Volume-3, Issue-8, August-2014 • ISSN No 2277 - 8160

Research Paper | Veterinary Science

Evaluating The Potential Toxic Effect of Aflatoxin and Ochratoxin on Serum Biochemical Parameters in Coloured (Raja Ii) Broilers

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ABSTRACT

The present experiment was conducted to evaluate the potential toxic effect of long term exposure of aflatoxin and ochratoxin in coloured broilers. Experimental birds were exposed to AFB1 and OTA as individual and combined mycotoxins @ 0.5 and 1ppm (low and high dose) for period of six weeks. Birds were observed daily for any change in clinical signs, mortality and morbidity throughout the experimental period. Blood samples were collected weekly for serum biochemical analysis. Birds exposed to combined aflatoxin and ochratoxin showed potential toxic effect on growth performance, mortality, morbidity, serum biochemical parameters than birds exposed to individual mycotoxins. Coloured broilers are also equally susceptible to combined mycotoxicosis and combination of aflatoxin and ochratoxin have potential toxic effect.

KEYWORDS : Combined mycotoxins; Coloured broilers; Potential toxic effect; Serum biochemical

Introduction

Although, mycotoxins have old history but till today have world global problem as they are ubiquitous abiotic hazards and not only affects the animal and human health but also, have an impact on world trade [1]

Different types of mycotoxins produced by a single or several fungal species may be occurring simultaneously on various agriculture commodities [2], thereby adding more potential risk of mixed mycotoxins interaction which leads to severe economic losses for poultry industry and also includes a reduction agricultural production, healthcare, veterinary and regulatory costs [3, 1]. Toxicity of individual mycotoxins may be potentiated or reduced when they occur in mixed mycotoxins contaminated feed [4,5], also toxicity may vary with age, dose and duration of exposure, strain, nutrition etc.

Aflatoxin B1 and ochratoxin A are most common mycotoxins in poultry feed, have detrimental effects on both animal and human health, and were classified as potent human carcinogen Group 1B and possible human carcinogen Group 2B respectively [6]. AFB1 was produced mainly by Aspergillus species such as flavus, parasiticus etc [7] and are potent liver carcinogenic, mutagenic and immunosuppressive compounds [8].

Ochratoxin A is produced mainly by Aspergillus ochraceus (Aspergillus allutaceus var. allutaceus) [9] and is a potent nephrotoxic mycotoxins, which also has hepatotoxic, teratogenic, embryotoxic, genotoxic, neurotoxic, immunosuppressive and carcinogenic effects [10].

The coloured broilers are named as RAJA-II and obtained by crossing PB1 cock with the PB2 hen. These birds are ideal for small scale and backyard farming, resemble local birds and do have high adaptability, survivability and better carcass quality. There is no any literature available on the toxic effect of mycotoxins in these birds. Hence, by considering all these points the present study was conducted in coloured broilers to know the potential toxic effect of individual and combined mycotoxins on various serum biochemical parameters.

Materials and Methods

Mycotoxin production

Culture of Aspergillus flavus (MTCC 2798) and Aspergillus ochraceus (MTCC 10276) obtained from Institute of Microbial Technology (IMTECH), Chandigarh), were inoculated on Potato Dextrose Agar (PDA) slants and incubated at 28°C for 7 days separatly. These aflatoxin and ochratoxin cultures were inoculated on rice and wheat for the production of AF and OTA as described method [11,12] respectively.

Experimental birds and diet

The coloured broilers and feed were procured from the Department of Poultry Science, Veterinary College, Bangalore. Necessary approval from the Institutional Animal Ethics Committee (IAEC), Veterinary College, Bangalore, KVAFSU, India was obtained (No. LPM/IAEC/127/2012) before conducting the present experiment.The basal diet was formulated and compounded to meet the nutritional requirements of commercial broilers based on the recommendations of Bureau of Indian Standards during the starter and finisher period.

Experimental design

Day old chicks were individually weighed (BW of 47 ± 0.5 g), wing banded and randomly assigned to six experimental groups (Group

2-7) and the negative control group (Group1) each group consist of 66 birds with four replicates of 16-17 birds each and exposed to dietary treatment for six weeks with water and feed ad libitum. All broilers were placed in wire-bottomed aluminum cages and housed in an environmentally controlled house equipped with central heating and chicks were vaccinated against Newcastle disease and Infectious Bursal disease.

Table 1. Different experimental groups and their treatment

Groups	Basal diet	AFB1 (ppm)	OTA (ppm)
G1	+	-	-
G2	+	0.5	-
G3	+	1	-
G4	+	-	0.5
G5	+	-	1
G6	+	0.5	0.5
G7	+	1	1

Parameters studied

Clinical signs, mortality and morbidity were recorded daily. Six birds from each group were selected randomly and sacrificed by cervical dislocation weekly. Blood samples were collected for serum biochemical analysis and was done by using clinical chemistry analyzer - Microlab 300 (Vitalab Scientific, The Netherlands) following the use of commercially available diagnostic kits from ERBA Mannheim (Transasia Biomedicals Ltd, HP).

Statistical analysis

Results were subjected to statistical analyses and were carried out using a software "GraphPad Prism" and Bonferroni post-test was applied for multiple comparisons (P<0.05). Mean values and standard deviation were calculated and all the values were expressed as Mean±SD.

Result and discussion

Clinical signs, mortality

Birds in Group 1 (negative control), remained normal, active and alert throughout the period of the experiment. Marked depression, reduced feathering, reduced growth rate, leg weakness, reduced feed intake, increased water consumption and manure moisture were observed in birds exposed to combined mycotoxins, ochratoxin followed by aflatoxin groups which were progressive and in a dose related manner.

Highest mortality rate was noticed in combined mycotoxins groups (12.21 and 21.21 % respectively) as compared to individual AFB1 and OTA groups (3.03 and 7.57 % respectively), highest mortality rate was noticed during first three weeks of experimental period in all treated groups, no mortality noticed in negative control group fed with basal diet only.

Clinical signs of mycotoxicosis and highest mortality rate was noticed in combined mycotoxins in the present study are due to toxic effect of aflatoxin and ochratoxin, also OTA being the most toxic during early life and could be the cause of mortality in young birds [13,14].

Serum biochemical parameters

The effect of individual and combined mycotoxicosis on serum biochemical parameters are presented in Table 2. Compared to control and individual mycotoxins groups, there was a significant (P<0.001) increase in enzyme activities of liver functional marker enzymes (SGPT and SGOT) in combined mycotoxin and this data are in agreement with similar findings of many workers with various dietary levels of aflatoxin and ochratoxin combinations [15,16,17,18].

Also significant (P<0.001) increase in GGT activity was noticed in birds fed with combined mycotoxins and was highly suggestive of bile duct injury, many authors reported increase in GGT activity in dietary aflatoxicosis [19,20,21] and ochratoxicosis of birds [22,23]. Consequently, increase in the activities of SGPT, SGOT and GGT are primarily indicating hepatic damage by aflatoxin and ochratoxin [24].

Significant (P<0.001) increase in serum creatinine and uric acid levels were noticed in combined mycotoxins groups and are supported by similar findings of many authors [25,26, 15,16, 20,17,18]. Increase in serum creatinine and uric acid may be suggestive of inflammatory or degenerative changes in the kidney [27,18].

In the present study, severe decrease (P<0.001) in the total serum protein level, albumin and cholesterol were noticed in combined mycotoxins groups and are in agreement with findings of [25,26,15,16,17,18] who reported similar results due to synergistic action of dietary aflatoxin and ochratoxin in broiler chicken.

Liver is the major site for synthesis of proteins including albumin, globulins. Decrease in total protein, albumin concentration was mainly due to severe binding of AF and ochratoxin to serum proteins, formation of adducts and severe damage to liver leads to inhibition proteins of synthesis in liver and increased protein loss seen in injured renal tubules [27,28,18]. Impairment in liver metabolism, leading to reduced synthesis of cholesterol and triglyceride, as was also evident in combined mycotoxins exposed birds in the present study are supported by similar findings of [26,15,16,17].

Conclusion

Aflatoxin B1 and ochratoxin A have more potential risk in coloured broilers (RAJA II) when exposed to combined mycotoxins than individual mycotoxins, hence control of combined mycotoxicosis is very necessary to check the severe economical loss to poultry industry.

Table 2. Individual and combined effect of aflatoxin and ochratoxin on serum biochemistry in 42 day old coloured broiler chickens (Mean±SD)

Parameter	G1	G2	G3	G4	G5	G6	G7
SGOT (U/L)	161.33 ± 3.9^{a}	190.06 ± 3.8^{b}	201.73 ± 5.3^{b}	180.48 ± 2.1^{b}	191.33 ± 6.5^{b}	219.08 ± 5.3^{c}	287.75 ± 3.4^{d}
SGPT (U/L)	16.13 ± 0.39^{a}	17.97 ± 0.24^{b}	18.36 ± 0.34^{b}	17.35 ± 0.1^{b}	17.64 ± 0.23^{b}	19.15 ± 0.29^{c}	19.48 ± 0.24^{c}
GGT (U/L)	23.01 ± 0.16^{a}	25.26 ± 0.65^{b}	26.04 ± 0.70^{b}	24.56 ± 0.45^{b}	25.91 ± 0.50^{b}	26.91 ± 0.60^{c}	28.25 ± 0.55^{d}
CR (mg/dL)	0.38 ± 0.04^{a}	0.41 ± 0.00^{b}	0.42 ± 0.00^{b}	0.43 ± 0.00^{bc}	0.46 ± 0.01^{c}	0.50 ± 0.01^{d}	0.55 ± 0.01^{e}
UA (mg/dL)	6.40 ± 0.00^{a}	6.51 ± 0.03^{b}	6.56 ± 0.02^{b}	7.48 ± 0.03^{d}	8.51 ± 0.04^{e}	9.50 ± 0.04^{f}	10.54 ± 0.01^{g}
TP (g/dL)	5.52 ± 0.14^{a}	4.82 ± 0.1^{b}	4.26 ± 0.2^{c}	4.90 ± 0.1^{d}	4.29 ± 0.3^{e}	3.57 ± 0.1^{b}	3.20 ± 0.1^{c}
CHOL (mg/dL)	128.5 ± 3.5^{a}	110.1 ± 1.4^{b}	98.4 ± 1.5^{c}	117.5 ± 1.5^{d}	104.3 ± 1.4^{e}	93.2 ± 4.5^{b}	78.4 ± 1.5^{c}
ALB (g/dL)	1.92 ± 0.15^{a}	1.40 ± 0.5^{b}	1.29 ± 0.4^{c}	1.48 ± 1.2^{d}	1.37 ± 1.5^{e}	1.05 ± 0.10^{b}	0.85 ± 0.05^{c}

Values with different superscripts within a row indicate significant differences (*P<0.05, **P<0.01, ***P<0.001)

Where, SGOT (serum gluatamate oxaloacetate tranferase), SGPT (serum glutamate pyruvatate transaminase,) GGT (gamma glutamyl transaminase), CR (creatinine) and UA (uric acid) concentration, TP (total protein), ALB (albumin) and CHOL (cholesterol)

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