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Original Article

Efficacy of Baker Yeast in Ameliorating Aflatoxicosis in Broiler Chicks Fed Aflatoxin-Contaminated Diet

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ABSTRACT

A total of 500 days-old Ross chicks with an average live weight of 53 g were used p to evaluate the effects of (Saccharomyces cerevisiae) backer yeast (BY) with or without Oxytetracycline (OTC) on the performance and nutrient digestibility of growing broiler chicks given contaminated aflatoxin (AFB1) basal diet. Birds were equally divided into five dietary treatment groups. Each group had 100 birds in five replicates (cages) and was assigned to one of the five dietary treatments in a randomized complete block design experiment. Treatments consisted of a control group maize-soybean meal basal diet and four test groups with aflatoxin AFB1(1% of moldy rice), AFB1 + baker yeast BY (0.003g/kg), AFB1+OTC (2.4g/Kg), and AFB1+BY+OTC respectively. Feed and water were provided ad libitum. Supplementation of BY and OTC or both BY+OTC was not statistically significant on feed intake between treatments during the 42 days experiment however, feed intake tended to be the lowest for the control group. Baker yeast and oxytetracycline added to the aflatoxin-containing diet significantly improved gain body weight; feed conversion ratio and digestibility of protein, fat and nitrogen-free extract efficiency, no significant differences were observed for fiber digestibility. Results of this research revealed that baker yeast as well oxytetracycline added to the aflatoxin containing diet improved the performance as well the digestibility of nutrients in broiler chickens. In general, The principal finding from this research is that the baker yeast (Saccharomyces cerevisiae) added to the aflatoxin contaminated diet in broiler diets could significantly relieve the negative effect of AFB1 on chicken's production performance and nutrient digestibility .

Keywords: Aflatoxin, baker yeast (Saccharomyces cerevisiae), performance, apparent digestibility, feed conversion ratio; gain body weight.

INTRODUCTION

The presence of fungal toxins in the feed is one of the main problems faced by the poultry industry because of the harmful effects of these toxins on the productivity of poultry (Zhang and Caupert, 2012). The harmful effect observed on poultry health, including poor performance (Magnoli *et al*., 2011; Rosa *et al*., 2012 and Forte *et al*., (2015); Liu *et al*,. 2018), liver and kidney lesions (Ortatatli *et al*., 2005; Oliveiraa, *et al*,. 2015), immunity damage (Marin *et al*., 2002; Keller *et al*., 2012; Forte *et al*., (2015); Liu *et al*., 2018), liver fibrosis and carcinogenic (Girish & Devebowda, 2006), loss of appetite, depression, bleeding, diarrhea and death (Zghini *et al*., 2005; Denli and O'Kan, 2006) decrease in egg production (Denli *et al*., 2004; Girish & Devebowda, 2006; Uttpatel *et al*., 2011) and hematology problems (Zhao *et al*., 2010). Effects of exposure to aflatoxin in large quantities may lead to mortality, but in the case of low levels of pollution is harmful if given to the animal over a long period (Ghahri *et al*., 2010; Yunus *et al*., 2011). Aflatoxins, a group of closely related and biologically active fungal toxins are produced by metabolites produced by some strains of Aspergillus flavus and Aspergillus parasiticus. Among the known mycotoxins, aflatoxin B1 (AFB1) is the most potent hepatotoxic and immunosuppressive in poultry (Martinez-de-Anda *et al*., 2010). Several detoxification strategies have been proposed-disrupting the activity of contaminated fungal toxins such as physical separation, thermal inhibition, irradiation, bacterial degradation and treatment with a variety of chemicals. The approach has been suggested to detoxify fungal toxins contaminated food such as bentonite, calcium, and sodium hydroxide aluminosilicate, zeolite, activated carbon, inorganic absorbent substances, and a mixture of organic acids and aluminosilicates have shown significant results in detoxification of aflatoxin in feed (Li *et al*. 2014; Lala *et al*. 2016).

There are many living organism strains used for detoxification purposes (Onifade, 1998), Progress in biotechnology has opened up a new area of study to prevent fungal toxicity. Yeast was considered as a promising in the detoxication of aflatoxins (Che *et al*. 2011). Aravind *et al*., (2003) found that live yeast has reduced the harmful effects of aflatoxin in poultry. When yeast is added as anti-aflatoxins for animals in the feed the positive effects are based mainly on the ability of yeast strains to stimulate growth, improve the animal intestines microflora and as immune system stimulant (Khan *et al*., 2017). A selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbial l, has beneficial effects upon the host health (Gibson *et al* 2010). Recently years, it has been recommended the use of Saccharomyces Cerevisiae yeast for the detoxification of aflatoxin in feeding broiler chickens. The susceptibility of animals to aflatoxins varies with species and age in general, younger animals are more susceptible than adult animals. A significant reduction in body weight and feed efficiency was reported in chicken broiler diets with AFB1 (Girish, and Devegowa 2006; Uttpatel *et al*., 2011; Chen *et al*., 2017). Due to the AFB1 toxicity the susceptibility of chicks to infections increases; it is advisable that antibiotics would be given to birds experiencing an infection along with a mycotoxicosis. Oxytetracycline is a therapeutic antibiotic in poultry, commonly used in the Libyan poultry industry as effective in controlling bacterial infections caused by Pasteurella multocida (fowl cholera) and Escherichia coli and in reducing shed of Salmonella typhimurium in turkey poults.

The aim of this study was to evaluate the efficacy of inclusion baker yeast (Saccharomyces cerevisiae), with or without Oxytetracycline in naturally aflatoxin contaminated diet on performance and nutrients digestibility of broiler chicks.

MATERIALS AND METHODS

Birds and management: A total of five hundred one-day-old broiler chicks (Rose) with an average live weight of 53 g were housed in a windowless metabolism room which was thermostatically controlled. The light was provided continuously, the room temperature was maintained by means of electrical, and gas heating at 37°c and gradually reduced to 27c° by the third week and it kept relatively constant in the range of 25-27°c throughout the experimental period of 42 days (6weeks). Chicks

were grouped in the twenties in each cage for five treatments of five replicates in a complete randomized block (CRB) design. The chicks were fed to one week of age ad-libitum on a standard based diet. Water was provided ad-libitum during the experimental period. Under each cage was placed a removable metal tray, which was used for collecting the excreta. Birds were vaccinated against Newcastle disease, infectious bronchitis, and infectious bursal disease.

Treatment Diets:- The standard maize-soybean meal basal diet (Table 1) with 22% crude protein was formulated to meet nutrient requirements according to NRC (1994) which was based on corn, soybean and fish meal (without added growth promoter or antibiotics) and fortified with the premix (table 2). The basal diet was then weighed into portions as needed for each treatment and the five treatment diets were formulated by supplementation the appropriate proportion of AFB1, BY, and OTC Treatment (1) a control diet without additives, treatment (2) with addition of aflatoxin AFB1(1% of moldy rice), treatment (3) AFB1 + baker yeast BY (0.003g/kg), treatment (4) AFB1+ OTC (2.4g/Kg), and treatment (5) AFB1+BY+OTC. The AFB1 production in rice was done according to the method reported by Shotwell *et al*., (1966). The AFB1 test diet was prepared by the addition of moldy rice at 1% and was adjusted in the feed formulation. Feeds were analyzed for aflatoxins by thin layer chromatography, according to Lin *et al*., (1988) and Oguz *et al*., (2011). The toxin was measured by spectrophotometric methods and it was estimated to be 180 µg/kg. Weekly feed consumption and body weights were recorded and weight gain was calculated. Feed conversion rate (FCR) was calculated as the amount of feed consumed per gain body weight. The methods used to determine the apparent digestibility of nutrients was the total collection method. The total experimental period was 42 days. Excreta were collected twice a day from each cage which represented the replicate. Excreta samples were placed in plastic bags, weighed and stored in a freezer at -20°C. At the end of the experimental period, feed intake and total effect of treatment supplementation were determined. The samples of the feed and excreta were analyzed according to the procedure described by methods of A.O.A.C. 2000 for crude protein, crude fiber, crude fat, moisture, and nitrogen-free extract. Because a part of nitrogen in excreta originates from uric acid, the fecal nitrogen was corrected for uric acid nitrogen. In this regard, the excreta were calculated as total nitrogen minus nitrogen in uric acid.

Data obtained from the experiment were calculated and expressed as Mean ± SE on all parameters. The results were subjected to statistical analysis of variance (ANOVA) using the general linear model (GLM) procedure of MINITAB (2015) and where significant F value for treatment effect was found, means were compared by Least Significant Difference (LSD). The tests were used to compare treatment means at ($P<0.05$) significant level.

Table 1: The Composition of diet:

Composition	**%**
Corn	60
Soybean	27
Fish meal	6
Vegetable Oil	2
Methionine	0.035
Dicalcium phosphate	2
Salt	1.62
Limestone	1
Premix	0.3
Determined Analysis	
Moisture	9.5
Crude protein	22.08
Ash	9.77
Ether extract	3.23
Crude fiber	2.67
Nitrogen-free extract	50.73
Calcium	1.00
Phosphrous	0.40

RESULTS

The means of feed intake, gain body weight, feed conversion ratio, apparent digestibility of protein, fat, fiber and nitrogen-free extract are presented in Table 2 and 3 respectively. Feed intake decreased by aflatoxin contamination but was not statistically different between treatments during the 42 days experiment, however, the results showed that aflatoxin supplementation decreased feed intake by 5.7% in comparison to the control. The gain body weights of birds in group given diet with BY or OTC or combination of BY and OTC were significantly ($P>0.05$) higher compared to the group of birds fed a contaminated diet with AFB1.

The results of this experiment showed that the gain body weight of birds in groups given diets with BY or OTC or BY+OTC increased by 18%, 14% 19.5%, respectively compared to the contaminated diet with AFB1. Birds in groups given diets with BY or OTC showed no difference in BGW compared to birds in the control group. BY and OTC supplementations improved the feed efficiency. The feed conversion ratio of birds in group given diet with BY or OTC or BY+OTC were significantly (P<0.05) lower compared to the group fed AFB1contaminated feed but was not statistically different (P>0.05) in comparison to the control group. The inclusion of BY, OTC, and BY+OTC reduced feed conversion rate by 10.9, 9 and 14%, respectively. The presence of aflatoxin showed a higher feed conversion rate, and the best conversion was seen with the treatment supplemented with BY+ OTC together. There were no deaths attributable to AFB1 within 42 days of the trial in any of the groups. Aflatoxin significantly (P <.05) reduced the apparent digestibility of protein, fat, and NFE. No significant effect was seen with the digestibility of fiber. On the other hand, the treatment with BY or OTC or the BY+ OTC significantly (P <.05) improved the digestibility of protein, fat, and NFE in comparison to the group of birds fed AFB1 contaminated feed. No significant improvement in the digestibility of protein, fat, and NFE in comparison to the control group, all the values are below that of the control.

Table 2: premix composition

Ingredients	%	Ingredients	%
Vitamin A	4000000 IU	Biotin	33333 mc. g.
Vitamin E	6.666mg	Choline	166 mg
Vitamin D3	833333 IU	Methionine	3331333 mg
Vitamin K	3666mg	Calcium	3333 mg
Vitamin B1	666 mg	Manganese	33333 mg
Vitamin B2	1666mg	Copper	2500 mg
Vitamin B5	10mg	Cobalt	33 mg
Vitamin B6	1000mg	Iodine	166 mg
Vitamin B12	5 mc. g.	Selenium	33 mg
Folic acid	333 mg	Iron	10 mg

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Table 3: Show BWG (the body weight gain *gm./day/bird*), FI (feed intake *gm./day/bird*) and FCR (feed conversion ratio *gain/feed*).

	Control	C+ AFB_1	C+ AFB_1+ BY	C+ AFB_1+OTC	C+ AFB_1+BY+OTC	LSD
BWG	80.08± 0.33 ^{a}	68.38±0.59^{c}	80.80± $^{0.62a}$	78.11±0.52 ^{b}	81.78±0.63 ^{a}	±1.5
FI	131.93±2.2	124.48±2.8	129.77±2.8	128.52±2.6	126.87±3.1	NS
FCR	1.65±0.03^{b}	1.82±0.05^{a}	1.62±0.04 ^{bc}	1.64±0.03^{bc}	1.56±0.04 ^{bc}	0.09

C (control), AFB1 (aflatoxin), BY (baker east), OTC (oxytetracycline), LSD Least significant difference, NS not significant.

Table 4: Show the percent apparent digestibility of protein, fat, fiber and NFE (mean±SE)

	Control	C+ AFB_1	C+ AFB_1+BY	C+ AFB_1+OTC	C+ AFB_1+BY+OTC	LSD
Protein	77.03±1.17 ^{c}	67.13±0.83 ^{a}	69.67±1.1 ^{b}	68.21±1.27 ^{ab}	69.33±1.27^{b}	±1.9
Fat	67.24±0.83^{c}	50.26±1.63 ^{a}	59.58±2.7 ^{b}	58.80±2.85 ^{b}	61.64±2.21^{b}	±3.5
Fiber	42.62±2.28	38.32±0.92	40.72±1.1	39.66±1.98	41.36±1.88	NS
NFE	59.7±1.87 ^{b}	46.28±2.46 ^{a}	56.78±2.7 ^{b}	53.4±1.34 ^{b}	55.26±1.61^{b}	±5.2

C (control), AFB_1 (aflatoxin), BY (baker east), OTC (oxytetracycline) and NFE (nitrogen- free extract**),** LSD Least significant difference, NS not significant.

DISCUSSION

Aflatoxins are important in the poultry industry because of the toxicity they may cause in the feed (Astoreca *et al*., 2011). Aflatoxin contamination causes significant losses in the animal economy, leading to a significant reduction in productivity, as well as loss of product quality such as meat and milk (Zhao *et al*., 2010.

Results obtained in this study showed that the AFB1-contaminated diet severely affected the broiler performance and that the addition of yeast showed significant improvement. There was a significant improvement in gain body

weight and feed conversation rate, although voluntary feed intake was not affected. The improvement in the performance of animals in the presence of aflatoxin may be due to several factors when the yeast was included in the diet according to studies and research published in the past (Giacomini *et al*., 2007; Forte *et al*., 2015). The improvement of the performance in yeast feeding can be attributed to the presence of selenium in the yeast which has a positive effect on growth through the role of thyroid hormone (He *et al*., 2013). Rottes *et al*. (1996) showed that the improvement in the performance of broiler chickens when fed yeast extracts may be due to the beneficial effects of nucleotides in the yeast extract and the presence of gluco-manganese, fructo-oligossaccharides in yeast. The results consent for feed intake with Kamalzadeh *et al*., (2009) who reported that feed consumption increased by the addition of Mycosorb, but was not statistically different between treatments during the 42 days experiment, however, tended to be lowest for the control group. This result also disagreed with that of Zhao *et al* (2010).who reported that feed intake was significantly ($P<0.05$) reduced with the addition of yeast in broiler diet .

No statistically significant differences were found in the productivity rates of chicks fed on an uncontaminated diet, and chicks fed diets containing yeast, indicating that the yeast is neutral and non-toxic. Similar results were obtained by Yalçinkaya *et al*., (2008), which evaluated the effects of feed supplemented by different percentages of managosaccharides manna from saccharomyces cerevisiae (0.05, 0.1, and 0.15 %). The current study showed that the presence of baker yeast has completely reverted growth performance to normal values, proposing such a protective effect of the yeast against aflatoxicosis.

The adverse effects of AFB1 have been linked to growth performance with reduced protein and fat digestion and NFE, possibly as a result of the degradation of the digestive system and the metabolism of birds that were in agreement with Denli *et al*., (2009). The digestibility of crude protein, crude fat and nitrogen-free extract in this study was consistent with Liu *et al*., (2018.)

It is believed that yeast has a very beneficial effect as potentiating adsorbing agents to isolate microbial toxins in the gastrointestinal tract and therefore plays an important protective role from the effect of mycotoxin. In this sense, yeast presentation in the diet may reduce the toxic effects of aflatoxin on animals because the AFB1-yest compound reduces the absorption of mycotoxin in the digestive system (Gratz *et al*., 2007). Magnoli *et al*., (2016) noted that the yeast strains tested in his study were potential adsorbents for AFB1 and therefore, had the beneficial advantage of animal performance and productivities.

Several studies have indicated a positive effect of yeast feeding on haematological indices indicating that yeast can promote red blood cells, haemoglobin concentration, packed cell volume, and mean cell volume (Nworgu, 2007, Nowaczewski and Kontecka, 2011). Some studies have shown that the broilers given the aflatoxin treatment diet have produced more WBC to fight infection suggesting that on feeding yeast have contributed to the enhancement of WBC (Mitruka and Rawnsky 1977).

One of the strategies for reducing the exposure to mycotoxin is to decrease their bioavailability by including various mycotoxin-adsorbing agents in the compound feed, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs. This strategy relies on the physical binding of the toxin during digestion, so that the toxins remain in the intestinal lumen and are then voided via faeces thus limiting toxin bioavailability (Firmin *et al*., 2010). The inactivation of the mycotoxin by two adsorbents yeast cell wall extract (YCW) and hydrated sodium calcium aluminosilicate (HSCAS) studied by Yiannikourisa *et al*., (2013), showed that YCW was more efficient inactivation of the mycotoxin and that it reduced the accumulation of toxin in the intestinal tissue by 40%. Yalcin *et al*., (2018) studied the effectiveness of binders as detoxification of aflatoxin stated that the organic, inorganic and mix toxin binders (TBs) were found to be effective to bind AFB1 among the TBs and could be applied to reduce the negative effects of AFB1 in poultry feeds.

The principal finding from this research is that the baker yeast (Saccharomyces cerevisiae) added to the aflatoxin contaminated diet improved the performance and the digestibility of protein, fat, and nitrogen-free extract in broiler chickens. This could suggest that baker

yeast could partly counteract some of the toxic effects of AFB1 in broiler chicks and that further investigations may be necessary for the use of viable yeast cultures in poultry diets

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