

Effects of probiotic feed additives on the growth performance and carcass criteria of broiler chickens exposed to an Aflatoxin B1 challenge

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Abstract

The purpose of this experiment was to examine the effects of probiotic feed additives on the growth performance, and carcass criteria of broiler chickens exposed to an aflatoxicosis challenge. A total of 256 Ross 308 broiler chicks were divided into four treatments which included eight replicates and eight birds in each over a 37-day period. Treatments included a negative control (without additions), a positive control with aflatoxin B1 for only the first 10 days (AFB1, 40 µg/kg), a negative control with probiotic (0.5 mg/kg), and a negative control with a combination of aflatoxin B1 and probiotic. Broiler chickens' body weight gain and feed conversion ratio were dramatically enhanced when their diets included probiotic supplements, either with or without Aflatoxin B1. In treated diets compared to the control group, protein, and ether extract digestibility increased. The broiler chickens fed probiotic additives in combination with AFB1 had a lower abdominal fat percentage compared to the control. Dietary treatments had no significant effect on the internal organs in broiler chickens. In conclusion, the present results indicated that probiotic supplements improved production sustainability and health status of broiler chickens exposed to the Aflatoxin B1 challenge.

Keywords: Aflatoxin; Broilers; Production; Probiotic; Sustainability.

1. Introduction

The natural environment contains a variety of biological toxins that could be harmful to both human and animal health. Currently, mycotoxins are regarded as some of the most harmful ones. They are toxic byproducts of several filamentous fungal species, primarily from the genera *Aspergillus*, *Penicillium* and *Fusarium* (Ostryl *et al.*, 2017). Aflatoxin B1 (AFB1), one of the 18 varieties of aflatoxins discovered, is the most


toxic and prevalent of them, followed by the less toxic aflatoxins G1, B2, and G2 (Meji-Teniente *et al.*, 2011; Freire and Sant, 2018). Aflatoxin B1 (AFB1) exposure at high doses can result in sickness, vomiting, and even death, while prolonged exposure at low doses can develop liver cancer (Sherif *et al.*, 2009). Although there are less ideal conditions for the production of mycotoxins in Europe than in North America or Asia, the issue of mycotoxins in cereals is still a major concern for many European nations. The current European Union standards for AFB1 in poultry feed is 20 µg/kg (European Commission, 2002). The impact of broiler food contaminated with high or low levels of aflatoxins on the health

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and sustainability of production, however, has not been thoroughly explored in the literature. Although most of the experiments produced modestly beneficial benefits, substantial outcomes were infrequent. There needs to be more investigation because there are practically infinite options for aflatoxin B1 dosage and length of exposure. Detoxification can happen as a result of mycotoxins being metabolically transformed by microbiota or as a result of their adhering to the cell walls of bacteria or yeast (Śliżewska *et al.*, 2019). Probiotic bacteria are known to bind various chemicals or complex structures, including mycotoxins, on the surface of cell walls (McCormick, 2013). Aflatoxin residues could be found in the tissues of animals fed contaminated feed, posing a risk to human health. Although adding probiotics to animal diets has been considered acceptable for contributing in the detoxification of aflatoxins (Chen *et al.*, 2019), the data on broiler chickens is still quite limited. Therefore, the purpose of this study was to examine the effects of probiotic feed additives on the growth performance and carcass criteria of broiler chickens exposed to an aflatoxicosis challenge.

2. Material and methods

2.1. Animals, diets, and experimental design

An experiment was conducted to study the effect of probiotic, with or without aflatoxin B1 supplementations on the growth performance, and carcass criteria of broiler chickens. A total of 256 Ross 308 broiler chicks were divided into four treatments which included eight replicates and eight birds in each over a 37-day period. Treatments included a negative control, a positive control with aflatoxin B1 (40 µg/kg), a negative control with probiotic (0.5 mg/kg), and a negative control with a combination of aflatoxin B1 and probiotic. In an open house, all chicks were raised

on the floor with a litter of wooden dust. Isonitrogen – isocalory diets were created to fulfill the NRC's recommendations (Table 1). Throughout the trial period, the experimental feed and clean drinking water were freely available. Throughout the experiment, there was a constant light source. Aflatoxin B1 used in the experiment was produced in the Mycotoxin Laboratory of the Animal and Poultry Production Department, Faculty of Agriculture, Minia University, as described by Lin and Dianese (1976). Probiotic was used based on company recommendations (AmPhiBact, American Pharmaceutical Innovations Company®) contains *Lactobacillus acidophilus* and *Bacillus subtilis*.

2.2. Performance indices

To evaluate body weight gain (BWG), the birds were weighted at the start of the experiment, 21 and 37 days later, and feed intake was estimated as the difference between the offer and residual feed for each replicate of age. At the same ages, the feed conversion ratio (FCR) and the rate of death were calculated.

2.3. Carcass criteria

At the marketing age of 37 days, the carcass weight of slaughtered broilers from each group was recorded, and the dressing % was computed by subtracting dressed weight from total live body weight. In proportion to carcass weight, fat percentages in the liver, gizzard, heart, and abdomen were calculated.

2.4. Statistical Analysis

All generated data were subjected to a one-way analysis of Variance (ANOVA) using SAS (2009). Pen was the experimental unit for growth performance, whereas other parameters were analyzed in individual birds. Treatment means were compared using Duncan's Multiple Range Test, and differences were considered significant at $P < 0.05$.

Table 1. Ingredients and chemical composition of diets

Ingredients, g/kg	Starter diet	Grower diet
Maize, ground	276	300
Sorghum, ground	276	300
Soybean meal (44% CP)	285	250
Corn gluten meal (60% CP)	95.0	60.0
Vit & Min. Premix ^a	3.00	3.00
Sunflower oil	30.0	55.2
Dicalcium phosphate	20.0	18.0
Limestone	10.0	10.00
Salt	3.80	3.80
DL-methionine	0.40	---
L-lysine HCl	1.00	---
Total	1000	1000
Analysis chemical composition, g/kg		
Dry matter	925	924
Crude protein	233	216
Ether extract	53.7	57.5
Crude fibre	25.8	37.8
Ash	67.4	61.8
Ca	13.22	12.84
P	7.05	7.21
GE, MJ/kg	18.55	19.18

^aSupplied vitamin-mineral premix contains per kg: 2400.000 IU vitamin A; 1000.000 IU vitamin D; 800 mg vitamin K; 16.000 IU vitamin E; 650 mg vitamin B1; 1.600 mg vitamin B2; 1.000 mg vitamin B6; 6 mg vitamin B12; 8.000 mg niacin; 400 mg folic acid; 3.000 mg pantothenic acid; 40 mg biotin; 3.000 mg antioxidant; 80 mg cobalt; 2.000 mg copper; 400 mg iodine; 1.200 mg iron; 18.000 mg manganese; 60 mg selenium; 14.000 mg zinc.

3. Results

3.1. Productive performances

Neither control birds nor birds fed diets containing 40 µg AFB1/kg during their first 10 days of life exhibited any mortality. In comparison to early contaminated AFB1 or control diets, broiler chickens' body weight gain and body weight gain were significantly improved when their diets contained probiotic

supplements, whether they were contaminated with or without AFB1 (Table 2). Feeding chickens a feed contaminated with 40 µg AFB1/kg for the first 10 days (1-10 days of age) had increased feed intake during 21-30 and 1-37 days of age and enhanced feed conversion ratio during 1-10, 11-20, 21-30, and 1-37 days of ages compared to birds fed the control diet (Table 3).

Table 2. Effect of Aflatoxin B1, probiotic and their combination on body weight and body weight gain of broiler chickens.

Items	Treatments				SEM*	P-Value
	Control	AFB1	Pro	AFB1+Pro		
Body weight, g						
1 day of age	41.33	41.67	41.0	42.67	0.298	0.231
10 days of age	282	259	294	289	3.015	<0.001
20 days of age	844	785	910	871	9.965	<0.001
30 days of age	1605	1603	1727	1700	14.09	<0.001
37 days of age	2129	2170	2293	2235	16.03	<0.001
Body weight gain, g						
1-10 days of age	241	217	253	246	3.033	<0.001
11-20 days of age	562	526	616	582	7.283	<0.001
21-30 days of age	761	818	817	829	11.17	0.123
31-37 days of age	524	568	567	543	6.238	0.024
1-37 days of age	2088	2129	2252	2192	16.03	<0.001

^{a-d} Means not sharing a common superscript in a row are significantly different ($P < 0.05$)

SEM: Standard error of the means

AFB1: 40 µg/kg Aflatoxin B1 supplemented to control diet.

Pro: 0.5 mg/kg probiotic supplemented to control diet

AFB1+Pro: 40 µg/kg Aflatoxin B1 and 0.5 mg/kg probiotic supplemented to control diet

Table 3. Effect of Aflatoxin B1, probiotic and their combination on feed intake and feed conversion ratio of broiler chickens.

Items	Treatments				SEM*	P-Value
	Control	AFB1	Pro	AFB1+Pro		
Feed intake, g						
1-10 days of age	340.3	318.7	329.3	323.3	4.130	0.288
11-20 days of age	781.3	773.7	759.3	759.7	6.18	0.536
21-30 days of age	1116.0	1239.2	1127.9	1089.2	15.89	0.001
31-37 days of age	879.3	908.7	927.9	888.6	11.14	0.440
1-37 days of age	3116.9	3240.3	3144.5	3060.8	21.88	0.019
Feed conversion ratio						
1-10 days of age	1.413	1.469	1.305	1.313	0.021	0.005
11-20 days of age	1.390	1.471	1.232	1.307	0.022	<0.001
21-30 days of age	1.468	1.522	1.385	1.319	0.025	0.013
31-37 days of age	1.683	1.601	1.636	1.636	0.016	0.385
1-37 days of age	1.494	1.523	1.396	1.396	0.014	<0.001

^{a-d} Means not sharing a common superscript in a row are significantly different ($P < 0.05$)

SEM: Standard error of the means

AFB1: 40 µg/kg Aflatoxin B1 supplemented to control diet.

Pro: 0.5 mg/kg probiotic supplemented to control diet

AFB1+Pro: 40 µg/kg Aflatoxin B1 and 0.5 mg/kg probiotic supplemented to control diet

3.2. Carcass criteria

In comparison to the control group, feeding chicken's diets containing probiotic supplements, whether they were contaminated with or without AFB1, significantly increased the weight of

dressing percentage and decreased fat (Table 4). The weights of the liver, gizzard, heart, kidneys, spleen, intestine, and cecum of broiler chickens did not change when the probiotic, AFB1, and their combination were added to the control diet.

Table 4. Effect of Aflatoxin B1, probiotic and their combination on carcass criteria and internal organs of broilers.

Items %	Treatments				SEM*	P-Value
	Control	AFB1	Pro	AFB+Pro		
Dressing	76.35	76.18	78.19	78.51	0.285	0.032
Liver	2.046	2.070	2.005	1.898	0.036	0.379
Heart	0.411	0.430	0.478	0.539	0.042	0.057
Gizzard	1.372	1.512	1.577	1.548	0.034	0.131
Spleen	0.088	2.657	0.095	0.118	0.648	0.455
Pancreas	0.388	0.351	0.370	0.386	0.028	0.074
Fat	1.422	0.795	0.786	0.627	0.104	0.005
Small intestine	3.313	3.368	3.182	3.662	0.122	0.621
Small intestine, cm	153.3	172.3	180.0	163.3	4.350	0.139
Cecum	0.668	0.736	0.692	0.795	0.028	0.446
Cecum, cm	16.00	17.00	18.33	19.33	0.432	0.042

^{a-d} Means not sharing a common superscript in a row are significantly different ($P < 0.05$)

SEM: Standard error of the means

AFB1: 40 µg/kg Aflatoxin B1 supplemented to control diet.

Pro: 0.5 mg/kg probiotic supplemented to control diet

AFB1+Pro: 40 µg/kg Aflatoxin B1 and 0.5 mg/kg probiotic supplemented to control diet.

4. Discussion

The loss of productive performance is one of the most significant economic effects of AFB1 in broiler chickens. Feeding chickens a feed contaminated with 40 µg AFB1/kg for the first 10 days (1-10 days of age) had improved body weight gain and feed conversion ration compared to birds fed the control diet. This study's findings are in line with those that have already been published (Suganthi *et al.*, 2011; Yunus *et al.*, 2011). At the end of the 21-day feeding period, the administration of 2 ppm AFB1 in poultry diets significantly reduced BW and BWG and increased FCR; however, the addition of curcumin (0.2%) or Cellulosic polymers (0.3%) to the diet containing AFB1 significantly reduced its adverse effects on these performance parameters (Solis-Cruz *et al.*, 2019). Furthermore, in the present study, Feeding chicken diets with probiotic supplements, whether they were contaminated with or without AFB1, significantly increased the weight of dressing percentage and decreased fat compared to the control group. The findings demonstrate that contamination of feed with 2 ppm of AFB1 led to a significant increase in the relative weight of the liver and spleen, a considerable decrease in the relative weight of the bursa of Fabricius, and

no significant difference in the relative weight of the intestine. These findings are in line with those of other investigations (Aravind *et al.*, 2003; Indresh *et al.*, 2013), which found that lipid accumulation causes an increase in the relative weight of the liver and resulting in the characteristically enlarged, friable, and fatty liver (Tung *et al.*, 1983). Additionally, the histological findings, which concur with earlier research, support the conclusions regarding the relative weight of the liver (Fan *et al.*, 2015; Tessari *et al.*, 2006).

When compared to controls, broilers fed 0.5g/kg of aflatoxin B1 showed lower weight in the gizzard, liver, and pancreas of the carcass (Nazarizadeh *et al.*, 2019). Similar findings were made by Alam *et al.* (2020), who discovered that broilers given diets contaminated with 200 and 400 ng/g aflatoxin B1 had a significantly lower dressing percentage of carcass than the control group. Furthermore, Tessari *et al.* (2006) discovered that broiler hens fed diets enriched with aflatoxin B1 at 50 and 200 g/kg of feed had significantly larger relative weights of the heart while the weights of the liver and spleen were unaffected. Broilers fed contaminated feed with 1g/kg aflatoxin B1 had considerably increased relative weights of the liver, but not the spleen (Denli *et al.*, 2009). In broiler fed feed treated

with aflatoxin at 2.5 g/g compared to control group, the relative weights of the spleen, liver, and kidney were significantly increased (Huff *et al.*, 1986). Additionally, liver weight was considerably larger in broilers fed a diet containing 3 mg/kg of aflatoxin than in the control group, although heart and pancreas weight were unaffected (Santurio, 1999). Raju and Devegowda, (2000) also reported that broilers fed diets enriched with aflatoxin B1 at 0.3 mg/kg had considerably higher weights for the liver and kidneys than the control group. In addition, Khaleghipour *et al.* (2019) discovered that broiler Japanese quail fed 2.2 mg/kg aflatoxin B1 throughout the period from 7 to 35 days of age had considerably lower liver and spleen percentages than the control group. Broiler chicks fed a contaminated diet containing 0.5 mg/kg of aflatoxin B1 from 1 to 42 days of age had considerably larger livers than the control group, but their weights of the spleen, abdominal fat, and pancreas were unaffected (Saei *et al.*, 2013). Similar findings were made by Solis-Cruz *et al.* (2019), who found that broilers given a feed contaminated with 2ppm aflatoxin B1 over the period from 1 to 21 days of age had significantly larger relative weights of the liver and spleen than the control group.

5. Conclusion

In conclusion, the present results indicated that probiotic supplement improved production sustainability of broiler chickens exposed to Aflatoxin B1 challenge.

Authors' Contributions

All authors are contributed in this research.

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Institutional Review Board Statement

All Institutional Review Board Statements are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable.

Conflicts of Interest

The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

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