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Evaluation of the Effectiveness of Yeast, Zeolite and Active Charcoal as Aflatoxin Absorbents in Broiler Diets

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Abstract: A total of 630 day old chicks were used in a completely randomized design to investigate the effects of yeast, zeolite and active charcoal, alone or in combination, in aflatoxin B1 contaminated diets on the performance, biochemical traits and the internal organ weights of broilers. Three pen replicates with 30 chicks in each pen were assigned into each of the 7 dietary treatments comprising: 1) basal diet containing 200 ppb of aflatoxin B1; 2) basal diet + %0.5 yeast; 3) basal diet + %1.5 zeolite; 4) basal diet + %0.5 yeast + %1.5 zeolite; 5) basal diet + %0.5 yeast + %1.5 active charcoal; 6) basal diet + %1.5 zeolite + %1.5 active charcoal; and 7) basal diet + %0.5 yeast + %1.5 zeolite + %1.5 active charcoal. The addition of absorbents to diets improved weight gain, feed intake and feed conversion ratio of chicks. Serum concentration of glucose, cholesterol, triglyceride, albumin and uric acid were also increased in chicks fed on absorbent added diets. Supplementation diets with absorbents reduced the relative weights of proventriculus, gizzard, liver, spleen and pancreas of broiler chicks, but their relative bursa weight were increased. In conclusion, results of the present study indicated that the mixtures of the tested absorbents were more effective for reducing the signs of aflatoxin B1 toxicities in growing broiler.

Key words: Aflatoxin · Biochemical traits · Liver · Performance

INTRODUCTION

Aflatoxins, a group of extremely toxic chemicals, are secondary metabolites produced by fungal species of the genus *Aspergillus (A. flavus and A. parasiticus*) and have been detected as contaminants of crops during harvest, storage and after processing and also in feed ingredients which are routinely used in poultry diets. The B1, B2, G1, G2 and the M1 are the major forms of aflatoxin with the aflatoxin B1 being the most common biologically active component of it [1-3]. Severe economic losses in poultry and livestock industries caused by the aflatoxin toxicity were reported. Aflatoxins cause a wide variety of negative effects including poor performances, liver pathology, immunosuppression and changes in the relative weights of organs [2, 4], mortality and increased susceptibility to environmental and microbial stresses [5]. Adverse clinical signs depend on the nature and concentration of mycotoxin, duration of exposure, animal species, age and the nutritional and health status of birds at the time of exposure to the contaminated feeds [6].

Removing aflatoxin from contaminated food and feedstuffs remains a major problem and there has been a great demand for an effective decontamination technology [7]. Recently, many researches looked toward ways to minimize the losses associated with aflatoxin in poultry diets and the use of several methods has been suggested. Mold inhibitors, microbial (bacteria and yeast) inactivation, physical separation, thermal inactivation, irradiation, chemical treatments (ammonia, sulfur dioxide and hypochlorite and ozone degradation), feed additives (vitamins and minerals) and absorbents were used to decrease aflatoxin negative effects [2, 7]. Many of these techniques are costly, time consuming, impractical, ineffective and potentially unsafe. The most popular method, due to its easy applicability, is the use of inorganic absorptive compounds in the diet to reduce aflatoxin absorption from the gastrointestinal tract. These compounds limit aflatoxin absorption from the digestive tract by binding with aflatoxin in feed irreversibly and allowing the mycotoxin to pass harmlessly through the gut.

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Yeast and its cell wall components (manna oligosaccharide) were used in poultry diets to minimize the adverse effects of aflatoxin. Santin *et al*. [8] reported that the cell wall of *Saccharomyces cerevisiae* improve the intestinal mucosa aspects. They suggested that it might be the explanation for the improvement in performance of broilers fed on the aflatoxin contaminated diets supplemented with cell wall of *Saccharomyces cerevisiae.* In addition, laboratory and field trials have shown that modified and esterified glucomannan compounds from the cell wall of *Saccharomyces cerevisiae* have a high affinity for the mycotoxins, both *in vitro* and within the intestinal tract [7]. Zeolites were used to minimize the negative effects of aflatoxin in poultry diets. Kubena *et al*. [9] reported that the addition of zeolite to broiler diets improved their performances in the presence of aflatoxin contamination. Furthermore, dietary additions of activated charcoal have been reported to improve the feed intake and weight gain of broilers fed on aflatoxin containing diets [10]. They announced that charcoal act as an insoluble carrier in diets which nonspecifically adsorb molecules and prevent their absorption. However, no improvement was observed in the performance of turkey poults when charcoal was used in aflatoxin containing diets [11].

Although, yeast, zeolite and activated charcoal were all used as a single aflatoxin absorbent in poultry diets previously, but a combination of them were rarely used. Different combinations of them were, therefore, used in the present work to further evaluate their effects on the performance, biochemical traits and the internal organ weights of broiler chicks when fed on the aflatoxin B1 contaminated diets.

MATERIALS AND METHODS

Six hundred and thirty Ross-308 day-old chicks were purchased from a local hatchery and randomly divided into 7 groups with 90 birds per each group. A completely randomized design was used with 3 pen replicates of 30 chicks (equal numbers of males and females with uniform average body weights (BW) in each group. The 7 dietary treatments were as follows:

- Basal diet (containing 200 ppb of aflatoxin B1) with no absorbent (control diet)
- Basal diet supplemented with only%0.5 yeast (Y diet)
- Basal diet supplemented with only %1.5 zeolite (Z diet).
- Basal diet supplemented with %0.5 yeast + %1.5 zeolite (Y+Z diet).
- Basal diet supplemented with %0.5 yeast + %1.5 active charcoal (Y+C diet).
- Basal diet supplemented with %1.5 zeolite + %1.5 active charcoal (Z+C diet).
- Basal diet supplemented with %0.5 yeast + %1.5 zeolite +%1.5 active charcoals (Y+Z+C diet).

Table 1: Ingredient and chemical composition of experimental diets (g/kg)

	Diets*		
Ingredients	Starter	Grower	Finisher
Yellow corn	590.1	641.2	672.4
Soybean meal	290.1	229.1	201.2
Fish meal	58.0	50	35.0
Limestone	11.0	10.3	9.9
Dicalcium phosphate	4.9	4.7	5.1
NaCl	2.0	2.0	2.0
Min. & Vit. Premix [a]	6.0	6.0	6.0
DL-Methionine	0.9	0.2	-
Absorbent[b]	0-35	0-35	0-35
Sand	0-35	0-35	0-35
Total	1000	1000	1000

*Diets: Starter (1-21 d), Grower (22-35 d), Finisher (36-49 d).

[a]Each kilogram of mineral premix provides: 397g I, 80 g Se, 20 g Fe, 4 g Cu, 33.88 g Zn and 39.68 g Mn. Each kilogram of vitamin premix contains: Vitamin A (36000 IU); vitamin D3 (7.2 g), vitamin K3 (7.3 g), Vitamin B12 (6 mg), thiamin (0.71 g), riboflavin (2.64 g), biotin (40 mg), folic acid (0.9 and 0.6 mg), pantothenic acid (0.4 g), niacin (11.88), pyridoxine (1.176 g) and choline (100 g).

[b]Yeast, active caracole and zeolite were included at levels of 0.5, 1.5 and 1.5% to make experimental diets and sand was use to adjust the weight of diets to 1000g.

Chicks were reared on litter through the experimental period and fed on a standard starter (from d0 to d21), grower (from d22 to d35) and finisher (from d36 to d49) diets formulated to meet their NRC [12] recommended nutrient levels (Table 1). The basal diet was tested for the residual aflatoxin before the commencement of trial according to the Trucksess *et al*. [13] methodology and the concentration of aflatoxin B1 in all diets was adjusted at 200 ppb by using maize containing known levels of aflatoxin. Chicks had free access to water and their relative diets during the experiment. Initially, the room temperature was maintained at 32°C with continuous lighting and then was reduced by 3°C/wk until reaching to 21°C at which the temperature maintained up to the end of experiment.

The live body weight (LBW) and the feed intake (FI) of birds were recorded for each of the starter, grower and finisher periods and their body weight gain (BWG) and feed conversion ratios (FCR) were then calculated based to these records. Two birds from each pen (6 birds per diet group, 3 birds of each sex) were randomly selected at 49 days of age and blood samples were taken from wing veins to measure the serum glucose, cholesterol, triglyceride and albumin and uric acid contents of them. These birds were then slaughtered and their proventriculus, gizzard, liver, spleen, pancreas and bursa of fabricius weights were measured.

Data were all analyzed using the GLM procedure of SAS [14] and the differences among the mean values were detected by the Duncan's multiple range tests at P< 0.05.

RESULTS

The effects of the absorbent added diets on the performance of broiler chicks are presented in Table 2. Broilers fed on the control diet had lower BWG weight gain, FI and LBW than birds in other groups for the starter, grower and finisher periods, as well as for the entire 49 days of experiment (p< 0.05). Compared to the other groups, chicks fed on the Y+Z+C diet had the highest BWG and the most suitable FCR during the starter, grower and finisher periods, as well as for the total 49 days of experiment (p< 0.05). No significant differences were observed between the FI of birds fed on the Y+C, Z+C and Y+Z+C diets during the starter, grower and finisher periods.

For the overall rearing period, chicks in Y+Z+C diet group consumed more feed than the other groups (p< 0.05). Compared to the Y or Z and their combination added diets, the Y+C and Z+ C supplementation in diets improved FI, BWG and FCR of chicks during the starter, grower and the entire experimental period (p< 0.05). The final LBW of birds in Y+Z+C diet group was higher than that in birds of other treatment groups (p< 0.05).

As Table 3 shows, the relative proventriculus, gizzard, liver, spleen and pancreas weights (g per 100 g of body weight) of birds were decreased by the absorbent utilization in diets (p< 0.05). Compared to the chicks in other groups, the lowest relative proventriculus, gizzard and pancreas weights were observed in birds fed on the Y+C, Z+C and Y+Z+C diets. However, no significant differences were observed between these traits for chicks of the three latter groups. In comparison to the control and other treatment groups, the lowest relative liver and spleen weights were measured for the chicks in Y+Z+C diet group (p< 0.05).

Table 2: Effects of yeast, zeolite and active charcoal on the body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and the live body weight (LBW) of broiler chicks fed on diets containing 200 ppb of aflatoxin B1

	Diets*							
Periods and items	Control	Y	Z	Y+Z	Y+C	Z+C	Y+ Z+C	SEM
Starter (0-21 d)								
BWG (g)	367.3^{e}	399.8^{d}	518.9^{c}	519.2^{c}	630.8^{b}	634.3^{b}	658.7^{a}	6.26
FI (g)	599.9^{d}	642.7^{c}	745.3^{b}	763.3^{b}	846.2^{a}	848.1^{a}	857.97^{a}	8.35
FCR	1.63^{a}	1.61^{a}	1.44^{b}	1.47^{b}	1.34^{c}	1.34^{c}	1.30^{c}	0.014
Grower(21-35 d)								
BWG (g)	656.7^{c}	722.2^{b}	722.9^{b}	739.2^{b}	853.6^{a}	856.0^{a}	865.4^{a}	6.70
FI (g)	1384.3^{c}	1448.4^{b}	1426.9^{b}	1434.4^{b}	1551.0^{a}	1558.7^{a}	1562.3^{a}	7.75
FCR	2.11^{a}	2.01^{b}	1.97^{bc}	1.94^{c}	1.82^{d}	1.82^{d}	1.81^{d}	0.015
Finisher (36-49 d)								
BWG (g)	656.4^{d}	993.9^{c}	1003.9^{c}	1053.4^{b}	1094.2^{a}	1092.5^{a}	1104.7^{a}	7.65
FI (g)	1790.5^{d}	2595.9^{c}	2619.2^{c}	2654.0^{b}	2706.4^{a}	2702.3^{a}	2722.8^{a}	10.61
FCR	2.73^{a}	2.61^{a}	2.61^{b}	2.52^{c}	2.47^{cd}	2.47^{cd}	2.46^{d}	0.015
Total (0-49 d)								
BWG (g)	1680.7^{f}	2114.2^{e}	2247.0^{d}	2312.9^{c}	2576.6^{b}	2575.1^{b}	2627.8^{a}	8.24
FI (g)	3778.1^{f}	4685.3^{e}	4791.4^{d}	4850.7^{c}	5106.2^{b}	5089.7^{b}	5144.4^{a}	9.79
FCR	2.25^{a}	2.22^{b}	2.13^{c}	2.10^{d}	1.98^{e}	1.98^{e}	1.96^{f}	0.006
LBW (g)	1725.7^{f}	2159.2^{e}	2292.0^{d}	2357.9^{c}	2608.3^{b}	2620.1^{b}	2672.8^{a}	7.26

*Diets, comprising of: Control (basal diet = diet contaminated with 200 ppb of aflatoxin); Y (basal diet supplemented with only 0.5% yeast); Z (basal diet supplemented with only 1.5% zeolite); Y+Z (basal diet supplemented with 0.5% yeast+1.5% zeolite); Y+C (basal diet supplemented with 0.5% yeast+ 1.5% charcoal); Z+C (basal diet supplemented with 1.5% zeolite+1.5% charcoal); Y+Z+C (basal diet supplemented with 0.5%.yeast+1.5% zeolite + 1.5% charcoal).$^{a–f}$ Row Means with different superscripts differ significantly (P< 0.05). SEM: Standard error of means.

Table 3: Effects of zeolite, active charcoal and yeast on relative weights of digestive organs (g /100 g BW) of broiler chicks fed on diets containing 200 ppb of aflatoxin B1

	Diets*							
Digestive organs	Control	Y	Z	Y+ Z	Y+C	Z+C	Y+ Z+C	SEM
Proventriculus	0.69^{a}	0.57^{b}	0.56^{b}	0.50^{c}	0.47^{d}	0.47^{d}	0.47^{d}	0.1503
Gizzard	4.45^{a}	3.84^{b}	3.48^{c}	3.50^{c}	3.03^{d}	3.03^{d}	3.06^{d}	0.0059
Liver	3.93^{a}	3.17^{b}	3.19^{b}	2.75^{c}	2.57^{d}	2.54^{d}	2.44^{e}	0.0026
Spleen	0.16^{a}	0.14^{b}	0.12^{c}	0.11^{d}	0.10^{e}	0.10^{de}	0.09^{f}	0.0057
Pancreas	0.39^{a}	0.36^{b}	0.35^{b}	0.35^{b}	0.33^{c}	0.32^{c}	0.32^{c}	0.0210
Bursa	0.17^{d}	0.18^{d}	0.17^{d}	0.21^{c}	0.20^{c}	0.23^{b}	0.25^{a}	0.0039

*Diets, comprising of: Control (basal diet = diet contaminated with 200 ppb of aflatoxin B1); Y (basal diet supplemented with only 0.5% yeast); Z (basal diet supplemented with only 1.5% zeolite); Y+Z (basal diet supplemented with 0.5% yeast+1.5% zeolite); Y+C (basal diet supplemented with 0.5% yeast+ 1.5% charcoal); Z+C (basal diet supplemented with 1.5% zeolite+1.5% charcoal); Y+Z+C (basal diet supplemented with 0.5%.yeast+1.5% zeolite + 1.5% charcoal).
$^{a-f}$Row Means with different superscripts differ significantly (P< 0.05).
SEM: Standard error of means.

Table 4: Effects of zeolite, active charcoal and yeast on serum biochemical properties (mg/dl) of broiler chicks fed on diets containing 200 ppb of aflatoxin B1

	Diets*							
Traits	Control	Y	Z	Y+Z	Y+C	Z+C	Y+Z+C ++C	SEM
Glucose	138.8^{c}	157.5^{ab}	155.9^{b}	159.0^{a}	158.0^{ab}	160.5^{a}	158.9^{a}	0.93
Cholesterol	101.8^{c}	147.4^{b}	147.6^{b}	166.2^{a}	152.6^{b}	165.8^{a}	162.7^{a}	2.33
Triglyceride	91.5^{f}	91.8^{f}	101.5^{e}	128.6^{b}	112.0^{d}	133.8^{a}	123.1^{c}	1.65
Albumin	0.77^{d}	1.1^{c}	1.1^{c}	1.3^{a}	1.2^{b}	1.2^{b}	1.2^{b}	0.15
Uric acid	4.6^{d}	4.8^{d}	4.8^{d}	6.1^{b}	5.2^{c}	6.1^{b}	7.1^{a}	0.05

*Diets, comprising of: Control (basal diet = diet contaminated with 200 ppb of aflatoxin B1); Y (basal diet supplemented with only 0.5% yeast); Z (basal diet supplemented with only 1.5% zeolite); Y+Z (basal diet supplemented with 0.5% yeast+1.5% zeolite); Y+C (basal diet supplemented with 0.5% yeast+ 1.5% charcoal); Z+C (basal diet supplemented with 1.5% zeolite+1.5% charcoal); Y+Z+C (basal diet supplemented with 0.5%.yeast+1.5% zeolite + 1.5% charcoal).
$^{a-f}$ Row Means with different superscripts differ significantly (P< 0.05). SEM: Standard error of means

A similar relative bursa weight was measured for the chicks in control, Y and Z diets, as well as it for the chicks in Y+ Z and Y+ C groups, by chicks in Z+C and Y+Z+C diets having different bursa weights with the heaviest weight of bursa for the Y+Z+C diet group (p<0.05). Dietary absorbent supplementation against aflatoxin B_1 toxics tended to increase the glucose, cholesterol, triglycerides, albumin and uric acid concentrations in serum of broiler chicks (Table 4).

Compared to the birds in control group, serum glucose and cholesterol levels were high in other groups with the highest levels of them for the serum of birds in the Z+C and Y+ Z diet groups, respectively (p< 0.05). The highest triglyceride level was observed for the serum of birds in Z+C diet group (p< 0.05). The y diet had no significant effect on the serum triglyceride levels. Birds receiving Y+Z and Y+Z+C diets had the highest serum albumin and uric acid levels, respectively (p< 0.05). The Y and Z diets had no significant effects on the uric acid concentration of serum.

DISCUSSION

The supplementation of aflatoxin B1 contaminated diets with yeast, zeolite and activated charcoal, alone or in combination, affected the performance, biochemical traits and the internal organ weights of broilers in the present study.

The negative effects of aflatoxin B1 on the BWG, FI and FCR of chicks observed are consistent with previous reports [15, 16]. The addition of yeast alone to the aflatoxin B1 contaminated diet for 49 days of experimental period increased BWG, FI and LBW of broilers by 19.3, 20.5 and 20.1 percentages, respectively. Stanley *et al.* [17] reported that the addition of *Saccharomyces cerevisiae* to an aflatoxin containing diet had ameliorative effects on the broiler performances. The beneficial effects of yeast culture when supplemented to aflatoxin contaminated diets in Japanese quail were also clearly indicated by Yildiz *et al.* [7]. Several hypotheses explain the ability of yeast to alleviate the aflatoxicosis effects. Raju and

Devegowda [18] and Yildiz *et al*. [7] reported that esterified glucomannan compounds, located in the cell wall of *Saccharomyces cerevisiae*, have a high affinity for aflatoxins in both *in vivo* and *in vitro* situations. It is possible that *Saccharomyces cerevisiae* represses the aflatoxicosis severity via chelating aflatoxin transported and eliminated via intestinal tract. Furthermore, it has been reported that the addition of yeast to an aflatoxin containing diet causes an increase in enzymes that modify the aflatoxin effects by stimulating bio-transformation of them, thus altering the duration and intensity of the toxic [10].

Results of this study showed that Z diet improved the broiler performances when compared with the control group. However, when Z and Y diets were compared, Z diet was more effective in improvement of growth parameters indicating that zeolite has more ability to act as an active absorbent in reducing the aflatoxin negative effects and form a stable complex with mycotoxin and thus reduces the toxic effects of aflatoxin B1 in favorite of the performance of broilers. These results are supported by the work done by Miazzo *et al*. [19] who reported that aflatoxin contaminated diet decreased body weight gain of broilers by about 10 percentages, whereas the addition of zeolite to diet resulted in only a one percent reduction. Pervious studies have demonstrated that zeolite has ability to absorb mycotoxins with high affinity. *In vitro* results suggested that zeolite is a strong binder for aflatoxins. An inert, stable and insoluble complex between zeolite and aflatoxin was assumed to be responsible for the preventing of toxin absorption in the intestine [20]. Also, these results are in agreement with the previous reports indicating the protective effects of zeolite compound against the effects of aflatoxin on the body weight and feed conversion ratio of broiler chicks [2, 9, 15] and turkeys [11].

The performance of birds fed on Y+Z diet was more different from that in birds consuming Y or Z diets. The Y+ C or Z+ C diets resulted in higher BWG and more suitable FCR in broilers. These results are in agreement with the findings of Dalvi and McGowan [10], Hesham *et al*. [6] and Kubena *et al*. [15], which they reported a trend in improvement of body weight gain and feed intake of chickens when activated charcoal was added to the aflatoxin contaminated diets. Rosa *et al*. [21] reported that charcoal acts as an insoluble carrier that adsorbs molecules nonspecifically, thereby eliminates their absorption from the intestinal tract. The protective effect probably involves the sequestration of the toxic molecules in the gastrointestinal tract of broilers and the chemisorptions to the charcoal [15].

Compared to the other groups, the Y+Z+C diet resulted in higher BWG and LBW and more suitable FCR in broilers. These effects were more pronounced during the starter period and less for the grower and finisher periods. Results indicated that the combinations of these materials could have higher ability to act as better absorbents for aflatoxins. In other words, it might be thought that the combinations of yeast, zeolite and active charcoal could have a kind of synergetic effect in alleviating the aflatoxicosis in growing broilers.

The liver is the primary target organ of toxins. In addition, increases in the relative weights of liver, proventriculus, pancreas [9], spleen and gizzard [2, 16, 20] of broilers fed on aflatoxin contaminated diets have also been reported previously. In poultry, the relative weight of liver was reported to be increased more than any of the other organs by the presence of aflatoxin in diet [22]. The increase in relative weight of gizzard may be due to the result of severe inflammation and thickening of the mucosal layer which caused by toxic effects of aflatoxin [23]. The increase in the relative weight of spleen might be a compensatory mechanism for the drop in weight (and activity) of the bursa of fabricius [6]. A decrease in relative weight of bursa of fabricius in chicken fed aflatoxin contaminated diet was also reported by Kubena *et al*. [9] which could be attributed to an immunosuppressive effect of aflatoxin. In our study, dietary aflatoxin absorbent supplementation tended to decrease the relative weights of liver, proventriculus, gizzard, spleen and pancreas and increase the relative weights of bursa of fabricius. Kubena *et al*. [2] reported that adsorbents have been effective in preventing or ameliorating changes in organ weights of chicks fed on aflatoxin added diets. These results indicate that the combination of yeast, zeolite and activated charcoal in the forms of Y+ C, Z+ C or Y+ Z+ C can be more effective for reducing the negative effects of aflatoxin on the internal organs.

Blood biochemical parameter changes in chicks fed on aflatoxin contaminated diets in this study were more or less similar to those reported in the literature. Decreases in the serum total protein and albumin contents [21] and the globulin, cholesterol and urea nitrogen [9] have been observed in chicks suffering from aflatoxicosis. In our study, there was variation on effectiveness of different absorbents and their combinations in the preventing of changes in serum biochemical parameters. The reasons for these variations are unknown, but they appear to have beneficial effects to the alleviate toxicity of the aflatoxin in diets. Therapeutic effect of activated charcoal on urea nitrogen and glucose concentrations in turkey poults fed aflatoxin added diet was reported by Edrington *et al*. [11]. In contrast to our finding, concentrations of albumin, cholesterol and triglycerides were not improved when activated charcoal was added to the aflatoxin contaminated diets [2, 11]. The amelioration in plasma albumin changes observed in chicks fed on Z diet in our study has been also reported by other researchers [21, 24, 25].

This study seems to be one of the rare reports describing the effects of different combinations of yeast; zeolite and active charcoal added to the broiler aflatoxin contaminated diets. Results indicated that the mixtures of the tested absorbents were more effective for reducing the signs of aflatoxin B1 toxicities in growing broilers. Further studies are, however, required to evaluate different levels of these absorbents to alleviate the negative effects of other mycotoxins.

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