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Experimental Afla and Ochratoxin Induced Mixed Mycotoxicosis in Broilers and its Amelioration with Herbomineral Toxin Binder 'Toxiroak Gold'

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Abstract: A study was conducted in 75 dayold Vencobb broiler chicks to evaluate toxic effects of aflatoxin B1 and ochratoxin A and efficacy of herbomineral toxin binder product (Toxiroak Gold) in preventing co-mycotoxicosis. Chicks were randomly divided into three groups of 25 each. Group I served as healthy control (C) and given standard basal ration and no treatment, Group T_0 and T_1 comprised healthy birds fed standard basal diet and mycotoxicated with 100 ppb each of aflatoxin B1 and ochratoxin A from 0-42 days. Group T_0 is not given any treatment and served as positive control; however, mycotoxicated group T_1 was administered herbomineral toxin binder product Toxiroak Gold@1kg/tonne of feed for 6 weeks. Mycotoxin adversely affected body weight gain, feed consumption, feed efficiency, haematobiochemical profile. However, supplementation of herbomineral toxin binder feed supplement has provided amelioration in mixed mycotoxicosis in broilers.

Key words: Aflatoxin, broiler, performance, ochratoxicosis, herbo-mineral, toxin-binder

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, characterized 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 summarized 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. In addition to this, another important mycotoxin is Ochratoxins (OTA), which are 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). 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% aqueous extract of artichoke and *Curcuma longa* (Turmeric) powder at 0.5 g/kg feed reduces the toxic effect of ochratoxin A and aflatoxin B1 respectively, in chicks. Hence the present investigation was carried out to study the protective role and efficacy of herbal toxin binder product in broiler during induced combined aflatoxicosis and ochratoxicosis.

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MATERIALS AND METHODS

Seventy five (75) day old broiler chicks were purchased and randomly divided into three identical groups (C, T_0, T_1) each comprising 25 chicks and reared up to 42 days. All the three groups were housed under identical managemental and environmental conditions. Standard poultry feed free from aflatoxin and ochratoxin (basal ration) was purchased for all the three groups. The required quantity of ration for feeding to Control group-C (Healthy negative control) was kept separately. The remaining feed was incorporated with 100 ppb of aflatoxin B1 and 100 ppb of ochratoxin A for feeding the birds belonging to groups T_0 and T_1 from 0-42 days. Chicks of group T_0 was offered afla and ochratoxin contaminated feed without any mycotoxin binder product from 0-42 day. Treatment groups T_1 was given mycotoxin binder product Toxiroak Gold@1 kg/tonne of feed from 0-42 days alongwith the afla and ochratoxicated feed from 0-42 days. All the birds were vaccinated as per routine farm practices.

Production of aflatoxin B1: A known aflatoxin B1-producing strain of *Aspergillus parasiticus* (NRRL 3240) maintained on Sabouraud's dextrose agar 2% (w/v) and aflatoxin B1 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 B1, 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 x 10^9 spores/ml and was used as inoculum immediately. Two hundred and fifty g crushed soya DOC 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°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 B1 was semiquantified according to Tapia (1985) using thin layer chromatography.

Production of ochratoxin A: Ochratoxin A (OA) was produced on crushed soya DOC 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. OA standard (3 µg/mL) was used for quantification of OA, according to Tapia (1985).

Different parameters evaluated were growth, performance, haematological parameters, biochemical, enzymatic and gross pathology. Among growth promotion parameters, mean weekly body weight, feed consumption, Feed Conversion Ratio (FCR), mean body weight at the end of experiment were recorded for indvidual birds per group. Blood samples were collected from five representative birds from each group twice during six week experimental period i.e. at the end of 3rd and 6th week to estimate haematobiochemical parameters. Haematological parameters included Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocytic Count (TEC), Total Leukocytic Count (TLC) and biochemical parameters included serum total proteins, albumin, globulin, lipid profile i.e. Total cholesterol, triglycerides, High Density Lipids (HDL), Low Density Lipids (LDL), Very Low Density Lipids (VLDL), serum creatinine, serum uric acid, SGOT, SGPT. All the parameters were statistically analysed by the method given by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Growth and performance parameters: Average weekly body weight of broilers in various treatment groups is presented in Table 1. Gradual and significant ($p<0.01$) decrease in average body weight was observed in mycotoxin fed group T_0. Significant ($p<0.05$) improvement and higher average body weight (1882 g) was observed in induced mycotoxicated groups treated with Toxiroak Gold (T_1) in comparision to mycotoxicated and untreated group T_0 (1753 g), during 1st, 3rd and 5th week of the experiment and found well comparable with the average body weight of healthy birds of control group C (1952 g). 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 significant ($p<0.01$) improvement in the body weight of treated group with herbal toxin binder product during induced mycotoxicosis. Earlier, Godbole *et al.* (2001) also reported a significant improvement in the performance of cockerels due to supplementation of mycotoxin binder product 'Growell' during induced aflatoxicosis.

Average weekly FCR of broiler is presented in Table 2. Significantly lower FCR was observed in prophylactically treated group T_1 (1.94) during than untreated and mycotoxated group T_0 (2.15) at 6th week of experiment and found nearer to the FCR of healthy birds of control group (1.91°C), indicating efficacy of the herbal toxin binder in ameliorating the toxic effects of the mycotoxin in the broilers.

Haematological parameters: Average haematological values of experimental broilers observed at 21st and 42nd day of experiment are presented in Table 3, respectively. Significant ($p<0.01$) reduction in values of Haemoglobin (Hb), PCV, TEC and TLC in mycotoxin fed group T_0 was observed as compared to control group C during both periods of experiment. Significant ($p<0.01$)

Table 1: Average weekly body weight (gm) of broilers from various treatment groups

	0 day	1st week	2nd week	3rd week	4th week*	5th week	6th week*
Group C	40.25±1.21	164.00±1.30^{a}	377.60±1.44	648.40±1.86^{a}	946.00±2.69^{a}	1437.00±1.39^{a}	1952.00±3.02^{a}
Group T_0	41.00±1.24	148.60±0.91^{b}	356.00±0.98	592.20±1.99^{c}	858.00±2.02^{b}	1306.00±2.12^{c}	1753.00±2.88^{b}
Group T_1	42.00±1.08	157.00^{ab}±1.37	363.80±1.71	618.00±2.02^{bc}	911.50±2.29^{a}	1350.00±2.01^{bc}	1882.00±2.71^{a}

Mean with different superscripts in a column differ significantly (p<0.05 or 5%)

Table 2: Average weekly FCR of broilers from various treatment groups

	1st week	2nd week	3rd week	4th week	5th week	6th week
Group C	1.02±0.21	1.29±0.55	1.49±0.31	1.62±0.54	1.75±0.05	1.91±0.81
Group T_0	1.13±0.34	1.45±0.32	1.58±0.27	1.69±0.71	1.83±0.18	2.15±0.74
Group T_1	1.10±0.51	1.38±0.09	1.54±0.11	1.65±1.01	1.78±0.15	1.95±0.61

Mean with different superscripts in a column differ significantly (p<0.05 or 5%)

Table 3: Hematological observations of blood collected from birds on 21st days (3rd week) of experiment

	Day 21st data			Day 42nd data		
Parameters	Group C	Group T_0	Group T_1	Group C	Group T_0	Group T_1
Hb (g %)	9.80±0.29^{a}	8.04±1.28^{b}	8.64^{b}±0.67^{cd}	10.16±1.24^{a}	7.92±1.11^{b}	8.84±0.21^{ac}
PCV (%)	31.20±1.04^{a}	25.40±1.63^{b}	28.00±0.81^{b}	33.00±1.69^{a}	26.00±0.24^{b}	29.20±1.22^{ac}
TEC (million/µl)	3.74±0.71^{b}	2.96±1.41^{a}	3.25±1.72^{b}	3.91±0.78^{a}	2.87±1.33^{b}	3.21±0.88^{ac}
TLC (Thousand/µl)	20.80±1.02^{a}	17.45±1.33^{b}	18.50±1.61^{bc}	22.15±0.31^{a}	17.00±1.20^{b}	18.60±0.97^{ac}

Mean with different superscripts in a column differ significantly (p<0.05 or 5%), Hb = Hemoglobin

Table 4: Biochemical estimates of serum sample collected from broilers on 21st day of experiment

	Day 21st data			Day 42nd data		
Parameters	Group C	Group T_0	Group T_1	Group C	Group T_0	Group T_1
SGOT (IU/L)	183.24±1.84^{b}	249.95±1.24^{a}	216.51±3.24^{b}	183.24±1.98^{b}	249.95±3.24^{a}	216.51±2.01^{b}
SGPT (IU/L)	12.74±2.20^{b}	20.47±1.24^{a}	15.16±0.24^{b}	12.74±1.93^{b}	20.47±0.91^{a}	15.16±0.24^{b}
Protein (g/dl)	3.77±2.02^{a}	3.02±1.02^{b}	3.40±0.64^{ab}	3.77±2.24^{a}	3.02±0.24^{b}	3.40±0.08^{b}
Albumin (g/dl)	1.66±1.94^{a}	1.31±1.04^{b}	1.45±1.29^{ab}	1.66±2.28^{a}	1.31±0.43^{b}	1.45±0.06^{ab}
Globulin (g/dl)	2.11±1.28^{a}	1.72±2.14^{b}	1.95±1.84^{ab}	2.11±0.24^{a}	1.72±1.25^{b}	1.95±0.24^{ab}
Cholesterol (mg/dl)	130.34±1.33^{a}	109.93±2.21^{b}	120.59±1.29^{b}	130.34±0.20^{a}	109.93±3.24^{b}	120.59±3.28^{ab}
Triglycerides (mg/dl)	105.14±1.53	96.13±2.24	103.17±1.27	105.14±2.26	96.13±1.25	103.17±3.22
HDL (mg/dl)	56.26±1.97	53.34±1.43	55.08±1.51	56.26±1.20	53.34±1.74	55.08±1.24
VLDL (mg/dl)	21.02±1.78	19.22±1.11	20.63±1.15	21.02±2.04	19.22±0.29	20.63±1.28
LDL (mg/dl)	27.84±1.81	23.55±1.24^{b}	27.46±1.11^{a}	27.84±1.84	23.55±2.04	27.46±1.45
Creatinine (mg/dl)	1.13±1.29^{a}	1.53±1.06^{b}	1.44±1.02^{ab}	1.13±0.04^{b}	1.53±0.04^{a}	1.44±1.21^{ab}
Uric acid (mg/dl)	5.02±1.44^{a}	6.67±1.04^{b}	6.39±1.03^{ab}	5.02±0.14^{b}	6.67±1.21^{a}	6.39±0.79^{ab}

Mean with different superscripts in a column differ significantly (p<0.05 or 5%)

improvement in hematological values were recorded in treatment group supplemented with Toxiroak Gold as compared to mycotoxin fed group T_0 during the experiment and the values were well comparable with healthy birds of control group C at both period of experiment. Significant reduction in Hb in broilers fed mycotoxin is in correlation with the earlier findings of Doerr and Huff (1980) and Mani *et al.* (1993) on aflatoxicosis and Mohiuddin *et al.* (1993) and Ramadevi *et al.* (2000) on ochratoxicosis. 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 also reported earlier. Aved *et al.* (1991) and Mohiuddin *et al.* (1993) recorded a decrease in TLC due to induced aflatoxicosis and ochratoxicosis. The reduction in Hb concentration observed during mycotoxicosis could be due to reduced protein synthesis, as observed in the present study. Supplementation of polyherbal toxin binder showed improvement in various haematological parameters during induced mycotoxicosis.

Biochemical parameters: Average serum biochemical values of experimental broilers observed at 21st and 42nd day of age are presented in Table 4. Significant reduction in serum total protein, albumin and globulin was observed in mycotoxicated positive control group (Group T_0) when compared to negative control group (group C) on both 21st and 42nd day of experiment.

The values of total protein, albumin and globulin were recorded to get normalized in prophylactically treated group with Toxiroak Gold and well comparable to healthy control and significantly higher than group T_0 indicative of efficacy of polyherbal formulation in ameliorating the toxic effects of mycotoxicosis on liver and normalizing the serum values. Significantly higher values of serum total

protein was found in the treated group T_1 than group T_0 and well comparable to the healthy chicks from control at both periods. Present findings are in agreement with Kalorey (1993), who recorded similar biochemical changes due to aflatoxin. 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, albumin and globulin values in polyherbal treated group T_1, in contrast to untreated groups, showed 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. Liver enzymes SGOT and SGPT were found to be significantly ($p<0.01$) elevated in induced combined aflatoxicosis and ochratoxicosis (Group T_0) when compared to negative control (Group C) at both the intervals. However, prophylactically treated group T_1 supplemented with polyherbal toxin binder product showed significant ($p<0.01$) reduction in SGOT and SGPT levels than group T_0 and found well comparable to healthy birds of group C leading to normalization of liver during mycotoxicosis indicating efficacy of Toxiroak Gold in ameliorating the toxic effects of mycotoxin on liver and keeping the liver in healthy state. Elevation in values of liver marker enzymes (ALT and AST) has been reported at various levels of aflatoxins (Borisava *et al*., 1987; Raina *et al*., 1991) and ochratoxins (Sawale *et al*., 2009). Only serum total cholesterol level was significantly ($p<0.05$) decreased in mycotoxin fed group T_0 while values of serum triglycerides, HDL, LDL and VLDL were found non significantly lowered when compared with control group C during 21st day of experiment. At 42nd day of experiment, serum total cholesterol, triglycerides and VLDL levels were found significantly ($p<0.01$) lowered in mycotoxin fed group T_0 in a comparison to healthy birds of control group, while serum values of HDL and LDL found non significantly lowered. Significant ($p<0.01$) improvement in serum cholesterol and non significantly higher levels of triglycerides, HDL, LDL and VLDL at 21st day of experiment in addition to significantly ($p<0.01$) increased levels of serum total cholesterol, triglycerides and VLDL alongwith non significant higher values of HDL and LDL at 42nd day of experiment were found in prophylactically treated groups with herbal toxin binder than mycotoxin fed group T_0 and found well comparable to healthy birds of group C indicating efficacy of herbal toxin binder in restoring the toxic effects of mycotoxin and normalizing the fat metabolism. Reduction in serum cholesterol during aflatoxicosis were 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 polyherbal toxin binders are indicative of their protective role during mycotoxicosis. 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%). Serum creatinine values were significantly ($p<0.05$ and $p<0.01$) higher in mycotoxin fed group T_0 than control group during both the periods of observation. Supplementation of polyherbal toxin binder during mycotoxicosis significantly ($p<0.01$ and $p<0.05$) prevented a rise in values of serum creatinine in treated group than group T_0 and found well comparable with serum creatinine values of healthy birds of control group C during both intervals of observation. Serum uric acid level in broilers was found to be significantly ($p<0.01$) elevated due to induced dietary mycotoxicosis in group T_0 than control group. However, significant reduction in levels were recorded in treated group T_1 receiving poly herbal toxin binder than group T_0 during mycotoxicosis and found well comparable with the values of control group at both the interval. 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 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. The findings in present study are also in corroboration with those reported by Sakhare *et al*. (2007) that Feeding of Toxiroak® to mycotoxicated broilers significantly prevents a rise in the creatinine value, indicating its protective effect on kidney during mycotoxicosis.

Gross pathology: Enlarged, swollen and pale kidneys were more pronounced in groups fed ochratoxin and aflatoxin-ochratoxin (T_0) (Fig. 1). Intensity of gross pathological changes was less in Toxiroak Gold treated group (Fig. 2). In co-mycotoxicated group, liver was enlarged, with yellowish discoloration and raised nodules (Fig. 3). Spleen, thymus and bursa of Fabricius

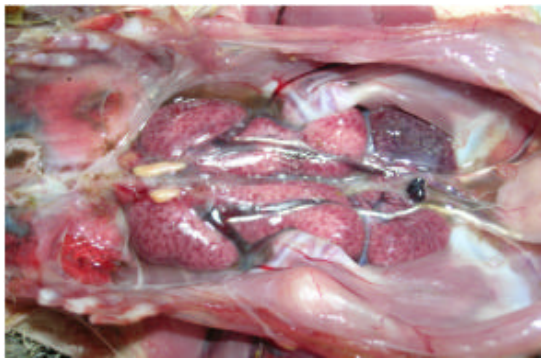

Fig. 1: Group T_0-Kidneys are swollen and shows nephrosis along with prominent ureter due to urates deposition on 42nd day

Fig. 2: Group T_1-Kidney is normal, not swollen but shows mild congestion on 42nd day

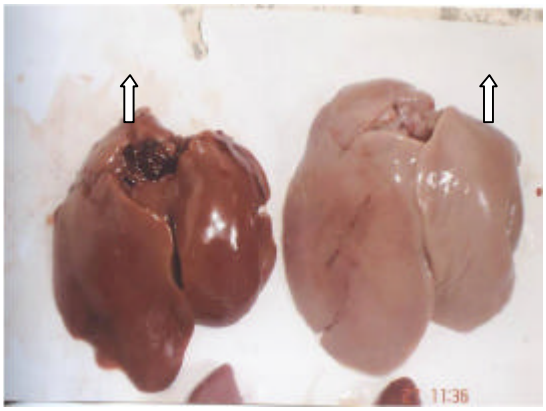

Fig. 3: Group T_0 - Extreme pale, yellowish, large and fragile liver in co-mycotoxicated group on 42nd day. Group T_1 - Normal lobular liver, reddish brown in colour in treated group on 42nd day

of all mycotoxin-fed groups also appeared to be atrophied. It can be inference that supplementation of mycotoxin binder product toxiroak gold.

Conclusion: There was a significant deleterious effect of mycotoxins on body mass, Feed Conversion Ratio (FCR), haematobiochemical parameters and body organs in broilers. Protection of changes in broilers supplemented with Toxiroak Gold® Gold @ 1 kg/tonne of feed for 0-42 days was recorded in terms of improving growth and performance parameters and normalizing the haematobiochemical profile in co-mycotoxicated broilers. The observed gross pathological changes on body organs; liver, kidney, spleen, bursa of fabricius and thymus of broilers were also alleviated by supplementation of Toxiroak Gold, as observed in the treatment group (T_1). It can be concluded that polyherbal toxin binder product "Toxiroak Gold" is efficacious in ameliorating mixed mycotoxicosis in poultry.

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