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EFFECT OF AFLATOXIN ON HAEMATOLOGICAL AND BIOCHEMICAL ALTERATION IN BROILERS

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Abstract: Experimental study in 90 day old broilers was undertaken by dividing them into three groups having thirty chicks in each group by feeding them with dietary Aflatoxin B1@ dose of 100 ppb & 150 ppb in groups II and III respectively and without aflatoxin in the feed serve as control I for a period of 45 days. The haematological study revealed that values of haemoglobin were 10.0±0.44, 9.12±0.38 g/dl, total erythrocyte count 3.33±0.04, 3.27±0.12x$10^{6/}$$mm^3$, total leukocyte count 22.10±0.69, 21.30±0.70 & biochemical levels of serum total protein 3.05±0.01, 2.97±0.11 g/dl, cholesterol 81.70±11.50, 62.03±3.88 mg/dl, and serum uric acid level was 7.18^{b} ± 0.17, 7.09^{b} ± 0.15 in the groups II & III respectively significantly decreased ($P < 0.01$) as compared with control diet (I). However, the activity of ALT 24.32±0.68, 26.02±1.0), AST 43.12±1.34, 45.52±0.93 & glucose 221±5.18, 230.94±8.8) in blood serum was significantly increased in groups II and III as compared with control I at the end of forty five days. Thus from above observations it was concluded that aflatoxin @ 100 & 150 ppb concentration have adverse effect on haematological & biochemical values which results into economical losses to farmers, consumers, animal products.

Keywords: Haematological, biochemical, AflatoxinB1, Broilers.

Introduction

Most of the fungi liberate, highly toxic principle called mycotoxins. These mycotoxins are low molecular weight, non-antigenic, heat stable metabolites. As per the FAO estimate of 1985, as much as 25 per cent of world's grain was contaminated with various harmful mycotoxins. The tropical and sub-tropical countries like India with moderate to high ambient temperature and high relative humidity levels, the ill effects of mycotoxins are widely experienced [4]. Mycotoxins lower the profitability due to high economic losses in broiler industry by decreasing the growth rate, feed conversion efficacy, carcass yield, carcass quality and increase susceptibility to other diseases caused due to their

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immunosuppressive effect [18]. Among these fungal metabolites, AflatoxinB1 (AF) is most important and is one of the common mycotoxins encountered in feedstuff at alarming concentrations. AflatoxinB1 (AF) are a group of heterocyclic toxic metabolites of toxigenic fungi Aspergillus flavus and A. parasiticus. The mechanisms of action of AflatoxinB1 involve their metabolism to reactive intermediates, which bind to macromolecules with consequent disruption of transcriptional and translational processes [8]. There are four major Aflatoxins type B_1, B_2, G_1 and G_2. Amongst these, AFB1, AFB2, 2, 3-oxide, AFM1 and AFM2 are having toxicological significance [6]. AflatoxinB1 are highly hepatotoxic causing acute and chronic liver damage, having carcinogenic, teratogenic and severe performance depressing effects along the impairment of immunologic mechanism and native defense mechanism in poultry [10]. However, proportionate increase in the level of AflatoxinB1 may leads to toxicosis. Serum proteins contents decreased by AflatoxinB1, these effects are more pronounced at 21st days of age. Blood haemoglobin decreased by AflatoxinB1 and T-2 [1]. Keeping these points, the present study was carried out to study hematological & biochemical alteration in broilers.

Materials and Methods

Mycotoxin production

Culture of Aspergillus flavus (MTCC 2798) obtained from Institute of Microbial Technology (IMTECH), Chandigarh, were inoculated on Czapeck Dox Agar and Sabaraud's Dextrose Agar slants and incubated at 28°C for 7 days separately. This AflatoxinB1 cultures were inoculated on rice and wheat for the production of AflatoxinB1 as described method of [21].

Experimental birds:

Day-old 'Vencobb- 300' broiler chicks procured from M/s, Vaishnavi hatchery, Girvali, Tq. Ambejogai, Dist. Beed, M.S. were weighted individually and reared in deep litter system under optimum conditions of brooding and management.

Experimental feed:

Broilers starter (21% crude protein, 2800 metabolic energy) and finisher (20% crude protein, 2900 metabolic energy) rations, procured from local market and tested to be mycotoxin free, were offered to broiler chicks from 0 - 21st day and 22 - 45th day of age, respectively. Chicks were provided feed and fresh *ad-libitum* feed and fresh drinking water throughout the experiment. Powered AflatoxinB1 was incorporated in the normal feed so as to obtain a level of 100 ppb & 150 ppb in the feed.

Experimental procedure:

A total of ninety, day-old chicks, were randomly divided into three dietary treatment groups each containing 30 chicks. The group I represent healthy control birds, fed with normal diet. The birds in groups II and III were fed with AflatoxinB1 @100 ppb and AflatoxinB1 @ 150 ppb respectively.

Haematology:

Ten birds from each group were sacrificed on 15^{th}, 30^{th} and 45^{th} days of the experiment. Prior to sacrifice, blood was collected in heparinized vials by cardiac puncture for haematological & biochemical studies. The haemoglobin (Hb, Sahli's acid haematin methods), packed cell volume (PCV, Microhaematocrit method), total erythrocyte count (TEC, Neubaur's chamber), total leucocyte count (TLC, Neubaur's chamber) & differential leucocyte count (DLC, Wright's stain) estimations were carried out [14].

Serum biochemistry:

Blood samples were also collected at each interval in non-heparinized tubes. The sera were separated and analyzed for the total protein (Biuret methods), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and uric acid using Automatic biochemical analyzer (Transasia Ebra Chem-5 plus, Transasia Bio-medicals Ltd., Andheri (E) Mumbai, India. All biochemical estimations carried out within 24 hours of collections.

Statistical analysis:

The Data was analysed statistically [20].The probability $P<0.01$ was accepted as significant.

RESULT AND DISCUSSION

Haematological changes: Average haematological values in broiler chicks of various experimental groups observed at 15^{th}, 30^{th} and 45^{th} days of experiment are presented in Table 1and 2. The mean values of Hb, PCV, TEC & DLC were significantly reduced at all intervals in groups II and III chicks as compared with that of the controls. It indicated that AflatoxinB1 treated chicks developed anaemia, similar to those reported previously by [13, 15]. Reduction in haemoglobin concentration during in aflatixicated fed birds observed in the present in the study was in accordance with the reports of earlier workers [17]. AflatoxinB1 mainly inhibits protein synthesis and even a very low level of AflatoxinB1 (100 & 150 ppb) was reported to cause significant decrease in the haemoglobin concentration during a period of 45 days in broilers. The reduction in haemoglobin concentration during aflatoxicosis could be due to impaired iron absorption combined with suppression of haematopoiesis [1].

Campbell reported similar results indicating an aflatoxicated induced microcytic hypochromic anaemia [5]. Due to depressing effect on a haemopoietic tissue might have resulted into decreases in RBCs production [3, 6]. Erythrocytes from aflatoxicated treated broiler are more sensitive to osmotic changes. Anaemia with significant reduction in TEC values have been described by [9, 13 & 16] in broiler fed with different of aflatoxin in the diet. Reduction in total leucocytic count in aflatoxicosis was almost in agreement with several previous reports of [19, 23] who observed leucocytopenia in broilers at relatively lower level of aflatoxicosis (100 ppb & 150 ppb), respectively than used in the present study [7, 12].

Table 1. Haematological values (Mean±SE) in experimental chicks of different groups at various intervals

Parameters	Intervals (days)	I	II	III	CD
Haemoglobin (g/dl)	15th day	8.40 [a] ± 0.20	7.47 [a b] ± 0.21	7.38 [b] ± 0.44	0.96
	30th day	10.30 [a] ± 0.30	8.42 [b] ± 0.12	8.10 [b] ± 0.14	0.65
	45th day	12.10 [a] ± 0.62	10.0 [b c] ± 0.44	9.12 [b] ± 0.38	1.13
TEC ($X10^6$ cumm)	15th day	3.80 [a c] ± 0.13	3.29 [b c] ± 0.12	3.02 [b] ± 0.12	0.40
	30th day	4.20 [a] ± 0.36	3.24 [b c] ± 0.11	3.19 [b c] ± 0.08	0.68
	45th day	3.90 [a] ± 0.10	3.33 [b] ± 0.04	3.27 [b] ± 0.12	0.31
TLC ($X10^6$ cumm)	15th day	21.02[a] ± 1.60	18.90 [abc] ± 0.50	18.25 [bc] ± 103	2.76
	30th day	22.43[ac] ± 0.80	20.09[bc] ± 0.45	19.29[b] ± 0.62	1.69
	45th day	24.96[ac] ± 0.50	22.10[bc] ± 0.69	21.30[b] ± 0.70	1.98

Mean bearing different superscripts shows significant differences between the groups among the rows ($P<0.01$)

Table 2. Differential leucocytic count (Mean±SE) in experimental chicks of different groups at various intervals.

Parameters	**Intervals (days)**	**I**	**II**	**III**	**CD**
Heterophils (%)	15th day	28.0 [a] ± 1.06	38.20 [b] ± 1.16	42.30[c] ± 1.11	3.55
	30th day	27.20 [a] ± 0.88	35.27 [b] ± 1.66	40.83 [c] ± 1.33	3.75
	45th day	28.96 [a] ± 0.88	37.80 [b] ± 1.62	42.03 [c] ± 1.31	3.53
Lymphocyte (%)	15 days	62.90 [a] ± 1.45	52.68[b] ± 2.43	51.59[b] ± 2.38	6.50
	30 days	64.86 [a] ± 2.29	56.73 [b] ± 2.72	50.67 [b] ± 1.62	6.27
	45 days	61.72 [a] ± 1.70	53.15 [b c] ± 1.19	48.48 [c] ± 2.67	6.28
Monocyte (%)	15th day	5.30a ± 0.19	4.02b ± 0.02	3.98 [b] ± 0.02	0.39
	30th day	4.99a ± 0.01	3.94 [b c] ± 0.06	3.62 [b] ± 0.20	0.48
	45th day	5.36a ± 0.20	4.22 [b] ± 0.16	4.02 [b] ± 0.02	0.50
Eosinophil (%)	15th day	3.80 [a] ± 0.16	4.96 [b] ± 0.17	5.08 [b] ± 0.25	0.61
	30th day	3.12 [a] ± 0.12	4.38 [b] ± 0.24	5.0 [c] ± 0.26	0.56
	45th day	3.96 [a] ± 0.04	5.03 [b c] ± 0.03	5.20 [b] ± 0.16	0.32

Mean bearing different superscripts shows significant differences between the groups among the rows (P<0.01).

Biochemical Changes: Average serum biochemical values of broiler chicks in various experimental groups observed at 15th, 30th and 45th days of the experiment are presented in table 3. Significant reduction in the mean values of total serum protein, cholesterol was observed in group III chicks at all the intervals as compared with that of the controls. Reduction in total serum protein due to aflatoxicosis has been reported earlier [12]. Low serum protein might be due to inhibition of protein synthesis. AflatoxinB1 inhibits the protein synthesis, through competitive inhibition of phenylalanine t-RNA synthesis with phenylalanine. Due to disturbances in fat mobilization, metabolism and its utilization in the body resulting in hepatic damage. Reduced cholesterol level during aflatoxicosis in chickens had been reported by [2]. Significantly elevated levels of alanine transaminase (ALT) & aspartate transaminase (AST) levels observed in the present investigation might be due to damage to the hepatocyte and consequent release of enzyme in the aflatoxicated (II and III) chicks, which have also been reported earlier in broiler birds during experimental aflatoxicosis [22]. Significant increase in the uric acid level was also observed at all the study intervals in group III as compared with that of the controls. Increased in serum uric acid in group III birds is indicative of impaired renal excretory function during aflatoxicosis. Similar finding have also been reported earlier [11, 12 & 16]. Hepatotoxic action of aflatoxin causes hepatic damage by destruction of hepatocyte in hepatic lobules. On the basis of present finding, it can be concluded that aflatoxicosis @ 100ppb and 150 ppb in feed, adversely affected the haematological & biochemical parameters of broiler birds which results into economic losses of poultry farmers, Industry & nations.

Table 3. Biochemical values (Mean ±SE) in experimental chicks of different groups at various intervals

Parameters	Intervals	I	II	III	CD
Total Protein (g/dl)	15th day	3.36 [a] ± 0.02	2.39 [b] ± 0.04	2.36 [b] ± 0.01	0.18
	30th day	3.64 [a] ± 0.05	2.90 [b c] ± 0.07	2.72 [b] ± 0.09	0.18
	45th day	3.90 [a] ± 0.10	3.05 [b] ± 0.01	2.97 [b] ± 0.11	0.30
Cholesterol (mg/dl)	15th day	234.38 [a] ± 10.18	43.72 [b] ± 8.34	43.50 [b] ± 7.49	27.78
	30th day	186.38 [a] ± 2.45	81.70 [b] ± 11.50	62.03 [b] ± 3.88	31.45
	45th day	204.67 [a] ± 9.14	62.17 [bd] ± 33.16	31.17 [b] ± 3.34	32.48
ALT (IU/ml)	15th day	17.95 [a] ± 0.25	20.33 [b] ± 0.13	22.28 [c] ± 0.29	0.79
	30th day	20.68 [a] ± 0.43	22.80 [a b] ± 0.68	24.02 [b] ± 1.25	2.24
	45th day	22.08 [a] ± 0.76	24.32 [a b] ± 0.68	26.02 [b] ± 1.07	2.45

AST (IU/ml)	15th day	40.98 [a] ± 1.45	46.98 [b] ± 1.45	48.54 [b] ± 1.71	4.86
	30th day	41.58 [a] ± 2.11	43.49 [a b] ± 1.26	47.12 [b] ± 2.45	5.14
	45th day	39.48 [a] ± 2.04	43.12 [a b] ± 1.34	45.52 [b] ± 0.93	4.61
Serum Uric acid (mg/dl)	15th day	8.54 [a] ± 0.15	7.39 [b] ± 0.17	7.06 [b] ± 0.21	0.56
	30th day	8.73 [a] ± 0.18	7.21 [b] ± 0.13	6.76 [b] ± 0.22	0.55
	45th day	8.62 [a] ± 0.13	7.18 [b] ± 0.17	7.09 [b] ± 0.15	0.40

Mean bearing different superscripts shows significant differences between the groups among the rows ($P<0.01$).

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All experiments using animals should be done under the approval of an Institutional Animal Experiment Committee.