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Evaluation Of Probiotic To Ameliorate The Toxicity Of Aflatoxin In Broiler Chickens.

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ABSTRACT: *This study was conducted to demonstrate the impact of yeast fractions on reducing aflatoxicosis on broiler. The effects of probiotic (Toyocerin) on toxicity of aflatoxin were evaluated using control and six dietary treatments on broiler chickens from 0-6 weeks of age. Aflatoxin (200, 400 and 800* µg /kg*) significantly reduced feed intake, weight gain and feed efficiency. Significant interaction between aflatoxin and probiotic were observed for their additive effects on body weight, feed intake, feed efficiency, apparent digestibility of protein, fat, fibre and nitrogen free extract (NFE). Probiotic significantly (P≤0.05) improved body weight, feed intake and feed efficiency increased apparent digestibility of protein, fat, fibre, nitrogen free extract, total digestible nutrients and packed cell volume. These results suggest that the addition of probiotic in case of aflatoxicosis may ameliorate and improve the performance as well the retention of nutrients with broiler chickens.*

Keywords: *Aflatoxin, Toyocerin, performance, apparent digestibility, feed conversion ratio, gain body weight, total digestible energy and packed cell volume.*

I. Introduction

Among the known mycotoxins, aflatoxin is the most important to poultry. Aflatoxin B_1 (AFB_1) is the most potent hepatotoxic and immunosuppressive. It has been reported as well to cause oral lesions and decreased feed intake in broiler chickens. This mycotoxin contaminated feedstuffs when consumed, produce a range of devastating effects on the general well-being and productivity of poultry (1).

Practical methods to detoxify mycotoxin contaminated grain on a large scale and in a cost effective manner are currently not available. At present, one of the most promising and practical approaches is the use of adsorbent. Research indicates that a number of adsorbents are capable of adsorbing aflatoxin B_1 and reducing its toxic effects.

A natural product called glucomannan, a cell wall derivative of *Sacchromyces cerevisiae*, has received much attention in minimizing mycotoxins present in the contaminated diets of livestock and poultry (2,3,4). Some dairy strains of lactic acid bacteria were found capable of removing aflatoxin B_1 from contaminated liquid media via a rapid process involving the removal of approximately 80% of AFB_1 immediately upon contact without further incubation (5, 6). Furthermore, Audisio(7)claimed that chicks treated with probiotic Enterococcus faeciumcan protect newly hatched chicks from Salmonella Pullorum infection. Probiotics supplements have beneficial effects on animals that stimulate appetite (8), stimulate the immune system (9, 10), although the application of probiotics has demonstrated trends with respect to altered aspects of immune response, the underlying mechanisms by which that occurs are unclear (11), intestinal microbial balance (12), improves digestive enzymes (13), improving feed consumption in chickens(8, 14), improving feed conversion ratio (15). It has a beneficial effect on host health and has been recommended as a health and growth promoter for chickens and turkeys (16). Toyocerin, a preparation containing the bacterial strain Bacillus Toyoi, has shown beneficial effects when consumed by Japanese quail(17), chicken and turkey (18, 19). Toyocerin is used as vital microorganisms in nutrition to promote the growth and health of the digestive tract (20).

The objective of the present work was to evaluate the efficacy of probiotic (ToyoCerin) feed additives in aflatoxicosis on performance and nutrient digestibility.

II. MATERIAL and METHODS

A total of 420 day-old sexed commercial broiler chicks were divided at random into 7 groups each of 3 replicates of 20 chicks. The test diets treatments consisted of a control basal diet which was based on corn, soybean and fish meal (Table 1, Table 2) and the test diets were prepared by addition of moldy rice to the basal diet.All diets were formulated to cover the nutrient requirements of chickenNRC (21). Moldy rice powder was obtained by inoculating known amount of broken rice with Aspergillusflavus supplied by Agriculture Research Center Tripoli. AF production was carried out in accordance with the method based on that reported by Shotwell(22) et al. (1966). Quantified by AFLATEST, Probiotic (1000g/ton) (Tyocerin), a proprietary product of (Lohmann Animal Health GmbH & Co. KG Heinz-Lohmann-Straße 4 27472 Cuxhaven, Germany) were tested from 0-6 weeks of age. The birds were randomly assigned to the following treatment groups. Control diet without additives (treatment 1), AFB_1 200µg /Kg (treatment 2), probiotic +AFB_1 200 µg /kg (treatment 3), AFB_1 400 µg /kg (treatment 4), probiotic + $AFB_1$400 µg /kg, (treatment 5), $AFB_1$800 µg /kg (treatment 6), probiotic + $AFB_1$800 µg /Kg, (treatment 7), Feed and water were provided for *ad- libitum.* Feed intake and excreta output and weekly from 0-5weeks were measured. The samples of the feed and excreta were analyzed according to the procedure described by methods of A.O.A.C. (23) for crude protein, crude fiber, crude fat, moisture and nitrogen-free extract. Total fat contents of feed, and excreta were determined by extraction of samples with petroleum ether. The determination of nitrogen in feed and excreta was performed with the macro-Kjeldahl method. Because a part of nitrogen in excreta originates from uric acid, thefecalnitrogen was corrected for uric acid. The total digestible nutrients calculated as (TDN) = dig. Protein + dig.Fibre + dig.NFE + (dig. Fat x2.25) Packed Cell Volume (PVC) was determined by microhematocrite method which is quickest and most practical to study the status of birds. The data obtained were analysed with SAS (24), GLM procedure and means were compared with Duncan multiple range test.

Table 1. Feed composition

Item	**%**
Corn	60
Soy bean	27
Fish meal	6
Vegetable Oil	2
Methionine	0.04
Dicalcium phosphate	2
Salt	1.65
Limestone	1
Premix*	0.3
Determined Analysis	
Moisture	9.5
Crude protein	23.57
Ash	9.77
Ether extract	3.23
Crude fiber	2.67
Nitrogen free extract	50.73
Calcium	1.00
Phosphorus	0.40

*Premix as shown in Table(2)

Table 2 premix composition *

Item	%
Vitamin A	4000000 IU
Vitamin E	6.666mg
Vitamin D3	833333 IU
Vitamin K	3666mg
Vitamin B1	666 mg
Vitamin B2	1666mg
Vitamin B5	10mg
Vitamin B6	1000mg
Vitamin B12	5□ g
Folic acid	333 mg
Biotin	33333 □ g
Choline	166 mg
Methionine	3331333 mg

III. RESULTS

Aflatoxin individually depressed body weight, feed intake and feed efficiency (Table 2).The weight gain was lower significantly (<0.05) in chicks fed aflatoxin diets of (400and 800 µg /kg g /kg aflatoxin). The addition of the probiotic has dramatically improved significantly ($p<0.05$) the weight gain of groups fed 400and 800 µg/kg levels of aflatoxicosis diets (Table 3).Chicken fed aflatoxin diets 200, 400 and 800 µg /kg /kg supplemented with probiotic gained 23.5%, 28.7% and 21.7% respectively and ate 10.8%, 12.2 % and 13.8% more feed than birds ate AFB_1 contaminated feed from 1 to 42 days of age. Feed intake was found to decrease significantly ($P<0·05$) in the AFB1-administered group, compared with the control group. Chicken fed probiotics added to aflatoxin diets consumed significantly more feed than the AFB_1diets(Table 3).The feed: gain ratios were

significantly ($p<0.05$) poorer for groups fed aflatoxin diet (400and 800 µg /kg g /kgaflatoxin) as compared to the control group. The probiotics addition to the aflatoxin diets resulted in better feed conversion ratio (Table 3). Studying the effect of adding probiotic to aflatoxicosis on the digestibility of protein, lipid,fibre as well the nitrogen free extract are shown in table (4). However, the probiotic supplementation in the diets, resulted in an improvement in protein, lipid, fibre and nitrogen free extract digestibility in comparison to AFB_1 contaminated diet 200, 400 and 800 µg /kg. The apparent digestibility of protein, lipid, fiber and nitrogen free extract were found to decrease significantly ($p<0.05$) in groups received only AFB1 compared with the control group (Table 4).

Table (5) shows the effect of probiotic on total digestible nutrients (TDN) and packed cell volume (PCV). The results of TDN significantly ($p<0.05$) decreased by as much as 17% and 20% with chicken ate aflatoxin diets 400 and 800µg /kg respectively. PCV was lower with chicken fed the three levels of aflatoxin 200, 400 and 800 µg /kg a decrease of 5%, 3% and 2.8% respectively. The PCV values were slightly improved when probiotic was supplemented in the diets.

Table 3.Effect of treatments on weight gain, feed intake and feed conversion ratio (mean± SE).

Treatments	Weight gain (gm.)	Feed intake (gm.)	Feed conversion Ratio
Control	1954.3 ± 72.9	3141.2 ± 117.3	1.51 ± 0.05
AFB_1 (200 g/kg)	1445.7 ± 68.2**	2657.3 ± 125.3**	1.71 ± 0.08
AFB_1(200 g/kg)+Probiotic	1819.6 ± 83.1	2999.9 ± 137.1	1.54 ± 0.07
AFB_1(400 g/kg)	1135.4 ± 56.8**	2196.8 ± 109.7**	1.77 ± 0.09**
AFB_1(400 g/kg)+Probiotic	1455.8 ± 73.3**	2465.7 ± 124.2	1.59 ± 0.08
AFB_1(800 g/kg)	1083.1 ± 49.4**	2115.9 ± 96.5**	1.82 ± 0.08**
AFB_1(800 g/kg)+Probiotic	1318.2 ± 65.7**	2406.9 ± 119.9	1.69 ± 0.08
LSD±	212	372	0.24

AF- Aflatoxin, LSD -Least significant ratio, SE -Standard error.

Table 4. Effect of treatments on digestibility of crude protein (CP), crude fibre (CF), ether extracts (EE) and nitrogen free extract (NFE) (Mean±SE**).**

Treatments	CP %	EE %	CF %	NFE %
Control	56.49±2.11	69.83±2.61	50.07±1.86	50.45±1.88
AFB_1 (200 g/kg)	52.46±2.47	64.25±3.02	45.06±2.12	42.76±2.02
AFB_1(200 g/kg)+Probiotic	54.17±2.47	67.58±3.08	47.29±2.16	46.65±2.13
AFB_1(400 g/kg)	43.76±2.18**	60.09±3.00**	40.59±2.02**	33.79±1.68**
AFB_1(400 g/kg)+Probiotic	49.67±2.50	64.05±3.23	44.22±2.22	40.66±2.04**
AFB_1 (800 g/kg)	36.61±1.66**	49.24±2.24**	34.74±1.58**	21.40±0.97**
AFB_1(800 g/kg)+Probiotic	39.64±1.97	55.78±2.17	38.47±1.91	28.54±1.42
LSD±	6.9	8.9	6.24	5.56

AF- Aflatoxin, LSD -Least significant ratio, SE -Standard error.

Table 5. Effect of treatments on total digestible nutrients (TDN) and packed cell volume (PCV) (Mean±SE**).**

Treatments	TDN	PCV %
Control	67.52±2.52	32.40±1.21
AFB_1 (200 g/kg)	61.38±2.89	27.30±1.28**
AFB_1(200 g/kg)+Probiotic	65.98±3.01	29.20±1.33
AFB_1(400 g/kg)	49.90±2.49**	26.40±1.33**
AFB_1(400 g/kg)+Probiotic	58.40±2.94	27.20±1.37
AFB_1 (800 g/kg)	45.14±2.06**	24.80±1.13**
AFB_1(800 g/kg)+Probiotic	54.37±2.71	25.50±1.27
LSD±	8.37	3.9

AF- Aflatoxin, LSD -Least significant ratio, SE -Standard error.

IV. Discussion

Contamination of feeds with AFB_1 resulted in a significant decrease in growth,feed intake and in feed conversion ratio, in addition to a significant decrease in protein, fibre and NFE digestion rates, as well a decrease in TDN and PVC. Several studies indicate that mycotoxicity causes a decrease in the amount of water intake, feed consumption as well as the gain weight and generally causes inactivity in broiler chickens (28, 29, 30).Manafi, and Khosravinia (31) revealed in there study that 500 µg /kgAFB_1 significantly ($P<0.05$) reduced feed consumption, feed efficiency, egg production as well as egg weigh. One of the most important ways to prevent mycotoxicity is to add non-nutritional sorbents that bind to aflatoxin B_1 in the poultry's digestive system. These adsorbed materials for aflatoxin are compounds with molecular weights that have the property of binding to mycotoxins so that they can pass through the digestive system. The use of yeast and its extract of the cell wall that contains 1.3 - 1.6 D-glucan and Mannanoligosaccharide are among the most important feed additives to improve performance and growth rates for poultry in poultry feeding (25). It also has a positive effect on modifying the ecosystem of intestinal microbes and stimulating the immune system, which raises the body's resistance to diseases of the digestive system (26).

The addition of commercial probiotic in different doses from 0.5 to 1.5 g per 10 kg of feed was observed to give a significant improvement in the growth of birds (27).Most studies indicated that probiotic additives in poultry nutrition worked to improve the efficiency of feed conversion, yet some other studies have shown that there is no effect on the feed conversion ratio(32). Sharif and Dabbagh(33) explained that additives probiotics (Saccharomyces cerevisiae) significantly ($P <0.05$) increased in body weight, feed intake and feed conversion ratio. The results of the present study showed that the addition of probiotics significantly increased body weight gain, improved the efficiency of feed utilization and an increase in the retention rate of nutrients. These data are consistent with previous findings on the prophylactic effects of the bio-association factor compound (34).

The positive and beneficial results observed can be attributed to the growth-enhancing probiotic effect and its ability to delay the effect of mycotoxins, which in turn are reflected on the animal health (35). Hussein and Selim (36) indicated that the inclusion of probiotic beneficially affects the growth performance, meat composition, antioxidant capacity, and fatty acid profile of the meat. The study of Salem and others (27) concluded that the liver and kidneys in the group fed on feed contaminated with AFB_1 showed pathological changes in birds while the groups fed on Nutritox probiotic significantly reduced the levels of aflatoxin poisoning in the liver. In a study on the effect of feeding probiotic, it was found that there was no significant improvement in overall performance while a significant improvement was found in the digestion rates of proteins, calcium and phosphorous(37). Some results indicated that the probiotic compounds that act as binding action for aflatoxin toxins depend on the type and amount of mycotoxin, and therefore the Saccharomyces cerevisiae strains are the best candidate for the future as an additive of non-nutritional probiotic in poultry feeding to reduce fungal toxicity (38).

The intestinal bacterial flora for animals plays an important role in digesting and absorbing feed. It is involved in the metabolism process of nutrients such as carbohydrates, proteins, fats and minerals and in the synthesis of vitamins. It was concluded that the selected Saccharomyces cerevisiaeyeast strain had beneficial properties since it was able to survive during gastrointestinal passage, to colonize the intestines and to reduce the growth of pathogenic microbial (39).Nahanshon (40) observed that Lactobacilius cultures in corn / soybean or corn / barley / soybean feed stimulated appetite and increased levels of digestion of fats, nitrogen, calcium, phosphorous and manganese in layer chickens.

The significant reduction in packed cell volume came in agreement with Hussein and Salim(36). Most caged birds have an average of PCV 22% to 35%. A packed cell volume less than 22% indicates anaemia while the one greater than 35% suggest dehydration. In this study the feeding AFB1 at the level of 800µg / Kg could show that the birds were closer to the border line for anaemia stage.

Our results revealed that feeding chicks on a diet contaminated with AFB1, modified by adding probiotic (Toyocerin) leads to a significant increase in the weight of chicks compared to chicks feeding on a contaminated and untreated diet. Aflatoxin can be degraded in feed by some probiotic compounds by interacting with the active groups in mycotoxins, which leads to a break in the active ring of mycotoxins, which leads to the destruction of mycotoxins or their conversion to non-toxic compounds. Probiotics may provide an alternative, safer, and more effective method of synthetic chemicals to prevent the growth of fungi and the decomposition of fungal toxins produced in poultry food.

V. CONCLUSION

The results indicate that supplementation of probiotic (Toyocerin) is beneficial in preventing the individual toxicity of aflatoxin in commercial broilers.

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