Studies on nephrotoxicity due to combined effect of Aflatoxin and Ochratoxin in broilers and their amelioration with adsorbents

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Abstract

The present study was undertaken to study the combined effect of AF (Aflatoxin) and OA (Ochratoxin A) on kidney function parameters in broilers and their amelioration using adsorbents. AF and OA were mixed with the broiler diet to attain a concentration 1 ppm and 2 ppm respectively. Activated charcoal @0.4% level and lyophilized yeast culture@0.2 % level were selected for testing their efficacy in ameliorating the combined toxicity in broiler chicks. Total of 128 no., one day old broiler chicks were divided into 4 groups with 4 replicates of 8 birds each in a completely randomized design. Each group was fed with 4 different diets for 6 weeks to study the effect on kidneys function parameters. Group 1 is toxin free basal diet (Control). Group 2 is a mixture of basal diet, AF and OA. Group 3 is a mixture of basal diet, AF, OA and activated charcoal. Group 4 is a mixture of basal diet, AF, OA, activated charcoal and lyophilized yeast culture. The birds in group 2 recorded significantly higher serum uric acid and lower BUN level compared to control group. The birds in group 3 recorded significant improvement in kidney function parameters as compared to group 2 but still significantly varying from groups 4 and 1, indicating that activated charcoal @ 0.4.% level has only partial ameliorating effect on combined toxicity. Birds in group 4 have shown significant improvement in the kidney function parameters as compared to group 2 and 3 but there is still a significant difference from the control group. From the results it is observed that the addition of activated charcoal @0.4% has only partial ameliorating effect on combined toxicity where as a combination of activated charcoal @0.4% and yeast culture @ 0.2 % has complimentary effect in ameliorating the combined toxicity in broilers. However, combination of activated charcoal and yeast culture also could not completely ameliorate the combined toxicity in broilers.

Key words: Aflatoxin; Ochratoxin A; Activated charcoal; Yeast culture; Kidney function parameters; Broilers

Introduction

Contamination of food commodities and agricultural harvest by various toxigenic fungi is a serious problem all over the world. This problem still remains unsolved even after extensive research over decades (Srikanth *et. al.,* 2011). Aflatoxin are the toxic metabolites produced by Aspergillus species and penicillin species and are the natural contaminants of poultry feed (Jand *et.al.,* 1995). Livestock feed contamination alters the productivity of the live stock and most important food safety concern is the aflatoxin residue in the animal product (Chen *et. al.,* 2014). Both AF and OA are extremely toxic to poultry by interacting synergistically and have the potential to compromise poultry health and negative impact on poultry production. The physical and chemical methods are impractile in terms of safeguarding the nutritive quality of food and feed, while the biological methods like use of toxin binders showing promising results in amelioration of mycotoxins in poultry (Patil *et. al.,* 2014). In vitro studies reported that OA in contaminated diet was adsorbed to an extent of 81.3 % on addition of 0.4 % activated charcoal to the diet (Prasadarao *et. al.* 2004). Yeast culture @0.5% partially ameliorated the adverse effect due to aflatoxin in respect of weight gain, feed efficiency and total serum protein in broilers (Modirsanei *et. al.* 2004). Therefore the present study was undertaken to study 1) the combine effect of AF and OA on kidney function parameters and 2) the effect of activated charcoal and lyophilized yeast culture on amelioration of the combined toxicity of AF and OA in broilers.

Materials and Methods

Aflatoxin (AF) was produced by growing A. parasiticus NRRL 2999 cultured on broken rice using the method of Short well *et. al.,* (1966). Ochratoxin (OA) was produced by growing A. ochrateus culture obtained from IMTC, Chandigarh on wheat flaques. Aflatoxin was extracted and quantified by TLC method using modified Roomer's method (Romer, 1975). OA was extracted and estimated using TLC as per AOAC 1995 method (A.O.A.C. 1995).

Experimental design

A total of 128, one day old vencobb strain broiler chicks were randomly divided in to 4 groups . Toxins and adsorbents were added to the basal diet to prepare the following experimental diet.

Group 1: Basal diet (Control group)
Group 2: Basal diet+1 ppm AF + 2 ppm OA
Group 3: Basal diet + 1 ppm AF + 2 ppm OA +0.4% Activated charcoal
Group 4: Basal diet + 1 ppm Aflatoxin + 2 ppm Ochratoxin A +0.4% activated charcoal + 0.2% lyophilized yeast culture.

Blood was collected from the birds in each treatment by puncturing the wing vein on 14^{th}, 28^{th} and 42 days. Serum was separated by centrifugation and used for analysis of kidney function parameters: Serum Uric acid (Phosphotungstic acid method), Serum creatinine (Jaffe's method), and Serum BUN (GLDH-Urease method). ***Statistical analysis*:** The experimental data was analyzed using the one way ANOVA procedures of SPSS.

Results and Discussion

Serum Uric acid

Serum uric acid levels differed significantly ($P<0.01$) with the different experimental diets. The serum uric acid levels were higher in birds fed with diet 2 compared to other diets. The serum uric acid concentration of group 2 was higher among all groups and followed by group 3, 4 and 1 (Table-1). This is attributed to impaired renal excretory function due kidney damage mainly due to the affect of OA. Similarly there was an increase in uric acid concentration in broilers fed with AF @ 0.2 ppm OA @ 0.2 ppm either singly or in combination (Kalorey *et. al.,* 2005). Elevated serum uric acid levels were also reported in broilers fed with AF (1 ppm) and OA (2 ppm) by Verma *et. al.,* (2012) and OA @ level of 0.4 to 0.8 ppm (Elaroussi *et. al.,* 2008). Feeding of AF b1 @ 0.2 ppm in pekin ducklings has shown elevation in uric acid concentration (Chen *et. al.,* 2016). Serum uric acid levels in group 3 were higher than group 4 and 1 but lower than group 2. This indicates that activated charcoal in combination with yeast culture has more ameliorating effect than activated charcoal alone.

Serum Creatinine

There was a significant difference ($P<0.01$) in the groups with respective diets and periods of collection of blood. Highest serum creatinine concentration was recorded in group 2 followed by group 3, 4 and 1 (Tabel-1). This might be due to synergistic effect of the two toxins. The synergistic effect involves increased activity of creatinine kinase due to aflatoxin (Abo Norag *et. al.,* 1995) and damage to glomeruli, proximal convoluted tubules due to OA induced nephropathy and impaired renal function (Elaroussi *et al.,* 2008). Similar results were observed in broilers

fed with 100 ppb of AF b1 and 100 ppb of OA at 21st and 42nd days (Sawarkar *et al.*, 2011) and OA fed to broiler chicks @ 1ppm (Jayaramu *et. al.*, 2012.). The serum creatinine level in group 3 were lower than group 2 but slightly higher than group 4 indicating that activated charcoal has less protective effect against the toxins. Significantly lower levels of serum creatinine in group 4 compared to group 3 indicates activated charcoal and yeast culture had complementary effect in ameliorating the toxins. The results were in accordance with the findings of Verma *et al.*, 2012 where they have used combination of AF and OA at levels of 2 ppm and 4 ppm respectively for 42 days.

Serum BUN

Serum BUN values were significantly (P<0.01) different among the groups. The serum BUN levels were significantly lower in group 2 compared to other groups. This might be due to hepatic and kidney damage caused by both the toxins as confirmed by histopathology studies. Decreased serum BUN levels in birds during ochratoxicosis were reported by Pandey *et. al.*, 2003. There was an increase in serum BUN concentration in broilers fed with 0.5 and 1 ppm AF (Lakkawar *et. al.*, 2016), and there was no significant change in serum BUN levels by feeding AF @ 0.1 ppm (Matur *et. al.*, 2011). Serum Bun values in group 3 and 4 were higher than group 2 but much lower than group 1 (Table-1). This indicates that activated charcoal alone and in combination of activated charcoal and yeast culture could not completely ameliorate the depressant values of BUN due to toxins but only have a negligible effect in alleviating the effect of the combined toxins.

Table -1 Mean values of Serum Uric acid, Creatinine, BUN (mg%) in broilers as effected by different experimental diets

Group	Period (Days)			Overall Mean±S.E.
	14	28	42	
Mean values of Serum Uric acid (mg %) in broilers as effected by diff. exp. Diets				
1	8.72±o.75	9.96±0.88	10.98±1.23	9.89±0.58[a]
2	11.44±1.64	14.79±1.62	17.39±1.56	14.54±1.11[d]
3	10.35±1.34	12.93±1.24	15.12±1.13	12.80±0.87[c]
4	8.94±0.43	10.67±1.35	12.46±0.92	10.69±0.67[b]
Overall Mean±S.E.	9.86±0.59[a]	12.09±0.76[b]	13.99±0.84[c]	
Mean values of Serum Creatinine (mg %) in broilers as effected by diff. exp. Diets				
1	0.22±0.02	0.34±0.02	0.41±0.01	0.32±0.03[a]
2	0.31±0.01	0.47±0.02	0.89±0.01	0.56±0.07[d]
3	0.28±0.01	0.42±0.01	0.65±0.02	0.45±0.05[c]
4	0.27±0.01	0.38±0.03	0.50±0.01	0.38±0.03[b]
Overall Mean±S.E.	0.27±0.01[a]	0.40±0.02[b]	0.61±0.05[c]	
Mean values of Serum BUN levels (mg %) in broilers as effected by diff. exp. Diets				
1	6.81±0.01	7.75±0.02	8.53±0.02	7.70±0.21[d]
2	6.72±0.02	6.68±0.01	6.60±0.01	6.67±0.02[a]
3	6.74±0.04	6.79±0.02	7.03±0.03	6.85±0.04[b]
4	6.78±0.02	6.84±0.02	7.12±0.01	6.91±0.05[c]
Overall Mean±S.E.	6.76±0.01[a]	7.02±0.11[b]	7.32±0.19[c]	

[a to d] values baring different superscripts within row as well as column differ significantly P values :(P<0.01)

Group1: Basal Diet, Group 2: Basal diet +1 ppm aflatoxin+ 2 ppm Ochratoxin A, Group 3: Basal diet +1 ppm aflatoxin+ 2 ppm Ochratoxin A+ 0.4% activated charcoal. Group 4: Basal diet +1 ppm aflatoxin+ 2 ppm Ochratoxin A+ 0.4% activated charcoal+ 02% lyophilized yeast culture.

Conclusion

Combination of AF (1 ppm) and OA (2 ppm) showed deleterious effects on kidney. Based on above results it is concluded that activated charcoal @0.4 % level showed only partial protection against combined toxicity in broilers. Combination of activated charcoal @ 0 .4% levels and lyophilized yeast culture @ 0.2% level showed more ameliorating nature by acting complimentarily than activated charcoal alone against combined toxicity. However, the combination of activated charcoal and yeast culture also could not completely ameliorate the combined toxicity in broilers.

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