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Effect of activated charcoal and lyophilized yeast on aflatoxin and ochratoxin fed broiler's performance

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Abstract
Aflatoxin and Ochratoxin A are produced from *Aspergillus parasiticus* and *Aspergillus ochraceus* respectively and quantified using thin layer chromatography. The toxins, aflatoxin at the rate of 1 ppm and ochratoxin at the rate of 2 ppm, were mixed in broiler diet. Activated charcoal (0.4%) and/or lyophilized yeast culture (0.2%) were added as adsorbents. Broiler chicks were divided into four groups with four replicates of eight birds each in a completely randomized design based on diets - Group 1- basal diet (control), Group 2- basal diet added with aflatoxin (1 ppm) and ochratoxin (2 ppm), Group 3- basal diet added with aflatoxin (1ppm), ochratoxin (2 ppm) and activated charcoal (0.4%) and Group 4- basal diet added with aflatoxin (1ppm), ochratoxin (2 ppm), activated charcoal (0.4%) and lyophilized yeast culture (0.2%). These four diets were fed to four groups of broiler chicks for 6 weeks. The body weight gains, feed consumption and feed conversion ratio were significantly ($P< 0.01$) lower in group 2 fed with aflatoxin and ochratoxin. These values were significantly improved in group 3 fed with aflatoxin, ochratoxin and activated charcoal. Further significant improvement was recorded in group 4 on diet containing aflatoxin, ochratoxin, activated charcoal and lyophilized yeast culture but these values were significantly lower than that of control group. The result of the study indicates that mixture of adsorbents (activated charcoal and lyophilized yeast) were more effective in reducing the effect of aflatoxin and ochratoxin compared to activated charcoal alone.

Keywords: Aflatoxin, ochratoxin, lyophilized yeast, activated charcoal, broilers

Introduction
Mycotoxins are secondary metabolites of fungi. They are been detected in contaminated crops during harvest, storage and processing of diet especially in poultry (Khadem *et al.*, 2012) [12]. Like other environmental pollutants mycotoxins also adversely affect the health and productivity of animals and poultry (Katole *et al.*, 2013) [11]. Aspergillus, Penicillium, Fusarium etc. are the common genera which produce mycotoxins like aflatoxin, ochratoxin, citrinin etc. (Ramesh, 2011) [18]. Severe economic loss of poultry industry occurs due to poor performance, decreased weight gain, liver damage, immunosuppression (Nittin *et al.*, 2011 and Rajendra *et al.*, 2014) [14, 17] and even mortality of the poultry birds. Aflatoxin is hepatotoxic and leads to hepatic carcinogenesis (Punam *et al.*, 2015 and Rajendra *et al.*, 2014) [16, 17] and proved by alterations in the biochemical profile of liver function tests (Priyadarshini and Narasareddy, 2010) [15]. Ochratoxin is a mycotoxin produced by *Aspergillus ochraceusin* tropical regions and by *Penicillium verrucosumin* temperate climates (Shashi *et al.*, 2015) [20]. Ochratoxin is one of the most important mycotoxins of concern for human health. Removal of aflatoxins from contaminated feed is a major problem. Aflatoxin-contaminated diet fed to birds could result to increased mortality, lower egg production, lower feed consumption rate, impaired resistance to infectious diseases, reduced vaccination efficiency, and induced pathological damage to the liver and other organs (Kamalavenkatesh *et al.*, 2005) [8]. In other words, poultry feed contamination with aflatoxins results in serious economic losses to the animal industries (Awad *et al.*, 2016) [3]. To overcome the economic losses caused by these toxins, many scientists attempted many strategies like physical, chemical and biological treatments to reduce the effect of toxins and detoxify them. Even though many methods have come up but practically many are not economical. So the use of adsorptive materials binds to the mycotoxins and immobilizes them in the gastro-intestinal tract and thus reduces the bioavailability of toxin (Hesham *et al.*, 2004 and Yildiz *et al.*, 2004) [5, 25]. The adsorbents like activated charcoal reported to improve the dietary intake of feed and weight gain in aflatoxin contaminated feed (Rajendra *et al.*, 2014 and Wiliam and Richard, 2011) [17, 26]. Modified compounds of cell wall of *Sacharomyces cerevesiae* have high affinity to mycotoxins within

the intestinal tract (Karazhiyan *et al.*, 2016 and Yildiz *et al.*, 2004) [10, 25]. Hence the present study was undertaken to know the combined effect of activated charcoal and lyophilized yeast on broilers performance fed with aflatoxin and ochratoxin.

Materials and Methods

Aflatoxin was produced by growing *Aspergillus parasiticus* NRRL 2999 culture on broken rice using the method of Shotwel *et. al.*, (1966) [21]. Ochratoxin was produced by growing *Aspergillus ochraceus* culture obtained from IMTC, Chandigarh on wheat flakes. Aflatoxin was extracted and quantified by TLC method using modified Romer's method (Romer, 1975) [19]. Ochratoxin was extracted and estimated using TLC as per (AOAC 1995 method (AOAC, 1995)) [1].

One hundred and twenty eight, day old broiler chicks of Vencob strain were randomly distributed into 4 groups with 4 replications of 8 birds in each replication. The experimental design was completely randomized design with four groups of chicks which were fed the following experimental diets:

Groups	Feed
Group I (Control group)	Basal diet
Group II	Basal diet + 1appm aflatoxin + 2 ppm ochratoxin
Group III	Basal diet + 1ppm aflatoxin + 2 ppm ochratoxin + 0.4% activated charcoal
Group IV	Basal diet + 1ppm aflatoxin + 2 ppm ochratoxin + 0.4% activated charcoal + 0.2% lyophilized yeast culture

All the chicks were weighed individually every week using a digital electronic top pan balance. Weekly feed consumption was recorded replicate wise in all the experimental groups from one to six weeks of age and was expressed as feed consumption per bird. Feed conversion ratio was also calculated.

Results and Discussion

Body weight gain

The mean values of weight gains of broiler chicks in group 1, 2, 3 and 4 were 272.01, 181.30, 229.28 and 246.12 g respectively. The mean values of body weight gains per week from 1-6weeks were 82.50, 175.64, 266.75, 311.56, 229.17 and 327.45 g, respectively (Table 1). The weekly body weight gains were significant ($p< 0.01$) at all ages and diets but body weight gain in fifth week was reduced. Decreased body weight gains observed in fifth week may be due to reduced feed consumption resulted from the stress caused by the variations in temperature from 35 ^{0}C to 38.5 ^{0}C and relative humidity from 70% to 89% during this period. Increased body weight gains seen in sixth week may be due to adaptation of the broiler chicks to the changed environment. There was significant ($p< 0.01$) growth depression in group 2 birds which were fed with aflatoxin (1 ppm) and ochratoxin (2 ppm). Highest body weight was recorded in group 1 followed by group 4, group 3 and group 2. The body weights reduced leading to depression of growth upon feeding aflatoxin and ochratoxin leading to reno-hepatic disorder. Activated charcoal has adsorbent properties, where toxins adhere to gastrointestinal tract. The body weight gain was significantly decreased in aflatoxin and ochratoxin containing diet. Similar findings like depression in growth on feeding aflatoxin individually or in combination with other toxins were reported by the other scientists (Khadem *et al.*, 2014; Aravind *et al.*, 2003; Rajendra *et al.*, 2014 and and Jaysri and Srikanth, 2016) [14, 2, 17, 6]. The body weight gain of the birds on diet containing activated charcoal is slightly increased. This implies that depression of body weights caused by aflatoxin and ochratoxin was ameliorated by activated charcoal and lyophilized yeast was increased compared to the toxin affected group but lower compared to control diet. The body weight gain was significantly ($p< 0.01$) decreased in aflatoxin and ochratoxin fed group. Similar findings like depression in growth on feeding aflatoxin individually or in combination with other toxins were reported by the other scientists (Theophilusanandkumar and Balachandran, 2014) [20], which might be due to synergistic effect of toxins on growth. The body weight gain of the birds fed with diet containing activated charcoal (group 3) is slightly increased. This implies that depression of body weights caused by aflatoxin and ochratoxin was partially ameliorated by activated charcoal and the body weight gains of the birds on diet containing activated charcoal and lyophilized yeast (group 4) was increased compared to the toxin fed group but lower compared to control group. Similar ameliorative effect was recorded by combination of activated charcoal and lyophilized yeast culture (Khadem *et al.*, 2014; Aravind *et al.*, 2003; Das *et al.*, 2011; Rajendra *et al.*, 2014 and Jayasri *et al.*, 2017) [12, 2, 4, 17, 7].

Table 1: Mean values of weekly body weight gain (g) of different experimental groups

Period weeks	Groups				Overall mean ± S.E.
	1	2	3	4	
1	84.91±3.73	77.8±3.37	82.84±2.85	84.45±2.10	82.50±1.55^a
2	204.60±3.27	138.05±2.61	176.24±3.75	183.69±2.60	175.64±6.36^b
3	312.41±2.98	197.26±2.43	275.45±2.14	281.86±2.63	266.75±11.02^d
4	363.56±2.95	251.13±4.12	306.43±4.77	325.12±2.48	311.56±10.59^e
5	284.73±3.03	163.25±1.86	221.18±3.80	247.52±1.76	229.17±11.49^c
6	381.84±2.26	260.34±2.56	313.55±4.51	354.09±2.22	327.45±11.88^f
Overall mean ± S.E.	272.01±21.22^d	181.30±13.33^a	229.28±17.00^b	246.12±18.93^c	

$^{a-f}$ values bearing different superscripts within row as well as column differ significantly ($p< 0.01$)

Feed consumption

The mean values of feed consumption per week per bird for different groups were 421.77, 386.43, 399.32 and 409.89 g, respectively. The mean values of feed consumption per bird by the end of week 1, 2, 3, 4, 5 and 6 were 103.53, 263.27, 434.00, 540.16, 524.80 and 560.35 g, respectively (Table 2).The feed consumption was significantly increased gradually from 1-4 weeks which is a natural phenomenon in broilers as the age advances, but feed consumption was decreased in fifth week due to stress by sudden changes in temperature from 35 ^{0}C to 38.5 ^{0}C and humidity from 70% to 89%. Feed consumption was increased later in sixth week

most probably due to adaptation of broiler chicks to the changed atmosphere. The mean feed consumption in birds on diet 2 containing aflatoxin and ochratoxin (group 2) was least compared to all the diets and it may be because of stress due to toxins in diet. The feed intake was higher in group 3 compared to group 2. Feed consumption was further improved in group 4.This indicates that activated charcoal could ameliorate the effect of toxins partially (Khadem *et al.*, 2012; Jayasri *et al.*, 2017 and Yunana *et al.*, 2019) [12, 7, 27]. Further improvement was observed in group 4 on diet containing activated charcoal and lyophilized yeast culture which indicates that yeast culture had a complementary effect to activated charcoal in ameliorating the effect of aflatoxin and ochratoxin on feed consumption. (Aravind *et al.*, 2003; Das *et al.*, 2011; Kamalzadeh *et al.*, 2009; Mogadam and Azizzpoura, 2011 and Yldrm *et al.*, 2011) [2, 4, 11, 15, 26].

Table 2: Mean values of weekly feed consumption (g) of different experimental groups

Period weeks	Experimental groups				Overall mean ± S.E
	1	2	3	4	
1	101.10±7.86	102.46±7.71	105.13±5.16	105.44±6.05	103.53±3.07[a]
2	267.96±4.75	256.02±3.54	262.18±2.88	266.91±4.58	263.27±2.16[b]
3	451.67±13.72	423.43±8.90	427.73±3.29	433.16±3.43	434.00±4.72 [c]
4	552.80±4.53	521.52±2.26	539.27±2.50	5475.08±7.03	540.16±3.65[e]
5	561.71±11.93	490.84±24.88	516.50±5.77	530.15±3.05	524.80±9.15[d]
6	595.38±4.77	524.29±2.57	545.11±3.04	576.62±3.54	560.35±7.27[f]
Overall mean ± S.E.	421.77±37.72[d]	386.43±80.62[a]	399.32±34.22[b]	409.89±35.69[c]	

[a-f] values bearing different superscripts within row as well as column differ significantly ($p < 0.01$)

Feed conversion ratio

The mean values of feed conversion ratios for different groups i.e., group 1, 2, 3 and 4 were 1.49, 2.06, 1.68 and 1.60, respectively. The mean values of feed conversion ratio at weekly intervals of 1, 2, 3, 4, 5 and 6 weeks were 1.24, 1.52, 1.66, 1.75, 2.36 and 1.72, respectively (Table 3). The feed conversion ratios were significantly affected by diets as well as age of birds.

The mean FCR was highest in group 2 fed with aflatoxin and ochratoxin. Though the FCR in group 3 and 4 were significantly higher than that of control group, the value of group 4 was nearer to that of group 1 indicating that activated charcoal at 0.4% level partially ameliorated the toxic effect of aflatoxin and ochratoxin on FCR (Wang *et al.*, 2006) [21] and activated charcoal (0.4%) and lyophilized yeast culture (0.2%) ameliorated the effect of toxins to a greater extent (Aravind *et al.*, 2003; Das *et al.*, 2011; Kamalzadeh *et al.*, 2009 and Yldrm *et al.*, 2011) [2, 4, 9, 26].

Table 3: Mean values of weekly feed conversion ratio of different experimental groups

Period weeks	Experimental groups				Overall mean ± S.E
	1	2	3	4	
1	1.18±0.06	1.31±0.05	1.26±0.01	1.24±0.07	1.24±0.02[a]
2	1.30±0.02	1.85±0.01	1.48±0.04	1.45±0.02	1.52±0.05[b]
3	1.44±0.05	2.14±0.04	1.54±0.02	1.53±0.01	1.66±0.07 [c]
4	1.51±0.02	2.07±0.02	1.75±0.02	1.68±0.03	1.75±0.05[e]
5	1.97±0.02	3.0±0.14	2.33±0.06	2.14±0.01	2.36±0.10[f]
6	1.55±0.01	2.01±0.01	1.73±0.02	1.62±0.01	1.72±0.04[d]
Overall mean ± S.E.	1.49±0.05[a]	2.06±0.42[d]	1.68±0.07[c]	1.60±0.05 [b]	

[a-f] values bearing different superscripts within row as well as column differ significantly ($p < 0.01$)

Conclusion

Combination of aflatoxin (1 ppm) and ochratoxin (2 ppm) showed deleterious effect. 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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