Combined effect of aflatoxin and ochratoxin on liver enzymes of broilers and amelioration using adsorbents

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Abstract

The present study was undertaken to study the combined effect of Aflatoxin and Ochratoxin A on liver enzymes of broilers and the amelioration of the effects using adsorbents. Aflatoxin and ochratoxin A were mixed with the broiler diet to attain a concentration of 1 ppm and 2 ppm respectively. Activated charcoal at 0.4% and lyophilized yeast culture at 0.2% level were used as adsorbents for testing their efficacy in ameliorating the combined toxicosis in broiler chicks. Four diets for broilers were prepared. Diet-1 is toxin free basal diet (control). Diet-2 is a mix of basal diet, aflatoxin and ochratoxin A. Diet-3 is a mix of basal diet, aflatoxin, ochratoxin A and activated charcoal. Diet-4 is a mix of basal diet, aflatoxin, ochratoxin A, activated charcoal and lyophilized yeast culture. These diets were fed to 4 different groups of broiler chicks with 4 replicates of eight birds each in a completely randomized design for 6 weeks to study their effect on liver enzymes. The birds fed with diet 2 recorded significantly ($P<0.01$) higher AST, ALT and GGT compared to control diet. The birds on diet 3 containing aflatoxin, ochratoxin A and activated charcoal recorded significant ($P<0.01$) improvement in liver parameters as compared to diet 2 but significantly ($P<0.01$) still varying from diets 4 and 1, indicating that activated charcoal at 0.4% level has only partial ameliorative effect on combined toxicity. The birds fed on diet 4 containing aflatoxin, ochratoxin A, activated charcoal and yeast culture have shown significant ($P<0.01$) improvement in the liver parameters studied as compared to diets 2 and 3 but still significant ($P<0.01$) difference is observed from the control diet. The results revealed that inclusion of activated charcoal at 0.4% has partial protective effect against combined toxicity in broilers whereas activated charcoal at 0.4% and yeast culture at 0.2% in combination has complementary effect in ameliorating the combined toxicity in broilers. Though, the combination of activated charcoal and yeast culture is more effective in ameliorating the combined toxicity as compared to activated charcoal alone, they also could not completely ameliorate the combined toxicity in broilers.

Keywords: Aflatoxin; ochratoxin A; activated charcoal; yeast culture; liver enzymes; broiler

Introduction

Contamination of food commodities and agricultural harvests by various kinds of toxigenic fungi is a serious problem that cannot be neglected. Even after extensive research over decades this problem still remains challenging (Srikanth *et.al.*,2011). After insects, molds are the reason for damage during storage of grains (Richard *et.al.*, 1989). Aflatoxins are the natural contaminants of food stuffs and toxic metabolites produced by *Aspergillus* species. Livestock feed contamination effects the productivity of the livestock and the most important food safety concern is the aflatoxin residues in the animal products. (Chen *et.al.*, 2014). Ochratoxin A is a mycotoxin produced by *Aspergillus ochraceus*in tropical regions and by *Penicillium verrucosum*in temperate climates(Shashi *et al.*, 2015).

In natural conditions, there is every chance of poultry rations getting contaminated by more than one mycotoxins which show interactive effect on bird's health (Srikanth *et.al.*,2011). Therefore the present study was taken to study (i) combined effect of two mycotoxins *viz*., aflatoxin and ochratoxin A on liver enzymesand (ii) the effect of adsorbents on amelioration of the combined effect of aflatoxin and ochratoxin A in broilers.

Materials and methods

Production of Aflatoxin and Ochratoxin A, their extraction and quantification

Aflatoxin was produced by growing *A.parasiticus* NRRL 2999 culture on broken rice using the method of (Shotwell *et.al.*,1966). Aflatoxin was extracted and estimated by TLC method using modified Romer's method (Romer, T.R., 1975). Ochratoxin A was produced by inoculating flaked wheat with *Asperigillus ochraceus* culture. Ochratoxin A was extracted and quantified using TLC, as per AOAC 1995 method. (A.O.A.C., 1995).

Experimental design

The experimental design was completely randomized design with 4 groups of chicks, which were fed with the following experimental diets.
Group 1 – Basal diet (control group)
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 + 0.2% lyophilized yeast culture.

Liver Enzymes

Blood was collected from the birds in each treatment by puncturing the wing vein 14^{th}, 28^{th} and 42^{nd} days. Serum was separated by centrifugation after collection and was stored at -20^{o}C for subsequent analysis. The individual serum samples were analyzed for Liver enzymes Gamma glutamyl transferase (kinetic colorimetric method), Aspartate amino transferase (IFCC method, kinetic) and Alanine transaminase (IFCC method, kinetic).

Statistical Analysis

The experimental data were analyzed as per the procedures of (Snedecor and Cochran, 1969)

Results and Discussion

Gamma glutamyl transferase (GGT)

The serum GGT values (Table1) differed significantly ($P<0.01$) among the diets. The diet 2 recorded highest activity of GGT compared to other diets. The increased values indicate hepatic damage due to profound synergistic effect of the two toxins. The hepatocyte damage is probably responsible for the leakage of the enzyme into circulation (Indresh and Umakantha, 2013). The results are in agreement with the observations of (Raju and Devegowda, 2000). Inclusion of activated charcoal in single and in combination with yeast culture in the diets 3 and 4, respectively had only partial effect in adsorbing the toxins and thus resulted in significantly reduced levels of GGT on these diets compared to diet 2 but significantly higher levels compared to control diet.The serum GGT levels were significantly ($P<0.01$) different among periods of collection and increased gradually with age of the birds.

Table1: Mean values of serum GGT (IU/L) in broilers as affected by different experimental diets

Diet	**Period (Days)**			**Overall mean + S.E.**
	14	**28**	**42**	
1	6.90+0.16	8.01+0.70	10.63+0.32	8.51+0.53[a]
2	7.52+0.47	12.94+0.78	18.13+0.86	12.86+1.36[c]
3	7.32+0.16	10.03+0.26	13.85+0.11	10.40+0.81[b]
4	7.16+0.15	9.88+0.66	12.22+0.94	9.75+0.72[b]
Overall mean + S.E.	7.22+0.14[a]	10.22+0.54[b]	13.71+0.78[c]	

Values bearing different superscripts within row as well as column differ significantly at (P<0.01).

Aspartate amino transferase (AST)

The mean serum AST levels (Table.2) differed significantly (P<0.01) with different diets. The serum AST levels were highest on diet 2. This might be due to liver damage caused by synergistic effect of the two toxins. Continuous administration of the toxins might have caused severe cellular damage in the form of necrosis or some other modification leading to increased cellular permeability which in turn leads to leakage of enzyme into circulation. The results were in accordance with the findings of (Raju and Devegowda, 2000).Serum AST levels on diet 3 were significantly lower than on diet 2 but higher than on diets 4 and 1 indicating that activated charcoal was partially effective against combined toxicity with regards to serum AST levels. Significant improvement in serum AST levels on inclusion of activated charcoal at 200 ppm concentration in aflatoxicosis was reported by (Jindal *et.al.*, 1994).Serum AST levels on diet 4 were significantly less than on diet 1 but were within the normal range indicating that activated charcoal at 0.4% and yeast culture at 0.2% in combination alleviated the effect on combined toxicity on serum AST levels.The serum AST levels were significantly (P<0.01) different at different periods of collection and the levels increased with age of birds.

Table2: Mean values of serum AST (IU/L) in broilers as affected by different experimental diets

Diet	Period (Days)			Overall mean + S.E.
	14	28	42	
1	116.05+1.80	122.32+3.00	125.73+2.55	121.37+1.78[a]
2	125.17+3.13	154.59+2.43	162.63+1.68	147.46+5.03[d]
3	121.53+1.91	132.84+2.92	141.29+1.32	131.89+2.69[c]
4	119.45+1.17	128.20+2.12	130.10+0.86	125.91+1.60[b]
Overall mean + S.E.	120.55+1.28[a]	134.49+3.36[b]	139.94+3.77[c]	

Values bearing different superscripts within row as well as column differ significantly at (P<0.01).

Serum alanine transaminase (ALT)

The serum ALT levels (Table3) were significantly (P<0.01) different among the diets. The serum ALT levels were highest on diet 2. This may be attributed to extensive liver damage caused by synergistic effect of the toxins. The results were in agreement with increased serum ALT levels in aflatoxicosis reported by (Pandey*et al.*, 2003).

The serum ALT levels on diet 3 were significantly lower than on diet 2 but higher than on diet 4 and 1 indicating the partial ameliorative effect of activated charcoal. Serum ALT levels on diet 4 were slightly lower than on diet 3 but higher than on control diet indicating that activated charcoal in combination with yeast culture has almost the same or slightly more ameliorating effect when compared to activated charcoal alone in single, on increased serum ALT levels due to combined toxicity.The serum ALT levels were significantly different at different periods of collection and increased with age of birds.

Table.3: Mean values of serum ALT (IU/L) in broilers as affected by different experimental diets

Diet	**Period (Days)**			**Overall mean + S.E.**
	14	**28**	**42**	
1	29.15+0.87	33.41+1.68	40.65+0.70	34.14+1.55[a]
2	32.47+0.67	52.83+0.99	65.72+0.9	50.34+4.15[d]
3	30.01+0.87	41.62+1.68	53.25+0.70	41.58+1.55[c]
4	30.62+0.67	38.56+0.99	49.14+0.91	39.44+4.15[b]
Overall mean + S.E.	30.81+0.55[a]	43.12+2.58[b]	53.19+3.26[c]	

Values bearing different superscripts within row as well as column differ significantly at (P<0.01).

Conclusion

Combination of aflatoxin (1 ppm) and ochratoxin A (2 ppm) showed deleterious effect on liver enzymes and thereby liver health. It is concluded that activated charcoal at 0.4% level showed only partial ameliorative nature on combined toxicity in broilers; combination of activated charcoal at 0.4% and yeast culture at 0.2% showed more ameliorative nature by acting complementarily than activated charcoal alone on 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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