analyzer using diagnostic kits (Span Diagnostics, Mumbai). Assessment of immunological parameters involved cell mediated immune response, evaluated on day 43rd of experiment, by Di-Nitro Fluro Benzene (DNFB) contact skin sensitization test as per method of Thompson *et al.* (1975) and humoral immune response, according to method of Thaxton *et al.* (1974). Statistical analysis of data was carried out using complete randomized design as per the method of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

In the present study the impact of induced ochratoxicosis on various parameters of chicks were studied. An attempt was also made to ascertain the protective role of polyherbal preparation Toxiroak® during induced mycotoxicosis in broilers.

Growth studies: Results of the present study demonstrate that dietary inclusion of ochratoxin affects body mass and performance of broilers. Average body weight gain, feed consumption and FCR of different groups is presented in Table 1. Data revealed that ochratoxin A @ 1 ppm (group III and IV) has adversely affected these parameters. There was gradual and significant decrease in weight gain and feed consumption. Mean body weight gain (g) was significantly ($p \leq 0.05$) decreased in group III ochratoxicated birds (39.68±2.63) compared to negative control (I) (53.35±2.86) and healthy birds treated with Toxiroak (56.06±2.78). These findings are in confirmation with those reported by Huff *et al.* (1992). Significant dose dependent decrease in body weight was also observed in chicken fed 1 ppm ochratoxin (Stoev *et al.*, 2000). Randall and Bird (1979) also reported decrease in body weight due to decreased feed consumption. However, ochratoxicated birds treated with Toxiroak exhibited higher weight gain, better FCR and feed consumption when compared to group III birds. Treatment with Toxiroak could significantly protect the effect of toxins on body mass and feed efficiency. Results in present study are also in concomitance with those reported by Stoev *et al.* (2004) and Sakhare *et al.* (2007).

Clinical signs and laying performance: The mycotoxicated birds showed ruffled feathers, dullness, depression, paleness of comb and wattle and occasional diarrhoea. Observations are in concomitance with those reported by Godbole (1998). However, no appreciable signs of ochratoxicosis were observed in birds of group IV, which indicate diminution of ochratoxin toxicity by addition of toxin binder product. Average egg production (per day/group) was calculated at weekly intervals. A significant ($p<0.05$) decrease in mean egg production (per day/group) was observed group III toxicated (9.7±0.41) birds as compared to control groups I (12.38±0.37) and II (12.26±0.36), respectively (Table 2). In contrast, birds fed Toxiroak showed significant ($p<0.05$) improvement in egg production (10.01±0.33) than group III. Ghosh *et al.* (1990) also reported drop in egg production by 5-10% and debilitation in mycotoxicated layers. Dose dependent drop in egg production in layers was also observed by Prior *et al.* (1981). It can be inferenced that herbal ingredients of Toxiroak act against ochratoxin in feed through its adsorption and thus reducing bioavailability in gastro intestinal tract.

Haematological study: Mean haematological values, recorded on day 0, 30th and 60th of experimental study is summarized in Table 3. A significant ($p<0.01$) reduction in value of Haemoglobin (Hb) was observed in mycotoxicated control group III (8.13±0.16) followed by aflatoxicated birds treated with toxiroak (group IV)

Table 1: Mean body weight gain, feed consumption and FCR of birds receiving Ochratoxin A plus toxin binder (n = 20)

Groups	I	II	III	IV
Body weight gain (g)	53.35±2.86[a]	56.06±2.78[a]	39.68±2.63[*]	38.98±2.46[*]
Feed consumption (g)	2203.98±57.56[a]	2208.63±59.58[a]	1980.81±82.44[b**]	2007.65±72.65[*]
FCR	30.98	29.54	37.43	38.62

Superscript may read row wise for comparision of means. Similar superscripts showing means do not differ significantly. *Significantly at (p<0.05) and **Significant at (p<0.01)

Table 2: Average (Mean±SE) egg production (per day per group) of laying hens receiving Ochratoxin A plus toxin binder (n = 20)

	Groups			
Weeks	I	II	III	IV
1	10.42±1.28[a]	10.14±1.27	8.42±0.67[a]	8.57±1.28[a]
2	10.85±0.98[a]	10.57±0.81[a]	8.42±1.06[a]	10.28±0.33[a]
3	11.85±0.87[a]	11.28±0.81[a]	11.00±1.21[a]	11.57±0.85[a]
4	14.0±1.78[a]	12.42±1.23[a]	13.71±1.62[a]	13.14±0.94[a]
5	14.14±0.65[a]	13.85±0.79[a]	11.42±0.92[b**]	11.85±1.13[b**]
6	15.28±1.28[a]	15.00±0.63[a]	9.00±1.34[b**]	7.71±0.71[b**]
7	10.57±0.76[ac]	11.71±1.76[b]	9.85±1.57[bd]	8.85±0.60[d]
8	12.42±1.28[a]	13.14±1.4[bd]	7.14±0.79[a]	9.28±0.88[dc]
9	11.50±0.77[a]	12.25±1.43[a]	7.25±1.47[a]	8.00±1.31[b**]
Mean	12.38±0.37[ac]	12.26±0.36[a]	9.70±0.41[a]	10.01±0.33[b**]

Superscript may read row wise for comparision of means. Similar superscripts showing means do not differ significantly. *Significantly at (p<0.05) and ** Significant at (p< 0.01)

(8.25±0.17) as compared to group I (8.96±0.11) and group II (8.92±0.09). A slight but non-significant decrease in the mean PCV values of four groups was recorded, however values were lowest for ochratoxicated group III followed by group IV. Similar trend was observed for mean TLC and DLC values, a significant (p<0.01) decrease in total TLC and DLC count was recorded in group III followed by IV as compared to control group II and I. The results of decline in TEC and PCV values are in congruence with those reported by Doerr and Huff (1980); Aved *et al.* (1991) and Mohiuddin *et al.* (1993). Stoev *et al.* (2000) also reported leukocytosis in chicks maintained on dietary ochratoxin A. The present findings are also in accordance to Sakhare *et al.* (2007). Results of the present study indicate that the level of ochratoxin used in the present study, might not have induced bone marrow toxicity. The reduction in haemoglobin concentration observed during mycotoxicosis could be due to reduced protein synthesis, as observed in the present study (Table 4). Supplementation of Toxiroak® was seen to resist the change induced by mycotoxins on the studied haematological parameters.

Biochemical study: A significant (p<0.01) decrease in Total Serum Protein (TSP) and albumin in ochratoxicated group III and IV were recorded in comparision to group I and II (Table 4). Manning and Wyatt (1984), Ramadevi *et al.* (2000) and Stoev *et al.* (2000) reported decreased serum proteins during induced ochratoxicosis in broilers. Doerr and Huff (1980) and Huff *et al.* (1992) also reported reduction in serum total protein due to ochratoxin contamination in chicks. Reduction in serum total protein and serum albumin induced by mycotoxicosis could be due to pathological changes in liver, as was observed in the present study. In this experiment, higher total serum protein and serum albumin values in Toxiroak®-treated groups suggests the restorative role of preparation. A significant (p<0.01) rise in liver enzymes AST and ALT (IU/L) was observed in group III (195.76±2.82 and 8.31±0.54), However, supplementation of toxiroak lead to normalization of liver enzymes in group IV (186.76±2.23 and 7.78±0.54), well comparable to control groups I (164.23±2.34 and 5.380.25) and group II (163.41±2.37 and 5.45±0.27), (Table 4). Elevation in values of liver marker enzymes (ALT and AST) has been reported at various levels of aflatoxins by many scientists (Borisava *et al.*, 1987 and Raina *et al.*, 1991). Supplementation of Toxiroak significantly prevented a rise in values of liver enzymes. Serum cholesterol level was significantly (p<0.01) decreased in birds of group IV in comparision to group III, II and I (Table 4). However, birds administered ochratoxin and toxiroak exhibited significant improvement in serum cholesterol than group III birds. The findings of present study are in concomitance with those of Johri and Beura (2000) with Avsorb+ and Jindal *et al.* (1993) with HSCAS (0.5%). Reduction in serum cholesterol level during induced mycotoxicosis reflects impaired liver metabolism, leading to reduced synthesis of cholesterol, as was also evident in the present study. The significant improvement in serum cholesterol level of mycotoxicated broilers supplemented with Toxiroak® is indicative of its protective role.

Immune response: A significant (p<0.01) decrease in HA titre in birds of ochratoxicated group III (3.73±0.22) was observed as compared to control group I (5.0±0.29) and II (5.2±0.36). However, group IV birds supplemented with Toxiroak recorded slightly higher HA titre (3.93±0.30) than group III birds (Table 5). The

Table 3: Average (Mean ± SE) Haematological findings (different intervals) of laying hens receiving Ochratoxin A plus toxin binder (n = 5)

		Days			
Parameters	Groups	0	30	60	Mean
Hb (g%)	I	9.18±0.86^{a}	8.93±0.55^{a}	8.70±0.79^{ac}	8.96±0.11^{a}
	II	9.10±0.62^{a}	9.0±0.70^{a}	8.74±0.76^{a}	8.92±0.09^{a}
	III	9.10±0.82^{a}	8.10±0.78^{b*}	7.73±0.81^{b}	8.13±0.16^{b**}
	IV	9.08±0.74^{a}	8.22±0.80^{bc}	7.84±0.91^{d}	8.25±0.17^{bc**}
PCV (%)	I	30.8±1.35^{a}	31.0±1.45^{a}	30.4±1.23^{a}	30.68±0.38^{a}
	II	31.0±0.83^{a}	31.2±1.38^{a}	30.6±1.34^{a}	30.92±0.35^{a}
	III	31.4±1.07^{a}	28.8±1.14^{b*}	27.4±1.06^{b**}	28.92±0.42^{b**}
	IV	30.8±0.80^{a}	28.8±1.21^{b*}	27.4±1.29^{b}	29.00±0.42^{b**}
TEC (million/µl)	I	2.59±0.05^{a}	2.65±0.04^{a}	2.70±0.07^{a}	2.70±0.03^{a}
	II	2.59±0.06^{a}	2.63±0.02^{a}	2.71±0.05^{a}	2.68±0.03^{a}
	III	2.62±0.04^{a}	2.48±0.03^{b**}	2.44±0.02^{b**}	2.50±0.02^{b**}
	IV	2.61±0.07^{a}	2.51±0.04^{bc*}	2.50±0.02^{b*}	2.53±0.01^{b**}
TLC (Thousand/µl)	I	18.51±0.17^{a}	18.37±0.28^{a}	16.02±0.80^{a}	17.62±0.26^{a}
	II	18.59±0.18^{a}	18.20±0.43^{a}	16.01±0.63a	17.62±0.25^{a}
	III	18.84±0.18^{a}	16.13±0.30^{b**}	13.13±0.24^{b**}	16.20±0.41^{b**}
	IV	18.73±0.19^{a}	16.59±0.24^{b**}	13.56±0.25^{b**}	16.38±0.38^{b**}

Superscript may read row wise for comparision of means. Similar superscripts showing means do not differ significantly. *Significantly at (p<0.05) and **Significant at (p<0.01)

Table 4: Average (Mean±SE) serum biochemical findings (different day intervals) of laying hens receiving Ochratoxin A plus toxin binder (n = 5)

		Days			
Parameters	Groups	0	30	60	Mean
TSP (g/dl)	I	5.91±0.20^{a}	5.71±0.46^{a}	6.32±0.35^{a}	5.91±0.14^{a}
	II	5.99±0.37^{a}	5.74±0.10^{a}	6.09±0.48^{a}	5.92±0.15^{a}
	III	5.62±0.36^{a}	4.67±0.39^{b*}	4.10±0.37^{b*}	4.74±0.22^{b**}
	IV	5.31±0.52^{a}	4.41±0.13^{b}	5.11±0.28^{b}	4.85±0.15^{b**}
Albumin (g/dl)	I	2.58±0.9^{a}	2.53±0.13^{a}	2.57±0.12^{a}	2.53±0.05^{a}
	II	2.59±0.19^{a}	2.58±0.18^{a}	2.51±0.04^{a}	2.53±0.05^{a}
	III	2.57±0.12^{a}	1.95±0.13^{b*}	1.70±0.19^{b*}	2.14±0.09^{b**}
	IV	2.51±0.20^{a}	2.0±0.18^{b}	1.63±0.13^{ab}	2.17±0.10^{b**}
AST (IU/L)	I	174.0±2.91^{a}	155.7±4.37^{a}	153.2±3.58^{a}	164.23±2.34^{a}
	II	177.6±2.69^{a}	151.2±2.52^{a}	152.4±2.65^{a}	163.41±2.37^{a}
	III	178.8±3.38^{a}	201.0±4.00^{b**}	189.0±1.87^{b**}	195.36±2.82^{b**}
	IV	176.4±5.31^{a}	195.4±3.26^{b}	177.0±2.54^{c}	186.76±2.23^{d**}
ALT (IU/L)	I	5.20±0.36^{a}	4.11±0.32^{a}	4.47±0.42^{a}	5.38±0.25^{a}
	II	5.15±0.32^{a}	4.06±0.39^{a}	4.67±0.20^{a}	5.45±0.27^{ac}
	III	5.12±0.44^{a}	6.68±0.32^{b}	7.60±0.64^{b**}	8.31±0.54^{b**}
	IV	5.80±0.20^{a}	7.09±0.28^{b}	6.54±0.92^{bc}	7.78±0.45^{b**}
Cholesterol (mg/dl)	I	138.4±1.91^{a}	123.80±1.74^{a}	134.34±3.71^{a}	133.90±1.77^{a}
	II	135.00±3.94^{a}	126.40±2.90^{a}	135.71±2.51^{a}	133..0±1.62^{a}
	III	138.12±3.36^{a}	96.50±2.21^{b**}	99.20±4.24^{b**}	106.32±3.54^{b**}
	IV	137.4±3.53^{a}	113.60±3.28^{c}	98.94±2.96^{b}	113.06±2.99^{d**}

Superscript may read row wise for comparision of means. Similar superscripts showing means do not differ significantly. *Significantly at (p<0.05) and **Significant at (p<0.01)

findings are in confirmation with those of Singh *et al.* (1990), Stoev *et al.* (2000) and Santin *et al.* (2002) who observed reduced immune response of chicks during ochratoxicosis. Campbell *et al.* (1983) observed reduced immune response of chicks during co-mycotoxicosis. Kalorey (1993) and Kurkure *et al.* (2000) also reported reduced immune response of chicks during mycotoxicosis, which can be accounted for reduced protein and globulin synthesis, impaired processing of antigen due to impaired phagocytosis during aflatoxicosis in poultry. There was partial protection of mycotoxin induced humoral immune-toxicity by supplementation of Toxiroak to chicks. Cell mediated immune response in birds was assessed by chemical contact sensitization with DFNB. A significant increase in mean skin thickness was observed in the birds of control groups I and II at 24 and 48 h post-challenge with DFNB as compared to birds of group III and IV (Table 6). Overall immunological investigations revealed a significant suppression in humoral and cell mediated immune response in mycotoxicated birds. However, administration of herbomineral toxin binder exhibited significant improvement owing to the constituents viz. sorbents like Hydrated Sodium Calcium Alumino-silicate (HSCAS) and protective herbs like *Azadirachta indica*, *Allium sativum*, *Solanum nigrum*, *Andrographis*

Table 5: Mean HA titre at different time intervals in layers receiving Ochratoxin A plus toxin binder (n = 5)

	Days			
Groups	5	10	15	Average
I	4.4±0.50^{a}	6.0±0.31^{a}	4.6±0.40^{a}	5.0±0.29^{a}
II	4.8±0.58^{a}	6.0±0.71^{a}	4.8±0.58^{a}	5.2±0.36^{a}
III	4.0±0.50^{a}	3.0±0.31^{b**}	3.2±0.37^{bc*}	3.73±0.22^{b**}
IV	4.2±0.83^{a}	5.0±0.44^{a}	3.6±0.64^{ab}	3.93±0.30^{b**}

Superscript may read row wise for comparison of means. Similar superscripts showing means do not differ significantly. *Significant at (p<0.05) and **Significant at (p<0.01)

Table 6: DNFB response (mean skin thickness in mm) of birds exposed to Ochratoxin A plus toxin binder (left side served as vehicle control and right side treated with DNFB (n = 5)

			After Sensitization	
Groups	Abdomen side	Before Sensitization	24 h	48 h
I	Left	0.98±0.08^{A}	0.99±0.06^{A}	0.91±0.03^{A}
	Right	0.93±0.06^{a}	4.47±0.18^{a}	4.14±0.29^{a}
II	Left	0.86±0.02^{A}	0.90±0.02^{A}	0.88±0.04^{A}
	Right	0.87±0.02^{a}	4.56±0.14^{a}	4.33±0.23^{a}
III	Left	0.81±0.05^{A}	0.87±0.04^{A}	0.85±0.04^{a}
	Right	0.96±0.03^{a}	3.17±0.26^{b**}	3.12±0.24^{b*}
IV	Left	0.85±0.05^{A}	0.92±0.05^{A}	0.90±0.06^{A}
	Right	0.91±0.07^{a}	3.52±0.28^{b*}	3.05±0.14^{b*}

Superscript may read row wise for comparison of means. Similar superscripts showing means do not differ significantly. *Significant at (p<0.05) and **Significant at (p<0.01)

paniculata etc. and ameliorated detrimental effect of ochratoxin A on immune system in layers.

Conclusion: Ochratoxin A (1 ppm) adversely affects body weight gain, feed consumption, laying performance of hens besides haematobiochemical disturbances and severe immunosupression. However, supplementation of herbomineral toxin binder feed supplement Toxiroak® has provided a moderate amelioration in Ochratoxin toxicity.

ACKNOWLEDGEMENT

Authors are thankful to Dean, College of Veterinary and Animal Sciences, Anjora, Durg, India for providing research facilities and to Ayurvet Limited, Baddi, India for facilitating samples of Toxiroak for the experimental study.

REFERENCES

Abdel-Wahhab, M.A., S.A. Nada and F.A. Khalil, 2002. Physiological and toxicological responses in rats fed aflatoxin-contaminated diet with or without sorbents materials. Anim. Feed. Sci. Technol., 97: 209-217.

Aved, I.A.M., R. Dafella, A.F. Yogi and S.E.I. Adam, 1991. Effect of ochratoxin A on Lohaman type Chicks. Vet. Human Toxicol., 33: 357-360.

Borisava, L., M. Duparinova, M. Aleksondrov, T. Tacheva, A. Dzhurov, D. Tikhova, D. Chatinsk and A. Tsuetkov, 1987. Experimental reduction of aflatoxicosis in broilers. Veterinarnomeditisinsxi Nauki, 24: 69-75.

Campbell, M.L., Jr., J.D. May, W.E. Huff and J.A. Doerr, 1983. Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. Poult. Sci., 62: 2138-2144.

Chang, C.F., W.E. Huff and P.B. Hamilton, 1979. A Leucocytopenia induced in chickens by dietary ochratoxin A. Poult. Sci., 58: 555-558.

Doerr, J.A. and R.B. Huff, 1980. Interactive effects of aflatoxin and ochratoxin A on some blood constituents in broiler chickens. Poult. Sci., 59: 1600-1602.

Dwivedi, P. and R.B. Burns, 1984. Pathology of ochratoxicosis A in young broiler chicks. Res. Vet. Sci., 36: 92-103.

Elaroussi, M.A., F.R. Mohamed, E.M. El Barkouky, A.M. Atta, A.M. Abdou and M.H. Hatab, 2006. Experimental ochratoxicosis in broiler chickens. Avian Pathol., 35: 263-269.

Ghosh, R.C., H.V.S. Chauhan and S. Roy, 1990. Immunosupression in broilers under experiemental aflatoxicosis. Br. Vet. J., 146: 457.

Gibson, R.M., C.A. Bailey, L.F. Kubena, W.E. Huff and R.B. Harvey, 1990. Impact of L-phenylalanine supplementation on the performance of three-week-old broilers fed diets containing ochratoxin A. 1. Effects on body weight, feed conversion, relative organ weight, and mortality. Poult. Sci., 69:414-419.

Godbole, S.M., 1998. Immunomodulation by Growell during experimental aflatoxicosis in chicks. M.VSc thesis submitted to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.).

Huff, W.E., L.F. Kubena, R.B. Harvey and T.D. Phillip, 1992. Efficacy of hydrated sodium calcium aluminosillicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poult. Sci., 71: 64-69.

Huff, W.E., L.F. Kubena and R.B. Harvey, 1988. Progression of ochratoxicosis in broiler chickens. Poult. Sci., 67: 1139-1146.

Jindal, N., S.K. Mahipal and N.K. Mahajan, 1993. Occurance of aflatoxin B1 toxicity in broilers by dietary supplementation of lysine, methionine and choline chloride. In. J. Anim. Sci., 63: 71-73.

Johri, T.S. and C.K. Beura, 2000. Efficacy of Avsorb+ feed supplement in alleviating adverse effects of Aflatoxins in broilers. CART Contract Research Report. Poult. Sci. India, 4: 2-4.

Kalorey, D.R., 1993. Effect of Aflatoxin on humoral immune system of chicks. Dissertation Faculty of Veterinary Science. Dr. Punjabrao Krishi Vidyapeeth, Akola, India.

Kalorey, D.R., N.A. Shete, V.C. Ingle, N.V. Kurkure and S.D. Harne, 2000. *In vitro* evaluation of antifungal and antitoxic activity of herbomineral premix. Proceedings of XXI Worlds Poultry Congress, Montreal, Canada.

Kubena, L.F., R.B. Harvey, T.D. Phillip and B.A. Clement, 1993. Effect of hydrated sodium calcium aluminosilicate on aflatoxicosis in broiler chicks. Poult. Sci., 72: 651-657.

Kumar, A., N. Jindal, C.L. Shukla, R.K. Asrani, D.R. Ledoux and G.E. Rottinghaus, 2004. Pathological changes in broiler chickens fed ochratoxin A and inoculated with Escherichia coli. Avian Pathol., 33: 413-417.

Kurkure, N.V., S.P. Pawar, S.M. Kognole, A.G. Bhandarker, A.G. Ganorker and D.R. Kalorey, 2000. Ameliorative effect of turmeric (Curcuma longa) in induced aflatoxicosis in cockrels. Ind. J. Vet. Pathol., 24: 26-28.

Manning, R.O. and R.D. Wyatt, 1984. Toxicity of Aspergillus ochraceus contaminated wheat and different chemical forms of ochratoxin A in broiler chicks. Poult. Sci., 63: 458-465.

Mohiuddin, S.M., S.M.A. Warasi and M.V. Reddy, 1993. Haematological and biochemical changes in experimental ochratoxicosis in broiler chicken. Ind. Vet. J., 70: 613-617.

NRC, 1998. Nutrient requirements of swine. 10th rev. ed. National Academy Press, Washington D.C. 189pp.

O'Brien, E. and D.R. Dietrich, 2005. Ochratoxin A: the continuing enigma. Critical Reviews in Toxicol., 35: 33-60.

Page, R.K., G. Stewart, R. Wyatt, P. Bush, O.J. Fletcher, and J. Brown, 1980. Influence of low levels of ochratoxin A on egg production, egg-shell stains, and serum uric acid levels in Leghorn- type hens. Avian Dis., 24: 777-780.

Politis, I., K. Fegeros, S. Nitsch, G. Schatzmayr and D. Kantas, 2005. Use of Trichosporon mycotoxinivorans to suppress the effects of ochratoxicosis on the immune system of broiler chicks. Br. Poult. Sci., 46: 58-65.

Prior, M.G., C.S. Sisodia and J.B. O'Neil, 1981. Effects of ochratoxin A on egg production, body weight and feed intake in white leghorn hens. Poult. Sci., 6: 1145-8.

Raina, J.S., K.S. Roy and B. Singh, 1991. Biochemical and histochemical studies in experiemental mycotoxicosis in chicks. Ind. J. Anim. Sci., 62: 1276-1281.

Raju, M.L.V.N. and G. Devegowda, 2000. Influence of esterified glucamannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to indvidual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). British Poult. Sci., 41: 640-650.

Raju, M.V. and G. Devegowda, 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). Br. Poult. Sci., 41: 640-650.

Ramadevi, N.R., R. Gopalnaidu and P. Ravikumar, 2000. An assessment of the protective effect of bentonite on ochratoxicosis in broiler with reference to certain haematological profile. Ind. Vet. J., 77: 303-306.

Randall, G.M. and F.H. Bird, 1979. The effect of exercise on the toxicity of aflatoxin B1 in chickens. Poult. Sci., 58: 1284-1288.

Sakhare, P.S., S.D. Harne, D.R. Kalorey, S.R. Warke, A.G. Bhandarker and N.V. Kurkure, 2007. Effect of toxiroak polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicosis in broilers. Veterinary Archiv, 77: 129-146.

Santin, E., A.C. Paulillo, P.C. Maiorka, A.C. Alessi, E. Krabbe and A. Maiorka, 2002. The effects of ochratoxin/aluminosilicate interaction on the tissues and humoral immune response of broilers. Avian Pathol., 31: 73-79.

Scott, P.M., 1978. Mycotoxins in feed and ingredient and their origin. J. Food Protection, 41: 385 -389.

Singh, G.S.P., H.V.S. Chauhan, G.H. Jha and K.K. Singh, 1990. Immunosuppression due to chronic ochratoxicosis in broiler chicks. J. Comp. Pathol., 103: 399-410.

Snedecor, G.W. and W.G. Cochran, 1994. Statistical Methods. 8th Edn. Iowa State University Press, Ames, Iowa.

Stoev, S.D., D. Djuvinov, T. Mirtcheva, D. Pavlov and P. Mantle, 2002. Studies on some feed additives giving partial protection against ochratoxin A toxicity in chicks. Toxicology Letters, 135: 33-50.

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

Stoev, S.D., M. Stefanov, S. Denev, B. Radic, A.M. Domijan and M. Peraica, 2004. Experimental mycotoxicosis in chickens induced by ochratoxin A and penicillic acid and intervention with natural plant extracts. Vet. Res. Comm., 28: 727-746.

Stoev, S.D., G. Aguelov, I. Ivanov and D. Pavlov, 2002. Influence of OA and an extract of artichoke on the vaccinal immunity and health in broiler chicks. Exp. Toxicol. Pathol., 52: 43-55.

Tapia, M.O., 1985. A quantitative TLC method for analysis of aflatoxins, OA, zearalenone, T2 toxin and other mycotoxins in food stuff. Revista Argentine de Microbiologia, 17: 183-186.

Thaxton, J.P., H.T. Tung and P.B. Hamilton, 1974. Immunosupression in chickens by aflatoxin. Poult. Sci., 53: 721-725

Thompson, C.D., S. Whittingham, L.R. Mackay, S.K. Khoo, B.H. Toh and R.J. Stagg, 1975. Quantitation of cell mediated immunity: response to dinitrochlorobenzene and ubiquitous antigen. Can. Med. J. Assoc. J., 112: 1078-1081.