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Effect of Toxiroak® polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers

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ABSTRACT

An experiment was conducted to study the protective role of polyherbal feed supplement (Toxiroak®) during induced mycotoxicosis in broilers. A total of 240 Vencobb one-day-old broilers were divided into eight equal groups. Group A served as control and was given plain feed. Groups B, D, F and H were given Toxiroak® at 0.75 g/kg of feed. Groups C, D, G and H were given dietary aflatoxin B_1 at 0.2 ppm, and Groups E, F, G and H were given ochratoxin A at 0.2 ppm in feed to study the effect of Toxiroak® on individual aflatoxicosis, ochratoxicosis and combined mycotoxicosis of broilers. Chicks were given their respective feeds from the 1st day to 6th week of age and were vaccinated at 7th and 28th days of age with a Lasota strain of Newcastle disease virus. There was a significant effect of mycotoxins, individually and in combination, on body mass of broilers. Toxiroak® protected the effect of individual mycotoxins on body mass. Feed conversion ration was highest in Group B birds, followed by Groups A, F, D, H, C, E and G. Significant restoration of haemoglobin and total leukocyte count values in broilers due to feeding of Toxiroak® in co-mycotoxicated and aflatoxins-fed groups, respectively, was observed. There was no significant effect of mycotoxin treatment on packed cell volume and total erythrocyte count in broilers. Due to single and combined mycotoxicosis there was a reduction in serum total protein, cholesterol and triglyceride and a rise in creatinine and uric acid levels. Supplementation of diets with Toxiroak® reduced the changes induced due to mycotoxins. There was a significant increase in the percentage organ weight of liver, and a reduction in that of spleen, bursa of Fabricius and thymus of broilers fed mycotoxins. Protection of changes in the percentage of organ mass of these organs by supplementation of Toxiroak® was recorded only in respect of bursa of Fabricius. The observed impaired immune response and histopathological changes in liver, kidney, spleen, bursa of Fabricius and thymus of broilers given mycotoxins was protected by Toxiroak® supplementation.

Key words: aflatoxicosis, broilers, herbs, ochratoxicosis

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Introduction

Aflatoxins are toxic secondary metabolites produced by fungi, namely *Aspergillus* spp. and *Penicillium* spp. High levels of aflatoxins have been recorded in ingredients of poultry feed soybean, sunflower, polished rice, cotton seed, etc. (JAND et al., 1995). The adverse effect of aflatoxins depends on age, species, nutritional status of birds as well as dose and period in which it is consumed. Chronic aflatoxicosis due to prolonged intake of low levels of aflatoxins retards growth, reduces feed conversion ratio and increases susceptibility of chicks to infectious diseases (BOONCHUVIT and HAMILTON, 1975; GIAMBORNE et al., 1978). Increased susceptibility of aflatoxicated chicks to infectious diseases indicates impaired immune responses. Aflatoxicosis leads to immunosuppression, characterised by decreased immune response (BAKSHI et al., 2000) and breakdown of vaccinal immunity (PANISUP et al., 1982). Similar effects of ochratoxin A with target organ kidney were summarised earlier by MARQUARDT and FROHLICH (1992).

Deleterious effect of aflatoxin could be overcome, or at least diminished, by adsorbents in rats (ABDEL-WAHHAB et al., 2002). Chemical adsorbents (KUBENA et al., 1993), Levamisole hydrochloride (KALOREY, 1993), glucomannan (RAJU and DEVEGOWDA, 2000) as well as Growell (GODBOLE et al., 2001) have been attempted with varying degrees of success to reduce toxicity and impairment of immune response during aflatoxicosis in birds. Use of adsorbents is of limited value in controlling ochratoxicosis in livestock (MARQUARDT and FROHLICH, 1992; SANTIN et al., 2002). STOEV et al. (2000) and KURKURE et al. (2000) recently reported that 5 percent aqueous extract of artichoke and *Curcuma longa* (Turmeric) powder at 0.5 g/kg feed reduces the toxic effect of ochratoxin A and aflatoxin B_1, respectively, in chicks. The protective role of herbal extracts during induced individual or combined mycotoxicoses is not well studied.

In the present study the protective role of polyherbal and mineral feed supplement Toxiroak® is studied during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. *In vitro* antitoxic and antifungal activity of the same was confirmed earlier (KALOREY et al., 2000)

Materials and methods

Toxiroak®, a polyherbal preparation prepared from extracts of *Allium sativum, Azarirachita indica, Solanum nigram, Emblica officinalis, Curcuma longa* and hydrated aluminosilicate, was procured from M/S Ayurvet Ltd, Sahibabad, India, for the present study.

Production of aflatoxin B_1. A known aflatoxin B_1-producing strain of *Aspergillus parasiticus* (NRRL 3240) maintained on Sabouraud's dextrose agar 2% (w/v) and aflatoxin B_1 standard of 1 µg/mL, available at the Department of Microbiology, Nagpur Veterinary

College, Nagpur, was used for production of aflatoxin and quantification of aflatoxin $B_{1,}$ respectively. The fungal spores were washed from the surface of agar slant with sterile Sabouraud's dextrose broth (SDB) containing an equal amount of 0.1% Tween® 80. The spore suspension was filtered through sterile muslin cloth and adjusted with SDB to a concentration of 1×10^9 spores/mL, and was used as inoculum immediately. Two hundred and fifty g broken rice was sterilized in a 1 L conical flask, and after cooling 25 mL of SDB was added to moisten the rice. One mL of the above mentioned inoculum was then added. It was then thoroughly mixed to ensure uniform distribution of spores, and incubated at 28 ± 1 ^{O}C for 15 days. The flasks were shaken twice a day to break up clumps. After incubation, flasks were autoclaved at 10 Lbs for 5 m. The aflatoxin B_1 was semiquantified according to TAPIA (1985) using thin layer chromatography.

Production of ochratoxin A. Ochratoxin A (OA) was produced on rice as per the procedure described above, using a known ochratoxin A-producing strain of *Aspergillus ocheraceus* (NRRL 3174) available at the Department of Microbiology, Nagpur Veterinary College, Nagpur. OA standard (3 µg/mL) was used for quantification of OA, according to TAPIA (1985).

Table 1. Details of experimental groups

Group	Description of group	Feed additionally supplied with		
		Aflatoxin B1 at the rate of 0.2 ppm	Ochratoxin A at the rate of 0.2 ppm	Toxiroak treatment at the rate of 0.75 g/kg of feed
A	Control	-	-	-
B	Toxiroak Control	-	-	+
C	Aflatoxin Control	+	-	-
D	Aflatoxin + treatment	+	-	+
E	Ochratoxin Control	-	+	-
F	Ochratoxin + treatment	-	+	+
G	Aflatoxin + Ochratoxin control	+	+	-
H	Aflatoxin + Ochratoxin + treatment	+	+	+

- Not supplied, + supplied

Feed. Broilers were given feed and water *ad libitum*. Broiler starter (22.60% CP, 2950 ME Energy) and Broiler finisher (21.60% CP, 2950 ME) was offered to broilers from 0-21st day and 22-42nd day of age, respectively. In order to achieve required toxin level in calculated feed, a quantity of fungus- infested rice was mixed in feed. The polyherbal preparation Toxiroak® was added to feed where required and the feed was given to birds from one-day-old to 42nd day of age.

Birds. A total of 240 Vencobb one-day-old broilers were purchased from a commercial hatchery and were divided into 8 equal groups. Group-wise treatment schedule of birds is presented in Table 1. Broilers were maintained on a deep litter system under standard management conditions from one-day-old to 42nd day of age. A separate pen was used for each group of birds. All chicks were vaccinated at the 7th and 28th day of age with the Lasota strain of Newcastle disease virus (NCDV).

Experimental observations

Growth and performance study. Ten birds from each treatment group were wing banded at one-day-old and thereafter weighed individually at weekly intervals. Also, feed offered to birds, and feed that was left uneaten, was recorded weekly in order to calculate cumulative feed conversion ratio (FCR).

Blood and serum collection. Blood was collected via jugular vein puncture at weekly intervals from 6 broilers per group for serum collection. For haematological study 1 mL blood was collected in heparinised vials at the 21st and 42nd day of age. Haematological parameters, viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC) were studied.

Biochemical observations. Serum collected on 21st and 42nd day was subjected to total protein, albumin, cholesterol, triglycerides, alkaline phosphatase, creatinine and uric acid level estimation, using commercially available kits (Span Diagnostics Ltd., India) on semi autoanalyser (Systronics, Clinical Chemistry Analyzer, 171).

Organ mass. At 21 and 42 days of age, body mass of six birds from each group was recorded, and they were then sacrificed. Liver, spleen, bursa of Fabricius and thymus were excised, blotted and weighed individually, and percentage mass of organs was calculated.

Immunological parameters. Humoral immune response of birds against NCDV post-vaccination was assessed by estimating haemagglutination inhibition (HI) titer at weekly intervals as per procedure recommended by ANONYM. (2000). Cell-mediated immune response was studied using contact sensitivity test, a delayed type of hypersensitivity reaction to 2-4-dinitrochlorobenzene, as recommended by TIWARI and GOEL (1982). Increase in skin fold thickness 24 h post-challenge was used as an indicator of cell-mediated immune response.

Gross and histopathological study. At the time of organ weighing, gross changes, if any, were recorded. Representative tissues were collected in 10% formal saline from above organs and processed for histopathological studies.

Statistical analysis. Statistical analysis of the experimental data generated was carried out as per SNEDECOR and COCHRAN (1967) using Completely Randomised Design on a particular day of observation.

Results

Growth studies. Average body mass (g) of broilers recorded at different age intervals in various treatment groups is presented in Table 2. There was no statistically significant effect of mycotoxins individually or in combination on body mass of broilers up to 4th week of age. Subsequently, there was a significant reduction in body mass in mycotoxicated groups as compared to control group. Treatment with Toxiroak® could significantly protect the effect of toxins on body mass at the 6th week of age. However, in combined toxin-fed groups it was still significantly less compared to control group. Feed conversion ratio (FCR) was highest in Group B, followed by Groups A, F, D, H, C, E and G. In general, herbal feed supplement improved the FCR of broilers better than was the case in their respective control groups.

Haematological study. Average haematological values of experimental broilers observed at the 21st and 42nd days of age are presented in Table 3. At the 21st day of age there was a significant ($P<0.01$) reduction in haemoglobin values of broilers of co-mycotoxicated Group G compared to control Group A. At the 42nd day of age there was a significant ($P<0.05$) reduction in haemoglobin values of broilers in the ochratoxin A-fed Group E and co-mycotoxicated Group G, as opposed to control Group A. Significant improvement in haemoglobin values was observed in broilers fed polyherbal preparation together with co-mycotoxin than the respective toxicated only groups at the 21st day of age. Total leukocyte count was significantly higher only in aflatoxins-fed Group C compared to control. The polyherbal preparation could protect this effect significantly. Other haematological parameters, such as packed cell volume and total erythrocyte count, were not significantly altered due to various mycotoxins and the polyherbal preparation used in the present study.

Biochemical observations. Average serum biochemical values of experimental broilers observed at the 21st and 42nd days of age are presented in Table 4. Significant ($P<0.01$) reduction in serum total protein was observed in aflatoxins-, ochratoxin- and co-mycotoxin-fed broilers compared to control at the 21st day of age. Among the mycotoxicated groups, feeding of Toxiroak® to broilers restored serum total protein values on a par with control in aflatoxin and ochratoxicated groups only. In both periods of observation there was no significant effect on serum albumin concentration in mycotoxin-fed groups compared to control. Serum cholesterol and triglyceride levels were reduced significantly ($P<0.01$) in both periods of observation in mycotoxin-fed Groups C, E and G. Supplementation of Toxiroak® to all mycotoxin-fed broilers could not significantly improve serum cholesterol and triglyceride levels. Serum creatinine level of mycotoxin-fed Groups C, E and G were significantly higher than control groups in both periods of observation. Supplementation of Toxiroak® significantly prevented a rise in values of

Table 2. Average weekly body mass (g) of broilers from various treatment groups.

Age in weeks	A	B	C	D	E	F	G	H	Sig.
1	93.20 ± 2.23	90.13 ± 1.51	94.00 ± 2.83	87.73 ± 3.25	91.33 ± 3.35	89.06 ± 1.98	93.73 ± 1.35	92.86 ± 2.35	NS
2	225.66 ± 8.35	219.66 ± 9.07	206.33 ± 7.49	206.66 ± 10.14	220.00 ± 11.07	212.66 ± 8.92	209.66 ± 5.88	211.00 ± 7.06	NS
3	396.66 ± 19.72	400.66 ± 13.58	385.00 ± 14.66	407.00 ± 22.08	393.66 ± 23.86	409.66 ± 20.38	392.33 ± 13.65	414.33 ± 16.95	NS
4	657.33 ± 11.93	632.66 ± 11.73	589.33 ± 15.85	656.00 ± 14.51	678.00 ± 14.72	650.66 ± 11.11	620.00 ± 24.00	634.00 ± 20.34	NS
5	978.00^{C} ± 21.86	970.66^{C} ± 21.82	813.33^{a} ± 27.34	900.66^{b} ± 24.94	849.26^{ab} ± 28.72	916.66^{bc} ± 22.99	848.66^{ab} ± 21.87	913.66^{bc} ± 18.09	*
6	1365.00^{d} ± 22.44	1372.66^{d} ± 34.68	1185.33^{ab} ± 33.16	1320.00^{cd} ± 32.74	1171.66^{ab} ± 35.77	1357.33^{d} ±37.29	1150.00^{a} ± 27.58	1246.00^{bc} ± 31.38	*
Feed conv. ratio	2.66	2.55	3.12	2.85	3.13	2.84	3.25	2.93	

NS = Not significant, * ($P<0.05$), **($P<0.01$). Means carrying same superscripts with in row do not differ significantly.

Table 3. Mean of haematological parameters in chicks from various treatment groups.

Age in days	A	B	C	D	E	F	G	H	
Haemoglobin (g/dL)									
21st day	9.40 ^{bc} ± 0.50	10.15 ^{bc} ± 0.32	9.36 ^{bc} ± 0.44	9.47 ^{c} ± 0.37	8.66 ^{b} ± 0.41	9.33 ^{bc} ± 0.40	7.16 ^{a} ± 0.25	8.40 ^{b} ± 0.18	**
42nd day	9.23 ^{c} ± 0.45	9.30 ^{c} ± 0.31	8.56 ^{abc} ± 0.47	8.23 ^{abc} ± 0.50	7.56 ^{a} ± 0.56	8.56 ^{abc} ±0.36	7.83 ^{ab} ± 0.31	8.76 ^{bc} ± 0.29	*
Packed cell volume (%)									
21st day	27.83 ± 2.22	27.50 ± 0.62	27.16 ± 1.87	28.50 ± 0.76	26.33 ± 1.59	31.50 ± 2.32	24.00 ± 1.67	29.66 ± 1.56	NS
42nd day	32.66 ± 1.54	31.00 ± 1.85	29.66 ± 2.20	30.83 ± 2.14	26.00 ± 1.44	31.33 ± 2.41	26.83 ± 0.8745	28.33 ± 3.03	NS
Total erythrocyte count ($\times 10^6$/cumm)									
21st day	2.21 ± 0.09	2.47 ± 0.14	2.67 ± 0.26	2.67 ± 0.07	2.90 ± 0.07	2.45 ± 0.16	1.92 ± 0.11	1.65 ± 0.08	NS
42nd day	2.49 ± 0.23	1.95 ± 0.46	2.55 ± 0.42	3.04 ± 0.35	2.86 ± 0.53	2.68 ± 0.43	2.98 ± 0.35	2.35 ± 0.26	NS
Total leucocyte count ($\times 10^3$/cumm)									
21st day	20.66 ^{ab} ± 1.33	23.33 ^{bc} ± 1.11	27.33 ^{c} ± 2.82	26.66 ^{c} ± 2.67	24.00 ^{bc} ± 2.31	19.33 ^{ab} ± 2.77	16.33 ^{a} ± 0.99	19.33 ^{ab} ± 1.23	*
42nd day	26.66 ^{a} ± 2.05	24.00 ^{a} ± 1.37	40.00 ^{b} ± 3.87	28.66 ^{a} ± 5.22	25.33 ^{a} ± 2.57	26.00 ^{a} ± 3.39	27.33 ^{a} ± 2.16	24.66 ^{a} ± 1.33	*

NS = Not significant, * ($P<0.05$), **($P<0.01$); Means carrying same superscripts with in row do not differ significantly.

Table 4. Mean of biochemical parameters in chicks from various treatments groups.

Age in days	A	B	C	D	E	F	G	H	Sign.
Serum total protein (g/dL)									
21st	4.98 c ± 0.22	4.98 c ± 0.50	2.48 a ± 0.11	4.80 c ± 0.10	3.47 b ± 0.24	4.94 c ± 0.15	2.10 a ± 0.28	2.47 a ± 0.30	**
42nd	3.81 ± 0.14	3.93 ± 0.06	3.27 ± 0.14	3.46 ± 0.30	3.32 ± 0.15	3.57 ± 1.14	3.12 ± 0.11	3.37 ± 0.22	NS
Serum albumin (g/dL)									
21st	0.98 ± 0.11	1.06 ± 0.12	0.92 ± 0.16	1.05 ± 0.18	0.93 ± 0.04	1.51 ± 0.12	1.08 ± 0.09	1.41 ± 0.07	NS
42nd	1.39 ± 0.03	1.34 ± 0.02	1.25 ± 0.13	1.32 ± 0.13	1.28 ± 0.02	1.22 ± 0.08	1.23 ± 0.13	1.31 ± 0.06	NS
Serum cholesterol (mg/dL)									
21st	143.00 b ± 15.82	145.00 b ± 11.08	105.23 a ± 1.66	123.60 ab ± 6.88	120.55 ab ± 16.77	139.98 b ± 7.50	94.20 a ± 14.00	94.22 b ± 14.00	**
42nd	142.76 d ± 7.78	140.29 d ± 7.06	73.07 ab ± 2.37	89.93 bc ± 4.52	83.92 bc ± 4.58	100.59 c ± 5.54	62.21 a ± 7.23	72.40 ab ± 6.00	**
Serum triglyceride (mg/dL)									
21st	100.81 d ± 8.42	98.04 cd ± 11.77	56.20 ab ± 8.67	82.69 bcd ± 10.89	42.98 a ± 10.15	55.99 ab ± 3.48	49.01 ab ± 7.38	64.60 bc ± 10.81	**
42nd	101.03 c ± 6.65	96.44 c ± 4.42	40.54 a ± 6.01	67.58 b ± 7.68	57.40 ab ± 4.70	62.34 ab ± 4.76	43.36 a ± 4.21	56.70 ab ± 9.13	**
Serum creatinine (mg/dL)									
21st	0.40 a ± 0.03	0.40 a ± 0.02	0.61 c ± 0.03	0.48 ab ±0.01	0.58 bc ± 0.02	0.45 a ± 0.01	0.61 c ± 0.02	0.60 c ± 0.03	**
42nd	0.48 a ± 0.04	0.45 a ± 0.06	0.50 ab ± 0.06	0.44 a ± 0.03	0.65 b ± 0.03	0.46 a ± 0.03	0.65 b ± 0.04	0.56 ab ± 0.04	**
Serum uric acid (mg/dL)									
21st	9.12 a ± 0.93	9.05 a ± 0.86	11.12 bc ± 0.81	9.84 ab ± 0.20	11.12 bc ±0. 43	9.59 ab ± 0.81	12.44 c ± 0.31	8.88 a ± 0.58	*
42nd	6.87 ab ± 1.70	6.50 a ± 0.61	9.82 c ± 0.48	6.87 a ± 0.94	8.86 b ± 0.44	6.55 b ± 0.61	10.12 c ± 0.90	8.37 abc ± 0.36	**
Serum alkaline phospatase (U/L)									
21st	273.27 ± 49.19	299.68 ± 33.72	338.98 ± 42.05	282.72 ± 41.04	379.21 ± 33.04	306.28 ± 38.92	347.67 ± 35.19	316.14 ± 18.56	NS
42nd	487.65 ± 22.51	503.59 ± 63.54	654.40 ± 51.25	562.47 ± 86.27	592.26 ± 73.16	419.27 ± 80.43	632.43 ± 80.43	488.30 ± 25.45	NS

NS = Not significant, * (P<0.05), **(P<0.01); Means carrying same superscripts with in row do not differ significantly.

serum creatinine due to mycotoxins. Serum uric acid level in broilers was found to be significantly elevated due to dietary mycotoxins, and the effect was more pronounced at the 21st day of age. Polyherbal preparation feeding inhibited the rise in serum uric acid levels significantly ($P<0.05$) at the 21st day of age. At the 42nd day of age this protective effect was observed only in aflatoxin-fed broilers, with respect to serum uric acid levels only. There was no significant effect of mycotoxins on the serum alkaline phosphatase levels of broilers.

Organ mass. The result of the effect of dietary mycotoxins on relative mass of organs is presented in Table 5. At the 42nd day of age there was a significant ($P<0.01$) increase in relative mass of liver of broilers receiving aflatoxins, both singly and in combination. Feeding of mycotoxins to broilers significantly ($P<0.01$) reduced relative mass of spleen in both periods of observation, and mass of bursa of Fabricius and thymus at the 42nd day of age. Toxiroak® neutralized this adverse effect only in the aflatoxin- and ochratoxin-fed group at the 42nd in respect of bursa of Fabricius only.

Immune responses. Result of humoral immune response as evaluated by HI titer against NCDV of broilers is presented in Table 6. It was observed that feeding of aflatoxin, ochratoxin individually or in combination, significantly reduced development of humoral immune response in broilers. Treatment with polyherbal preparation could partially protect the reduction in HI titers of mycotoxicated broilers. Dietary mycotoxin alone or in combination significantly ($P<0.01$) suppressed the cell-mediated immune response of broilers in both periods of observation compared to control (Table 7). Toxiroak®-treated birds revealed better CMI status than their respective controls, although the difference was not significant.

Gross pathology. In aflatoxin-fed Group C and co-mycotoxicated Group G, liver was enlarged, with yellowish discoloration and raised nodules. Spleen, thymus and bursa of Fabricius of all mycotoxin-fed groups appeared to be atrophied. Enlarged and pale kidneys were more pronounced in groups fed ochratoxin and aflatoxin-ochratoxin. Intensity of gross pathological changes was less in Toxiroak®- mycotoxin treated groups.

Histopathology. A section of Groups A and B revealed no histopathological changes in any of the organs in both periods of observation.

Liver. Sections of aflatoxin-fed Group C at the 21st day of age revealed degenerative changes in liver parenchyma. Most of the hepatocytes were swollen and vacuolated, indicating a moderate response of mycotoxin ingested by broilers. The area of necrosis was diffusely spread in liver parenchyma and was infiltrated with heterophils and lymphocytic aggregates. On the 42nd day of age, vacuolar degenerative changes were reduced. However, fibrous connective tissue proliferation, along with scattered infiltration of lymphocyte and heterophils, was prominent. Hepatocytes in the affected area revealed adenomatous arrangement. On the 21st day of age, a section of Group E revealed mild

Table 5. Average percent organ mass of chicks from various treatment groups

Age in days	A	B	C	D	E	F	G	H	Sign.
Liver									
21st	3.50 ± 0.23	3.80 ± 0.23	3.78 ± 0.33	3.72 ± 0.23	3.51 ± 0.17	3.19 ± 0.09	4.20 ± 0.14	3.69 ± 0.20	NS
42nd	2.90 a ± 0.17	3.12 ab ± 0.13	3.99 c ± 0.30	3.84 bc ± 0.24	3.20 ab ± 0.18	3.20 ab ± 0.12	3.77 bc ± 0.18	3.74 bc ± 0.10	*
Spleen									
21st	0.200c ± 0.036	0.200 c ± 0.029	0.140ab ±0.008	0.150bc ± 0.006	0.100a ± 0.004	0.120ab ±0.001	0.110ab ±0.012	0.110ab ±0.100	**
42nd	0.210 d ±0.012	0.170 c ±0.010	0.156bc ±0.007	0.170c ±0.010	0.130ab ±0.013	0.130 ab ±0.009	0.110a ±0.009	0.130 ab ±0.010	**
Bursa									
21st	0.260 ±0.033	0.240 0.020	0.250 ± 0.008	0.260 ± 0.011	0.250 0.022	0.270 ±0.010	0.220 ±0.030	0.260 ±0.022	NS
42nd	0.110bc ±0.012	0.130c ± 0.011	0.070a ± 0.006	0.110bc ±0.009	0.080a ±0.007	0.120c ±0.080	0.080a ±0.004	0.090ab ±0.007	**
Thymus									
21st	0.500 ± 0.021	0.590 ± 0.030	0.410 ± 0.030	0.440 ± 0.020	0.480 ± 0.036	0.420 ± 0.030	0.410 ± 0.27	0.590 ± 0.030	**
42nd	0.640b ±0.029	0.790c ±0.060	0.483a ±0.010	0.440a ±0.010	0.474a ±0.018	0.640b ±0.020	0.400a ±0.010	0.470a ±0.010	*

Table 6. Mean HI titer (log_2) against Newcastle disease virus in experimental chicks.

Age in weeks	A	B	C	D	E	F	G	H	Sign.
2	3.33 ± 1.22	3.33 ± 1.22	2.33 ± 0.42	3.00 ± 0.25	1.66 ± 0.36	2.33 ± 0.33	2.00 ± 0.25	2.33 ± 0.21	NS
3	5.16^{d} ± 0.16	5.33^{d} ± 0.21	3.50^{ab} ± 0.22	4.83^{cd} ± 0.25	3.08^{ab} ± 0.25	3.30^{ab} ± 0.61	2.83^{a} ± 0.16	4.00^{bc} ± 0.25	**
4	4.33 ± 0.21	4.33 ± 0.22	2.83 ± 0.40	4.00 ± 0.51	4.50 ± 0.42	4.60 ± 0.76	3.80 ± 0.30	3.83 ± 0.16	NS
5	5.33^{b} ± 0.42	5.33^{b} ± 0.21	4.33^{ab} ± 0.61	4.83^{ab} ± 0.16	4.50^{ab} ± 0.50	5.16^{b} ± 0.16	3.83^{a} ± 0.47	4.33^{ab} ± 0.42	**
6	4.50^{b} ± 0.22	4.66^{b} ± 0.33	3.00^{a} ± 0.51	4.00^{b} ± 0.44	2.83^{a} ± 0.30	4.16^{b} ± 0.16	2.50^{a} ± 0.42	4.16^{b} ± 0.30	**

NS = Not significant, * (P<0.05), **(P<0.01)
Means carrying same superscripts with in row do not differ significantly

Table 7. Cell mediated immune response by contact sensitivity test in chicks from various treatment groups

Age in days	A	B	C	D	E	F	G	H	Sign.
	Mean increase in skin thickness (in mm)								
30^{th}	2.30^{d} ± 0.18	2.29^{d} ± 0.19	1.79^{bc} ± 0.10	1.95^{bc} ± 0.10	1.56^{ab} ± 0.16	2.12^{cd} ± 0.05	1.19^{a} ± 0.03	1.52^{a} ± 0.15	**
45^{th}	3.59^{b} ± 0.12	3.40^{b} ± 0.40	2.91^{ab} ± 0.12	2.96^{ab} ± 0.31	2.64^{a} ± 0.54	3.05^{ab} ± 0.23	2.40^{a} ± 0.19	3.00^{ab} ± 0.12	*

NS = Not significant, * (P<0.05), **(P<0.01); Means carrying same superscripts with in row do not differ significantly.

degenerative changes and congestion in hepatocytes. On the 42nd day of age, lymphocytic and heterophilic infiltration in necrotic area was observed. On the 21st day of age, sections of liver in Group G revealed intensive granular and vacuolar degenerative changes in hepatocytes. Necrosis of liver parenchyma and areas of haemorrhages were more prominent compared to Group C. On the 42nd day of age, vacuolar degenerative changes, necrosis, increase in sinusoidal space and reduction in size of hepatocytes with a tendency to form lumen, was recorded. Proliferation of fibrous connective tissue in perilobular space was prominent. The liver section in Groups D, F and H revealed similar types of change. However, the changes were mild in degree. Marked degenerative changes in hepatocytes were observed, along with infiltration of lymphocyte and heterophils in hepatic parenchyma of Group H.

Spleen. At the 21st day of age spleen in Group C revealed necrotic areas in the germinal centre, together with sclerotic changes in central artery, and depopulation of lymphocytes. On the 42nd day of age spleenic arterioles showed hyperplasia of tunica intima and destruction of elastic fibres. A section of Groups E and G revealed changes similar to those observed in Group C on the 21st and 42nd day of age. However, there was evidence of necrotic patches in spleenic corpuscles. The spleenic section from Toxiroak®-treated Groups D, F and H also revealed changes similar to those of Toxiroak® non-treated groups. However, changes were mild in degree and necrotic patches in spleenic corpuscles were not seen in any of the sections.

Bursa of Fabricius. On the 21st day of age, sections from bursa of Groups C, E and G revealed depletion of medullary lymphocytes from bursal follicle. Among mycotoxin-treated groups, histopathological changes were more pronounced in co-mycotoxicated groups compared to single mycotoxin-fed groups. On the 42nd day of age, reduction in size of bursal follicles, and depletion and necrosis of lymphoid cells from follicle, with proliferation of fibrous connective tissue in inter-follicular space, was observed. The changes were more pronounced in Group G. Sections of bursa in Groups D and F revealed no appreciable changes at the 21st day of age. However, in Group H, depletion of lymphocytes from follicles was recorded. At the 42nd day of age mild atrophy of bursal follicle was noticed in these groups in contrast to the respective Toxiroak® untreated-mycotoxicated groups.

Thymus. Sections of Groups C, E and G showed depletion of lymphoid cells from medullary areas. A few areas of haemorrhage were also observed. The number of Hazel's corpuscles were increased in Group G compared to individual mycotoxicated Groups C and E. Toxiroak®-treated Groups D, F and H revealed no appreciable pathological changes in thymus at the 21st day of age. At the 42nd day of age, mild depletion of lymphoid cells from medullary areas was seen.

Kidney. On the 21st day of age a section of Group C showed degenerative changes in tubular epithelium, as well as condensation of nuclear material. On the 42nd day of age, some tubules showed necrosis, separation of epithelial cells from basement membrane, and areas of haemorrhage. On the 21st day of age, sections of Group E revealed swelling and degenerative changes in tubular epithelium, leading to occlusion of lumen. In some tubules, hyaline casts in lumen were prominent. At a later period of observation pathological changes in tubular epithelium were more pronounced. Additionally, there was infiltration of lymphocytes in renal parenchyma. Hyalinization of glomerular tuft, along with hyaline droplet in Bowman's capsule, was observed in this group. On the 21st day of age a section of Group G revealed necrosis of tubular epithelium, leading to extensive destruction of the tubules. Areas of necrosis were also prominent in glomerulus. On the 42nd day of age extensive destruction of tubular epithelium, with detachment of tubular cells from basement membrane, was observed. In some tubules deposition of urinary casts was seen. Toxiroak®-treated Groups D, F and H revealed mild changes, compared to respective untreated groups.

Discussion

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

Results of the present study demonstrate that dietary aflatoxin, ochratoxin, individually or in combination, affect body mass and performance of broilers. Similar observations due to feeding of aflatoxin and ochratoxin were noticed earlier by HUFF and DOERR (1981), GIAMBORNE et al. (1985), RAJU and DEVEGOWDA (2000), and STOEV et al. (2000). There was a slight improvement in the body mass of Toxiroak®-treated chicks. Earlier, GODBOLE et al. (2001) reported a significant improvement in the performance of cockerels due to supplementation of Growell during induced aflatoxicosis.

The observed significant reduction in haemoglobin in broilers fed mycotoxins confirms the earlier findings of DOERR and HUFF (1980), and MANI et al. (1993) with regard to aflatoxins, and MOHIUDDIN et al. (1993) and RAMADEVI et al. (2000) with regard to ochratoxin. There was no effect of mycotoxins on other haematological parameters studied in the present experiment. In contrast, reduction in TEC and PCV due to feeding of aflatoxin (SINGH et al., 1992), and ochratoxin (DOERR and HUFF, 1980; AVED et al., 1991; MOHIUDDIN et al., 1993) was reported earlier. AVED et al. (1991), and MOHIUDDIN et al. (1993) recorded a decrease in TLC due to induced aflatoxicosis and ochratoxicosis. STOEV et al. (2000) reported leukocytosis in chicks maintained on dietary ochratoxin A. Results of the present study indicate that the level of mycotoxin, individually or in

combination, 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.

Due to single or combined induced mycotoxicosis in broilers there was reduction in serum total protein and albumin compared to control chicks. Present findings are in agreement with KALOREY (1993), who recorded similar biochemical changes due to aflatoxoin. 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) reported reduction in serum total protein due to synergistic action of dietary aflatoxin and ochratoxin 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, in contrast to Toxiroak®-untreated groups, point to the restorative role of preparation as far as protein synthesis is concerned. Similarly, SONI et al. (1992) and KURKURE et al. (2000) reported that treatment of chicks with curcumin and *Curcuma longa* (0.5 g/kg feed) during aflatoxicosis help to maintain normal serum protein levels.

In the present study there was a significant reduction in serum cholesterol and triglyceride levels due to dietary aflatoxin, ochratoxin and feeding of these mycotoxins in combination. In respect of serum cholesterol during aflatoxicosis, a similar trend was reported earlier by MANI et al. (1993) and VASSAN et al. (1998), likewise by MANNING and WYATT (1984), RAMADEVI et al. (2000), and STOEV et al. (2000) during ochratoxicosis. Due to combined mycotoxicosis, similar results were recorded by HUFF et al. (1992). Reduction in serum cholesterol and triglyceride levels during induced mycotoxicosis reflects impaired liver metabolism, leading to reduced synthesis of cholesterol and triglyceride, as was also evident in the present study. The significant improvement in serum cholesterol and triglyceride levels of mycotoxicated broilers supplemented with Toxiroak® is indicative of its protective role. Similarly, histological changes in liver were also less intense in degree in these groups compared to mycotoxin-fed only groups.

Significantly elevated serum creatinine and uric acid levels in co-mycotoxicated, ochratoxicated, followed by aflatoxicated groups, were recorded in our investigation. The present findings are in agreement with those of MANNING and WYATT (1984), RAMADEVI et al. (2000), DOERR and HUFF (1980), and HUFF et al. (1992) in respect of ochratoxin and aflatoxin-ochratoxin combination, respectively. The increase in serum creatinine and uric acid may be attributed to the nephrotoxic effect of ochratoxin, as evident in the present study, leading to renal dysfunctions. Feeding of Toxiroak® to mycotoxicated broilers

significantly prevent a rise in these values, indicating its protective effect on kidney during mycotoxicosis.

During the present study, percentage of organ mass was altered due to dietary mycotoxins, individually, or in combination. The data indicate an increase in percentage mass of liver but a reduction in spleen, bursa of Fabricius and thymus due to feeding of mycotoxins. The results of experimental aflatoxicosis are in accordance with REDDY et al. (1984), and HUFF et al. (1992). With regard to aflatoxin, SINGH et al. (1990), and STOEV et al. (2000) reported similar changes during ochratoxicosis, whereas HUFF and DOERR (1981), and RAJU and DEVEGOWDA (2000) reported similar changes during simultaneous mycotoxicosis in broilers. Supplementation of Toxiroak® during mycototoxicosis partially protected changes in organ mass. Similarly, KURKURE et al. (2000), and STOEV et al. (2000) reported partial protection of organs by feeding of herbal extracts during aflatoxicosis and ochratoxicosis, respectively.

A marked reduction in the humoral immune and cell mediated response against NCDV was recorded in aflatoxins-, ochratoxin- and aflatoxin-ochratoxin fed broilers, compared to control. KALOREY (1993), and KURKURE et al. (2000) also reported reduced immune response of chicks during aflatoxicosis. SINGH et al. (1990), STOEV et al. (2000), and SANTIN et al. (2002) observed reduced immune response of chicks during ochratoxicosis. CAMPBELL et al. (1983) observed reduced immune response of chicks during co-mycotoxicosis. Reduced immune response observed in the present study could be accounted for by reduced protein and globulin synthesis (Table 4), impaired processing of antigen due to impaired phagocytosis, as reported earlier (SINGH et al., 1990; KALOREY, 1993) during ochratoxicosis, aflatoxicosis in poultry and direct lymphotoxic activity of ochratoxin A on lymphocytes, as suggested by LEA and FREDICK (1989).

There was partial protection of mycotoxin-induced humoral immune-toxicity by supplementation of Toxiroak® to chicks. This may be attributed to the protection of immune organs from the histotoxic effect of mycotoxins, as observed in the present study. KURKURE et al. (2000), and STOEV et al. (2000) also recorded better immune response of chicks due to supplementation of *Curcuma longa* and 5% extract of artichoke during experimental aflatoxicosis and ochratoxicosis, respectively.

Gross and histological findings observed during mycotoxicosis in the present study are in agreement with those of BALACHANDRAN and RAMKRISHNAN (1987), and KALOREY (1993), during aflatoxicosis. The observations made during ochratoxicosis are in accordance with DWIVEDI and BURNS (1984), and STOEV et al. (2000). SONI et al. (1992), and KURKURE et al. (2000) reported a reduction in liver lesions due to feeding of curcumin and *Curcuma longa* in duckling and chicks during aflatoxicosis, respectively. STOEV et al. (2000) reported partial protection of organs due to 5% aqueous extract of artichoke during ochratoxicosis in chicks. The observed tissue protection against dietary aflatoxin

and ochratoxin, individually or in combination, could be due to toxin neutralization of the herbal extracts (KALOREY et al., 2000; WARKE, 2001). However, the exact mode of the protective action of polyherbal preparation during mycotoxicosis in broilers needs to be investigated in detail.

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SAŽETAK

Izveden je pokus radi istraživanja zaštitne uloge višebiljnog dodatka hrani (Toxiroak®) u tijeku izazvane mikotoksikoze u tovnih pilića. Ustanovljena je znatno veća masa jetara te smanjena slezena, Fabricijeva burza i timus u pilića koji su dobivali mikotoksine. Zaštitna uloga pripravka Toxiroak®, s obzirom na masu organa, ustanovljena je samo za Fabricijevu burzu. Oslabljeni imunološki odgovor te patohistološke promjene u tkivu jetara, bubrega, Fabricijeve burze i timusa u pilića koji su dobivali mikotoksine bile su poboljšane dodatkom Toxiroak®.

Ključne riječi: aflatoksikoza, ohratoksikoza, tovni pilići, biljke