

Effect of Polyherbal Feed Supplement "Growell" 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 (Growell) during induced mycotoxicosis in broilers. A total of 240 Vencobb broilers were divided at day old stage into eight equal groups. Group A served as control and was given plain feed, group B, D, F and H were given Growell at 0.35 g/kg of feed. Group C, D, G and H were given dietary aflatoxin B₁ at 0.2 ppm and groups E, F, G and H were given ochratoxin A at 0.2 ppm in feed to study effect of Growell on individual aflatoxicosis, ochratoxicosis and combined mycotoxicosis of broilers. The chicks were given their respective feeds from 1st day to 6th week of age and were vaccinated at 7th and 28th day of age with Lasota strain of Newcastle disease virus. There was no statistically significant effect of mycotoxins individually or in combination on body weight of broilers. However, body weights were highest in group B and lowest in co-mycotoxicated group G. Feed conversion ratio was best in group B followed by A, D, F, E, H and G. Significant improvement in haemoglobin values was observed in broilers due to feeding of Growell in ochratoxin and co-mycotoxicated groups. There was no significant effect of mycotoxin treatment on PCV, TEC and TLC of broilers. Due to single and combined mycotoxicosis, there was reduction in serum total protein, albumin, cholesterol and triglyceride and rise in alkaline phosphatase, creatinine and uric acid levels. Supplementation of diets with Growell reduced the alterations induced due to mycotoxins. There was a significant rise in per cent organ weight of liver and reduction of that of spleen, bursa of Fabricius and thymus of broilers fed mycotoxins. Protection from alteration in per cent organ weight of these organ by supplementation of Growell was recorded. The observed impaired immune response and histopathological changes in liver, kidney, spleen, bursa of Fabricius and thymus of broilers given mycotoxins were protected by supplementation of Growell. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 3 : 375-383)

Key Words : Herbs, Aflatoxicosis, Ochratoxicosis, Broilers

INTRODUCTION

Aflatoxin is produced by fungi namely *Aspergillus* and *Penicillium*. High levels of aflatoxin have been recorded in ingredients of poultry feed soyabean, sunflower, rice polish, cotton seed etc. (Jand et al., 1995). The adverse effect of aflatoxin depends on age, species, nutritional status of birds as well as dose and period for which it is consumed. Chronic aflatoxicosis due to prolonged intake of low levels of aflatoxin retards growth, reduces FCR and increases susceptibility of chicks to infectious diseases (Boonchuvit and Hamilton, 1975; Giambrone et al., 1978). Increase susceptibility of aflatoxicated chicks to infectious diseases indicates impaired immune responses. Aflatoxicosis leads to immunosuppression, characterised by decreased immune response (Rao and Joshi, 1986; Bakshi et al., 2000) and breakdown of vaccinal immunity (Panisup et al., 1982; Ilgaz, 1987). 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 (Kalorey, 1993), Glucomannon (Raju and Devegowda, 2000) as well as Growell (Godbole et al., 2001) have been tried with varying success to reduce toxicity and impairment of immune response during aflatoxicosis in birds. Use of adsorbents are of limited value to control ochratoxicosis in livestock (Marquardt and Frohlich, 1992; Santin et al., 2002). Recently Stoev et al., (2000) and Kurkure et al. (2000) reported that extract of artichoke and *Curcuma longa* reduces the toxic effect of ochratoxin A and aflatoxin B₁ respectively in chicks. The protective role of herbal extracts during induced individual or combine mycotoxicoses is not studied well. Growell is a polyherbal herbomineral prenuix, found to improve the performance of broilers and immune system of broilers (Kalorey et al., 2001). Growell has been found to reduce the deleterious effect of aflatoxin in cockerels (Godbole et al., 2001). In present study, protective role of polyherbal feed supplement "Growell" is studied during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers.

MATERIALS AND METHODS

Growell, a polyherbal preparation prepared from extracts of *Operculina turpethum*, *Piper nigrum*, *Embllica ribes*, *Curcuma longa*, *Terminalia chebula*, *Phyllanthus*

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Table 1. Details of experimental groups

Group	Description of group	Feed additionally supplied with		
		Aflatoxin B ₁ at 0.2 ppm	Ochratoxin A at 0.2 ppm	Growell treatment at 0.35 g/kg of feed
A	Control	--	--	--
B	Growell control	--	--	++
C	Aflatoxin control	++	--	--
D	Aflatoxin+treatment	++	--	++
E	Ochratoxin control	--	++	--
F	Ochratoxin+treatment	--	++	++
G	Aflatoxin+ochratoxin control	++	++	--
H	Aflatoxin+ochratoxin+treatment	++	++	++

emblica, *Piper officinarum*, *Piero Rhiza Kurro Royle*, *Cyperus scariosus*, *Carum carvi*, *Boerhavia diffusa*, *Zingiber officinale*, *Piper longum*, *Cedrus deodara*, *Plumbago zeylanica*, *Terminalia belerica*, *Boliospermum montanum*, *Holarrhena antodyserterica*, *Pistacia integerima*, *Myrica nagi* (each 3.8%) with Iron as Iron oxide (24%) was procured from Growell India, Pune, India for the present study.

Production of aflatoxin B₁

A known aflatoxin B₁ producing strain of *Aspergillus parasiticus* (NRRL 3,240) maintained on Sabouraud's dextrose agar and aflatoxin B₁ standard of 1 µg/ml available at the Department of Microbiology was used for production of aflatoxin and quantitation of aflatoxin B₁ respectively. The fungal spores were washed from the surface of agar slant with sterile Sabouraud's dextrose broth (SDB) containing equal amount of 0.1% Tween 80. The spore suspension was filtered through sterile muslin cloth and adjusted with SDB to a concentration of 1.0×10^9 spores/ml and was used as inoculum immediately. A 250 g broken rice was sterilized in 1 liter conical flask and after cooling 25 ml of SDB was added to moisten them. Afterward 1 ml of above inoculum was added to it. It was mixed properly for uniform distribution of spores and incubated at $28 \pm 1^\circ\text{C}$ for 15 days. The flasks were shaken twice a day to break up the clumps. After incubation flasks were autoclaved at 10 Lbs for 5 min. The aflatoxin B₁ was quantified from above fungus infested rice as per 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 ochraceus* (NRRL 3,174) available in the department. OA standard (3 µg/ml) was used for quantitation of OA as per procedure adopted by Tapia (1985).

Feed : Broilers were given feed and water, *ad libitum*. Broiler starter (22.60% CP, 3,150 ME Energy) and Broiler finisher (21.60% CP, 3,250 ME) was offered to broilers

from 0-21st day and 22-42nd day of age, respectively. To achieve required toxin level in feed calculated, quantity of fungus infested rice was mixed in feed. A polyherbal preparation "Growell" was added in feed where ever required and the feed given to birds from day old to 42nd day of age.

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

Experimental observations

Growth and performance study : Ten birds from each treatment group were wing banded at day old stage and weighed individually at weekly intervals. Also feed offered to birds and left out was recorded weekly to calculate feed conversion ratio (FCR).

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

Biochemical observations : The serum collected on 21st day 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 weight : At 21st and 42nd day of age, body weight of six birds from each group was recorded and then were sacrificed. Liver, spleen, bursa of Fabricius and thymus were excised blotted and weighted individually and per cent organs weight were calculated.

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

Age in weeks	A	B	C	D	E	F	G	H
1	94.53±2.23	84.53±2.26	94.00±2.83	93.46±1.92	91.33±3.35	90.66±1.94	93.73±1.35	91.13±1.92
2	221.46±7.80	226.66±9.07	206.33±7.49	198.00±6.36	209.33±12.50	217.66±6.07	209.66±5.88	215.00±5.96
3	396.66±19.72	427.93±21.56	355.00±14.66	402.00±16.13	393.66±23.86	415.66±11.73	392.33±13.65	398.00±17.63
4	657.33±30.63	720.66±31.26	589.33±31.24	652.00±39.83	678.00±34.76	682.00±22.46	620.00±24.00	654.00±32.33
5	978.00±47.75	1023.33±33.30	849.33±46.39	938.66±40.47	948.33±48.49	963.33±28.33	850.00±31.64	932.66±46.94
6	1,416.33±58.24	1,460.00±54.31	1,268.66±42.49	1,399.33±44.93	1,217.00±49.78	1,403.33±51.87	1,191.33±34.37	1,390.66±56.54
Feed conversion ratio	2.09	2.00	2.30	2.18	2.24	2.16	2.40	2.25

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

Age in days	A	B	C	D	E	F	G	H	Significance
Haemoglobin (g/dl)									
21 st day	9.40 ^b ±0.50	9.53 ^b ±0.57	9.36 ^b ±0.44	8.83 ^b ±0.49	8.66 ^b ±0.41	9.20 ^b ±0.55	7.16 ^a ±0.25	8.30 ^{ab} ±0.39	*
42 nd day	9.23 ^{bc} ±0.45	9.36 ^{bc} ±0.41	8.56 ^{ab} ±0.47	9.16 ^{bc} ±0.44	7.56 ^a ±0.56	10.03 ^c ±0.30	7.83 ^a ±0.31	9.46 ^{bc} ±0.58	**
Packed cell volume (%)									
21 st day	27.83±2.22	30.00±2.30	27.16±1.87	26.83±0.60	26.33±1.59	29.50±1.46	29.66±1.45	31.83±1.85	NS
42 nd day	32.66±1.54	31.16±1.52	29.66±2.20	32.83±0.75	29.33±1.36	31.5±0.62	29.16±1.45	32.66±1.63	NS
Total erythrocyte count (×10⁹/cumm)									
21 st day	2.275±0.32	2.37±0.59	2.67±0.26	2.22±0.36	2.90±0.35	2.32±0.23	2.17±0.18	2.33±0.32	NS
42 nd day	2.49±0.23	2.67±0.40	2.71±0.50	2.49±0.51	2.56±0.53	2.15±0.13	2.98±0.35	2.48±0.21	NS
Total leucocyte count (×10³/cumm)									
21 st day	18.66±1.23	20.66±1.20	27.33±1.43	23.00±1.77	24.00±1.15	17.66±1.05	22.66±1.14	20.66±0.92	NS
42 nd day	26.66±2.05	20.66±2.76	33.33±1.23	32.00±3.06	25.33±2.57	25.33±2.67	27.33±2.16	22.66±1.61	NS

NS: not significant, * Significant at 5%, ** Significant at 1%.

Means carrying same superscripts with in row do not differ significantly.

Immunological parameters: Humoral immune response of birds against NCDV post vaccination was adjudged by estimating haemagglutination inhibition (HI) titer at weekly intervals as per procedure recommended by OIE (2000). Cell mediated immune response was studied using contact sensitivity test, a delayed type of hypersensitivity reaction to 2-4-dinitrochlorobenzene as recommended by Tiwary 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 weight, 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 analysed as per Snedecor and Cochran (1967) using Completely Randomised Design at particular day of observations.

RESULTS

Growth studies

The average body weight (g) of broilers recorded at different age intervals from various treatment groups is presented in Table 2. There was no statistically significant

effect of mycotoxins individually and in combination on body weight of broilers. However, body weights were highest in group B and lowest in co-mycotoxicated group G. The feed conversion ratio (FCR) was best in group B followed by A, D, F, E, H, C and G. In general herbal feed supplement improved the FCR of broilers than their respective control groups.

Haematological study

Average haematological values of experimental broilers observed at 21st and 42nd day of age are presented in Table 3. At 21st day of age there was significant ($p < 0.05$) reduction in haemoglobin values of broilers of co-mycotoxicated group G as compare with control group A. At 42nd day of age there was significant ($p < 0.01$) reduction in haemoglobin values of broilers from ochratoxin A fed group E and co-mycotoxicated group G in contrast to control group A. Significant improvement in haemoglobin values was observed in broilers fed polyherbal preparation along with ochratoxin and co-mycotoxin than respective only toxicated groups. Other haematological parameters like packed cell volume, total erythrocyte count and total leucocyte count were not significantly altered due to various mycotoxins and polyherbal preparation used in the present study.

Biochemical observations

Average serum biochemical values of experimental

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

Age in days	A	B	C	D	E	F	G	H	Significance
Serum total protein (g/dl)									
21st day	4.98 ^a ±0.22	5.68 ^d ±0.36	2.48 ^{ab} ±0.11	5.21 ^d ±0.55	3.47 ^{bc} ±0.24	4.30 ^c ±0.33	2.11 ^a ±0.21	5.48 ^d ±0.60	**
42nd day	3.97±0.14	4.09±0.55	2.94±0.18	3.44±0.50	2.83±0.16	3.69±0.53	2.82±0.17	3.02±0.35	NS
Serum albumin (g/dl)									
21st day	1.30 ^b ±0.16	1.54 ^c ±0.11	0.92 ^a ±0.16	1.47 ^c ±0.11	0.93 ^a ±0.04	1.32 ^{bc} ±0.90	1.08 ^{ab} ±0.09	1.28 ^{bc} ±0.13	**
42nd day	1.39 ^d ±0.03	1.55 ^d ±0.13	1.07 ^a ±0.11	1.11 ^{ab} ±0.07	1.05 ^a ±0.09	1.17 ^{abc} ±0.09	1.01 ^a ±0.04	1.37 ^{bcd} ±0.13	**
Serum cholesterol (mg/dl)									
21st day	143.00 ^b ±15.82	139.78 ^b ±6.16	102.16 ^a ±2.26	123.37 ^{ab} ±9.45	120.55 ^{ab} ±16.77	136.62 ^b ±6.39	94.20 ^a ±14.00	133.97 ^b ±8.02	*
42nd day	142.76 ^b ±7.78	160.68 ^c ±8.71	71.76 ^a ±3.15	137.31 ^b ±8.79	83.92 ^a ±4.58	150.31 ^b ±10.32	62.21 ^a ±7.23	128.25 ^b ±10.45	**
Serum triglyceride (mg/dl)									
21st day	123.46 ^b ±21.40	120.06 ^b ±59.53	56.20 ^a ±8.67	97.91 ^b ±11.17	50.31 ^a ±5.31	87.98 ^{ab} ±6.98	49.01 ^a ±7.38	96.03 ^b ±7.16	*
42nd day	101.73 ^a ±6.65	99.78 ^a ±10.93	40.89 ^a ±7.83	80.58 ^{bc} ±12.22	64.33 ^{ab} ±6.70	94.94 ^c ±17.42	37.66 ^a ±4.21	93.79 ^c ±8.57	**
Serum creatinine (mg/dl)									
21st day	0.380 ^a ±0.035	0.305 ^a ±0.060	0.610 ^b ±0.024	0.353 ^a ±0.068	0.581 ^b ±0.026	0.346 ^a ±0.59	0.695 ^b ±0.269	0.383 ^a ±0.052	**
42nd day	0.338 ^a ±0.33	0.340 ^a ±0.093	0.500 ^{ab} ±0.068	0.375 ^a ±0.078	0.691 ^b ±0.39	0.460 ^a ±0.74	0.656 ^b ±0.041	0.456 ^a ±0.74	**
Serum uric acid (mg/dl)									
21st day	6.04 ^a ±0.92	5.49 ^a ±1.16	11.12 ^b ±0.81	6.71 ^a ±1.43	11.12 ^b ±0.43	6.83 ^a ±1.37	12.44 ^b ±0.31	7.16 ^a ±1.17	**
42nd day	6.30 ^a ±0.70	6.50 ^a ±1.32	9.82 ^b ±0.48	6.91 ^a ±0.92	8.86 ^{ab} ±0.44	7.16 ^{ab} ±1.02	10.12 ^c ±0.90	7.85 ^{ab} ±1.45	*
Serum alkaline phosphatase (U/L)									
21st day	273.27 ^{ab} ±49.19	246.76 ^a ±45.96	474.85 ^{cd} ±67.51	326.73 ^{ab} ±77.01	414.67 ^{cd} ±10.03	320.41 ^{ab} ±31.98	481.95 ^d ±42.21	361.53 ^{ab} ±62.30	*
42nd day	487.65 ^{ab} ±42.51	440.17 ^a ±80.03	729.26 ^c ±54.04	480.25 ^{ab} ±97.06	652.60 ^{bc} ±50.98	452.67 ^{ab} ±81.62	713.14 ^c ±79.89	478.60 ^{ab} ±88.03	*

NS: not significant. * Significant at 5%. ** Significant at 1%.

Means carrying same superscripts with in row do not differ significantly.

broilers observed at 21st and 42nd day of age are presented in Table 4. Significant ($p < 0.01$) reduction in serum total protein was observed in aflatoxin, ochratoxin and co-mycotoxin fed broilers as compared with control at 21st day of age. Among the mycotoxicated groups, feeding of polyherbal preparation to broilers has restored the serum total protein values at par with control in aflatoxin and co-mycotoxicated groups only. At both the periods of observation there was significant reduction ($p < 0.01$) in serum albumin concentration in plain mycotoxin fed groups as compare to control. Statically significant improvement in serum albumin due to polyherbal preparation treatment was noticed in aflatoxin and ochratoxin fed chicks at 21st day of age and in simultaneously mycotoxicosis at 42nd day of age. Serum cholesterol and triglyceride levels were reduced significantly ($p < 0.05$ at 21st day and $p < 0.01$ at 42nd day) in mycotoxin fed groups C, E and G. Supplementation of polyherbal preparation to all mycotoxin fed broilers significantly improved the serum cholesterol and triglycerides levels which were at par with control. Similar trend was seen for serum alkaline phosphatase levels. However, at 42nd day of age protection due to herbal treatment was observed in aflatoxin and co-mycotoxicated broilers. Serum creatinine level of mycotoxin fed groups C, E and G were significantly higher than control groups at both the periods of observation. Supplementation of polyherbal preparation significantly prevented the rise in values of serum creatinine due to mycotoxins. Serum uric acid level in broiler was found to be elevated due to dietary mycotoxins and the effect was more pronounced at 21st day

of age. Polyherbal preparation feeding has inhibited the rise in serum uric acid levels significantly ($p < 0.01$) at 21st day of age. At 42nd day of age this protective effect was observed only in aflatoxin fed broilers.

Organ weight

The result of effect of dietary mycotoxins on relative weight of organs is presented in Table 5. At 42nd day of age there was significant ($p < 0.01$) increase in relative weight of liver of broilers receiving mycotoxin both singly or in combination. This effect was significantly ($p < 0.01$) protected by supplementation of polyherbal preparation and values were at par with that of control. Feeding of mycotoxins to broilers significantly ($p < 0.01$) reduced relative weight of spleen at both the period of observations. This adverse effect was neutralized by polyherbal preparation only in aflatoxin fed group at 21st day of age and in ochratoxin and co-mycotoxicated birds at 42nd day of age. At 21st day of age, reduction in weight of bursa of Fabricius was significant ($p < 0.01$) in aflatoxin and co-mycotoxicated groups. However at 42nd day of age there was significant ($p < 0.01$) reduction in all only mycotoxin fed groups C, E and G. Treatment with polyherbal preparation could restore the relative weight of bursa of Fabricius during active feeding of mycotoxins at both the periods of observations. There was no effect of various treatments on relative weight of thymus at 21st day of age. At later period of observation, there was significant ($p < 0.05$) reduction in relative weight of thymus in plain mycotoxicated groups (C, E and G) as compared with Growell supplemented group B.

Table 5. Average per cent organ weight of chicks from various treatment groups

Age in days	A	B	C	D	E	F	G	H	Significance
Liver									
21 st	3.49±0.23	3.88±0.11	3.78±0.33	3.55±0.23	3.51±0.17	3.83±0.17	4.20±0.14	3.47±0.11	NS
42 nd	2.90 ^{ab} ±0.17	2.62 ^a ±0.13	3.99 ^c ±0.307	3.29 ^b ±0.06	3.20 ^b ±0.18	2.68 ^a ±0.127	3.77 ^c ±0.18	2.911 ^a ±0.04	**
Spleen									
21 st	0.207 ^c ±0.036	0.176 ^{bc} ±0.029	0.140 ^a ±0.008	0.211 ^c ±0.0027	0.108 ^a ±0.004	0.120 ^{ab} ±0.006	0.116 ^a ±0.012	0.134 ^a ±0.01	**
42 nd	0.219 ^a ±0.012	0.208 ^{bc} ±0.11	0.156 ^{bc} ±0.007	0.174 ^c ±0.007	0.133 ^{ab} ±0.013	0.183 ^{cd} ±0.007	0.114 ^a ±0.009	0.158 ^{bc} ±0.008	**
Bursa									
21 st	0.356 ^{cd} ±0.022	0.351 ^{cd} ±0.046	0.252 ^{ab} ±0.021	0.371 ^d ±0.040	0.272 ^{abc} ±0.025	0.367 ^{cd} ±0.021	0.223 ^a ±0.038	0.319 ^{bcd} ±0.031	**
42 nd	0.123 ^{bc} ±0.004	0.146 ^c ±0.018	0.088 ^a ±0.006	0.130 ^b ±0.016	0.082 ^a ±0.008	0.121 ^{bc} ±0.012	0.086 ^a ±0.005	0.104 ^{ab} ±0.005	**
Thymus									
21 st	0.506±0.021	0.565±0.063	0.414±0.023	0.574±0.072	0.443±0.036	0.529±0.069	0.411±0.27	0.497±0.063	NS
42 nd	0.561 ^{ab} ±0.059	0.669 ^b ±0.058	0.483 ^a ±0.054	0.537 ^{ab} ±0.068	0.474 ^a ±0.018	0.596 ^{ab} ±0.012	0.472 ^a ±0.033	0.492 ^a ±0.042	*

NS: not significant, * Significant at 5%, Significant at 1%.

Means carrying same superscripts with in row do not differ significantly.

Table 6. Mean HI titer (log₂) against Newcastle disease virus in experimental chicks

Age in weeks	A	B	C	D	E	F	G	H	Significance
2	3.33±0.33	3.16±0.40	2.33±0.42	2.83±0.40	2.00±0.36	2.50±0.34	2.00±0.25	2.33±0.21	NS
3	5.16 ^b ±0.16	5.50 ^b ±0.22	3.50 ^a ±0.22	4.66 ^b ±0.45	3.00 ^a ±0.25	3.66 ^a ±0.55	2.83 ^a ±0.16	3.66 ^a ±0.42	**
4	4.33 ^{bc} ±0.21	4.50 ^c ±0.22	2.83 ^a ±0.40	2.83 ^a ±0.47	3.83 ^{abc} ±0.47	4.33 ^{bc} ±0.61	3.33 ^{ab} ±0.33	3.83 ^{abc} ±0.47	*
5	5.83 ^a ±0.40	7.50 ^a ±0.34	4.33 ^{ab} ±0.42	5.50 ^{cl} ±0.34	4.50 ^{ab} ±0.50	5.00 ^{bcd} ±0.44	3.83 ^a ±0.47	5.33 ^{bcd} ±0.21	**
6	4.50 ^{ab} ±0.22	5.00 ^a ±0.36	3.00 ^{ab} ±0.36	4.16 ^{cl} ±0.40	2.50 ^a ±0.42	3.66 ^{bcd} ±0.21	2.83 ^{ab} ±0.30	3.50 ^{bc} ±0.22	**

NS: not significant, * Significant at 5%, Significant at 1%.

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	Significance
	Mean increase in skin thickness (in mm)								
21	2.08±0.17	2.29±0.28	1.68±0.10	1.97±0.21	1.70±0.14	2.08±0.17	1.56±0.16	1.87±0.20	NS
42	5.25 ^c ±0.25	5.27 ^c ±0.35	3.26 ^{ab} ±0.22	3.77 ^{ab} ±0.25	3.33 ^{ab} ±0.42	4.03 ^b ±0.35	3.04 ^a ±0.08	3.5 ^{ab} ±0.41	**

NS: not significant, * Significant at 5%.

Means carrying same superscripts with in row do not differ significantly.

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 B₁, ochratoxin A individually or in combination significantly reduced the 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 at 42nd day of age as compared with control (Table 7). Polyherbal preparation treated birds revealed better CMI status than respective controls but 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 polyherbal preparation-mycotoxin treated groups.

Histopathology

Section of groups A and B did not revealed any histopathological changes at both the period of observations in any of the organs.

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

On 42nd day of age, lymphocytic and heterophilic infiltration in necrosed area was observed. On 21st day of age, sections of liver from group G revealed intensive granular and vacuolar degenerative changes in hepatocytes. Necrosis of liver parenchyma and areas of hemorrhages were more prominent as compare to group C. On 42nd day of age, vacuolar degenerative changes, necrosis, increase in sinusoidal space and reduction in size of hepatocytes with tendency to form lumen was recorded. Proliferation of fibrous connective tissue in peribular space was prominent. The liver section from group D, F and H revealed similar type of changes however, changes were of mild degree. Marked degenerative changes in hepatocytes were observed along with infiltration of lymphocyte and heterophils in hepatic parenchyma of H group

Spleen : Spleen at 21st day of age from group C revealed necrotic areas in germinal center along with sclerotic changes in central artery and depopulation of lymphocytes. On 42nd day of age splenic arterioles showed hyperplasia of tunica intima and destruction of elastic fibers. Section from E and G groups revealed similar changes as observed in C group on 21st and 42nd day of age. However, there was evidence of necrotic patches in splenic corpuscles. The splenic section from Growell treated groups D, F and H also revealed similar changes as that of Growell non treated groups, however, changes were of mild degree and in none of the sections, necrotic patches in splenic corpuscles were seen.

Bursa of fabricius : On 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 group as compare to single mycotoxin fed groups. On 42nd day of age, reduction in size of bursal follicles, 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 from D and F group did not revealed any appreciable changes at 21st day of age. However, in group H, depletion of lymphocytes from follicles was recorded. At 42nd day of age mild atrophy of bursal follicle was noticed in these groups in contrast to respective Growell untreated-mycotoxicated groups.

Thymus : Sections from groups C, E and G showed depletion of lymphoid cells from medullary areas. Few areas of hemorrhages were also observed. Number of Hazel's corpuscles were increased in G group as compared with individual mycotoxicated group C and E. Growell treated groups D, F and H did not revealed appreciable pathological changes in thymus at 21st day of age. At 42nd day, mild depletion of lymphoid cells from medullary areas was seen.

Kidney : Section of group C on 21st day of age showed degenerative changes in tubular epithelium as well as condensation of nuclear material. On 42nd day of age, some of the tubules showed necrosis, separation of epithelial cells from basement membrane and areas of hemorrhages. On 21st day of age, sections from group E revealed swelling and degenerative changes in tubular epithelium leading to occlusion of lumen. In some of the tubules hyaline casts in lumen were prominent. At 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 21st day of age section from group G revealed necrosis of tubular epithelium leading to extensive destruction of the tubules. Areas of necrosis were also prominent in glomerulus. On 42nd day of age extensive destruction of tubular epithelium with detachment of tubular cells from basement membrane was observed. In some of the tubules deposition of urinary casts was seen. Growell treated groups D, F and H revealed mild changes as compared with respective untreated groups.

DISCUSSION

In the present study impact of induced aflatoxicosis, ochratoxicosis and simultaneous mycotoxicosis on various parameters of chicks have been studied. An attempt was also made to adjudge the protective role of polyherbal preparation "Growell" during induced mycotoxicoses in broilers.

The results of present study demonstrate that dietary aflatoxin, ochratoxin individually or in combination affects the body weight and performance of broilers. Similar observations due to feeding of aflatoxin and ochratoxin have been noticed earlier by Huff and Doerr (1981), Giamborne et al. (1985) EL-Karim et al. (1991) Raju and Devegowda (2000) and Stoev et al. (2000). There was slight improvement in the body weight of polyherbal treated chicks. In contrast to this Godbole et al. (2001) reported significant improvement in 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), Mani et al. (1993) as regards of aflatoxin and Mohiuddin et al. (1993) and Ramadevi et al. (2000) as regards of ochratoxin. There was no effect of mycotoxins on other haematological parameters studied in the present experiment. In contrast to this reduction in TEC and PCV due to feeding of aflatoxin (Balachandran and Ramkrishnan 1986; 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 decrease in TLC due to induced aflatoxicosis and ochratoxicosis. Stoev et al. (2000) reported leucocytosis in chicks maintained on dietary ochratoxin A. The results of the present study indicates that the level of mycotoxin used in present study might have not induce bone marrow toxicity. The reduction in haemoglobin concentration observed during mycotoxicosis could be due to reduced protein synthesis as observed in present study (Table 4). Supplementation of polyherbal preparation was seen to resist the change induced by mycotoxins on the haematological parameters studied.

Due to single or combined induced mycotoxicosis in broilers there was reduction in serum total protein and albumin as compared with control chicks. Present findings are in agreement with Kalorey (1993) who recorded similar biochemical changes due to aflatoxin. Manning and Wyatt (1984), Sreemannarayana et al. (1989) and Ramadevi et al. (2000). 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 observed in the present study. Better total serum protein and serum albumin values in polyherbal treatment during mycotoxicosis suggest 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* during aflatoxicosis help to maintain normal serum protein levels.

In the present study there was significant reduction in serum cholesterol and triglyceride and rise in alkaline phosphatase due to dietary aflatoxin, ochratoxin and feeding of these mycotoxins in combination. In respect of serum cholesterol during aflatoxicosis similar trend was reported earlier by Mani et al. (1993) and Vassan et al. (1998), like wise by Manning and Wyatt (1984), Sreemannarayana et al. (1989) 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 and rise in alkaline phosphatase levels during induced mycotoxicosis reflects the impaired liver metabolism leading to reduced synthesis of cholesterol and triglyceride and was also evident in present study. Significant improvement in serum cholesterol and triglyceride levels of mycotoxicated broilers supplemented with polyherbal preparation indicates its protective role. Similarly the histological changes in liver were also of milder degree in these groups as compare to only mycotoxin fed groups.

Significantly elevated serum creatinine and uric acid levels in co-mycotoxicated, ochratoxicated followed by

aflatoxicated groups was recorded in present investigation. Present findings are in agreement with those of Manning and Wyatt (1984), Sreemannarayana et al. (1989), Ramadevi et al. (2000) as well as Doerr and Huff (1980) and Huff et al. (1992) in respect of ochratoxin and aflatoxin-ochratoxin combination respectively. Increase in serum creatinine and uric acid may be attributed to nephrotoxic effect of ochratoxin as evident in present study leading to renal dysfunctions. Feeding of polyherbal preparation to mycotoxicated broilers significantly prevent the rise in these values indicating its protective effect on kidney during mycotoxicosis.

During the present study per cent organ weight was altered due to dietary mycotoxins individually or in combination. The data indicates increase in per cent weight of liver while reduction of spleen, bursa of Fabricius and thymus due to feeding of mycotoxins. The results of aflatoxicosis are in accordance with Reddy et al. (1984) and Huff et al. (1992) as far as aflatoxin is concern. Sreemannarayana et al. (1989), 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. The supplementation of polyherbal preparation during mycotoxicosis has partially protected the changes in organ weight. 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.

Marked reduction in the humoral immune and cell mediated response against NCDV was recorded in aflatoxin, ochratoxin and aflatoxin-ochratoxin fed broilers as compared with control. Ilgaz (1987), Kalorey (1993) and Kurkure et al. (2000) also reported reduced immune response of chicks during aflatoxicosis. Singh et al. (1990), EL-Karim et al. (1991), Kozacynski (1994), 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 present study might be accounted for 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 and aflatoxicosis in poultry and direct lymphotoxic activity ochratoxin A on lymphocytes as suggested by Lea and Fredick (1989).

There was partial protection of mycotoxin induced immune-toxicity by supplementation of polyherbal preparation to chicks. This may be attributed to protection of immune organs from 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 present study are in agreement with that of Balachandran and Ramkrishnan. (1987) and Kalorey (1993) during aflatoxicosis. The observations during ochratoxicosis are in accordance with Dwivedi and Burns (1984). Sreemannarayana et al. (1989), Kozaczynski, (1994) and Stoev et al. (2000). Soni et al. (1992) and Kurkure et al. (2000) reported reduction in the lesions of liver due to feeding of curcumin and *Curcuma longa* in duckling and chicks during aflatoxicosis respectively. While, Stoev et al. (2000) reported partial protection of organs due to 5% aqueous extract of artichoke during ochratoxicosis in chicks. The tissue protection observed against dietary aflatoxin and ochratoxin individually or in combination might be due to the toxin neutralization of the herbal extracts (Kalorey et al., 2000; Warke, 2001). However the exact mode of protective action of polyherbal preparation during mycotoxicosis in broilers needs to be investigated in detailed.

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