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Effect of Xylo-oligosaccharide supplementation on some performance characteristics of broiler diets contaminated with Aflatoxin-B1

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ABSTRACT

Aflatoxins, toxic metabolites produced by fungi in grains, cause economic losses and health problems in the poultry industry. This study investigates the protective effectiveness of xylo-oligosaccharides (0.4% and 0.8% g/kg) in mitigating the adverse effects of aflatoxin B1 (AFB1; 3 mg/kg) contamination in broiler diets. Experimental groups included: no supplement, 3 mg/kg aflatoxin, 0.4% xylo-oligosaccharide, 0.8% xylo-oligosaccharide, 3 mg/kg aflatoxin + 0.4% xylo-oligosaccharide, and 3 mg/kg aflatoxin + 0.8% xylo-oligosaccharide. Weekly live weight, weight gain, feed intake, feed conversion ratio, bursa of Fabricius weight, and internal organ weights were evaluated. Results showed that AFB1-contaminated feed reduced weekly and final weight, weekly and final weight gain, and weekly feed intake, while increasing the feed conversion rate. AFB1 also had detrimental effects on internal organ weights. Xylo-oligosaccharide alone did not significantly improve performance and increased abdominal fat. However, the combined use of AFB1 and xylo-oligosaccharides mitigated the negative effects of AFB1. The addition of 0.8% xylo-oligosaccharide was particularly effective in reducing the adverse effects of AFB1 in broiler chickens.

KEY WORDS:

Aflatoxin B1, broiler, performance, xylo-oligosaccharide.

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تأثير إضافة سكر الزايلوز المعقد في أداء و بعض خصائص فروج اللحم المتغذي على عليقة ملوثة بسم الأفلا B1

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الخلاصة

تسبب الأفلاتوكسينات وهي مستقلبات سامة تنتجها الفطريات في الحبوب المهمة، خسائر اقتصادية ومشاكل صحية في صناعة الدواجن. تبحث هذه الدراسة في الفعالية الوقائية للسكريات المعقدة من الزايلوز (0.4% و 0.8% جم/كجم) في التخفيف من الآثار الضارة لتلوث الأفلاتوكسين B1 3 ملجم/كجم في علائق فروج اللحم. شملت المجموعات التجريبية: بدون إضافة، 3 ملغم/كجم من الأفلاتوكسين، 0.4% زيلو-أوليغوسكاريد، 0.8% زيلو-أوليغوسكاريد، 3 ملغم/كجم أفلاتوكسين + 0.4% زيلو-أوليغوسكاريد، و 3 ملغم/كجم أفلاتوكسين + 0.8% زيلو-أوليغوسكاريد. تم تقييم الوزن الحي الأسبوعي، وزيادة الوزن، وأستهلاك العلف، ونسبة التحويل الغذائي، و وزن جراب فابريشيوس، وأوزان الأعضاء الداخلية. أظهرت النتائج أن العلف الملوث بـ AFB1 قلل الوزن الأسبوعي والنهائي، وزيادة الوزن الأسبوعي والنهائي، وتناول العلف الأسبوعي، مع زيادة في معدل التحويل الغذائي. كان لـ AFB1 أيضًا آثار ضارة على أوزان الأعضاء الداخلية. لم يحسن Xylo-oligosaccharide وحده الأداء بشكل معنوي مع زيادة الدهون في البطن بشكل معنوي. ومع ذلك، فإن الاستخدام المشترك لـ AFB1 و-Xylo-oligosaccharides قلل من الآثار السلبية لـ AFB1. كانت إضافة 0.8% زيلو-أوليغوسكاريد كان فعال بشكل خاص في الحد من الآثار الضارة لـ AFB1 في فروج اللحم.

الكلمات الافتتاحية: سم الأفلا B1 ، فروج اللحم ، أداء إنتاجي ، سكر الزايلوز المعقد.

INTRODUCTION

One of the most significant sectors in animal protein production is the broiler industry. This industry plays a crucial role in providing sufficient quantities of high-quality animal protein (Castro *et al* ,2023: Darniati *et al* ,2024). To meet the consumption demands driven by the rapid growth of the human population, the broiler industry has expanded and become globally concentrated due to intense demand from local and international markets (Osuji,2019) . Nevertheless, the existence of anti-nutritional substances, agents, and toxins in feed has caused various issues in the broiler industry, such as slow growth rates, poor feed quality, and high mortality rates (Arif *et al* ,2020). Not harvesting and drying the grains to be used in poultry feed production with appropriate methods and storing them at temperatures higher than normal conditions cause the growth of fungal species such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* in the feed and the formation of secondary metabolites (Peltonen *et al* ,2001: Kumar *et al* ,2017). As a result, secondary fungal metabolites are associated with the production of mycotoxins, leading to aflatoxin contamination in feeds used for poultry nutrition (Arif *et al* ,2020).

Aflatoxin (AF) is a group of potent mycotoxins with mutagenic, carcinogenic, teratogenic, hepatotoxic, and immunosuppressive properties and is of great importance due to its adverse effects on animal and human health (Benkerroum, 2020: Okasha *et al* ,2024).

Aflatoxin B1 (AFB1), among the types of aflatoxins, is a mycotoxin with high toxicity commonly found in contaminated grains. It has been reported that exposure of various poultry species such as chickens, turkeys, ducks and quails to high doses of AFB1 for a certain period of time causes clinical symptoms such as increased mortality, decreased egg production and decreased body weight (Hoerr, 2020). AFB1 is usually detected at low concentrations in poultry feed, which can lead to subclinical effects such as decreased production, immunosuppression, and decreased vaccine efficacy (Murugesan *et al* ,2015).

Oligosaccharides are considered as natural, versatile, non-toxic, environmentally friendly new feed additives that can replace antibiotics under certain conditions (Stokniene *et al* , 2020). Studies have shown that oligosaccharides improve animal performance, regulate intestinal microflora and immunity, reduce cholesterol, have antioxidant, antimicrobial and anti-inflammatory effects ^{12,13}.(Camacho *et al* ,2019: Maochen *et al* ,2020).Currently, many studies are being conducted on the effects of oligosaccharides on animal production(Liu *et al* , 2018:Al-Khalaifa *et al* ,2020).

The purpose of this study was to assess the efficacy of xylo-oligosaccharides as dietary supplements for preventing AFB1 toxicity in broilers.

MATERIAL AND METHODS

Chemicals

Xylo-oligosaccharide powder (XOS-35P) was obtained from Heagreen (China) (<http://www.heagreen.com>).

Production, Extraction and Quantification of AFB1 Toxins.

Aflatoxin was produced from *A. parasiticus* NRRL 2999 culture by fermentation of rice according to the method of Shotwell *et al.* (1966) . The successfully fermented rice was then sterilized in an autoclave at 121 °C for 10 minutes to eliminate the fungus. It was dried for 24 hours at 55 °C in an incubator and pulverized. Aflatoxin extraction was performed according to the method reported by Liu *et al.* (2020). 2 g of feed sample was shaken in 10 mL of methanol:water (70:30, v/v) mixture for 10 min at 150 rpm. The extract was then filtered through Whatman No. 1 filter paper and diluted in PBST (phosphate buffered saline (PBS) + 0.05% Tween 20) for indirect competitive enzyme-linked immunosorbent assay (ic-ELISA). AFB1 content in rice powder was measured by ELISA (ELX800; BioTek Instruments, Winooski, VT).

Experimental design and dietary treatments

A growth performance study in floor cages was conducted at the Poultry Unit of the Department of Animal Production, Faculty of Agriculture, Tikrit University, Iraq. The chicks were fed with starter feed from the age of 1 day. Then, the chicks were randomly distributed into six groups as Group 1 (G1: No treatment was applied to the feeds in this group (control group)), Group 2 (G2: 3 mg/kg AFB1 was added to the feed), Group 3 (G3: 0.4% g/kg xylo-oligosaccharide was added to the feeds), Group 4 (G4: 0.8% g/kg xylo-oligosaccharide was added to the feeds), Group 5 (G5: 0.4% xylo-oligosaccharide and 3 mg/kg AFB1 are added to the feeds), Group 6 (G6: 0.8% xylo-oligosaccharide and 3 mg/kg AFB1 are added to the feeds). Xylo-oligosaccharide amounts

determined according to Xu *et al* (2003). Their nutrition was carried out in three ways; (i) starter feed from day 7 to day 14, (ii) growth feed from day 15 to day 21, and (iii) finisher feed from day 22 to the end of the experiment (Table 1).

Table 1. Composition of experiment diet %.

Ingredients	Starter diet	Grower diet	Finisher diet
Corn	46.18	50.08	53.88
Wheat	9.92	10.22	9
Soybean meal (%48)	36.6	33	29.5
*Protein Concentrate	2.5	2.5	2.5
Sunflower oil	2.8	3.1	4.2
Dicalcium phosphate	1	0.4	0.3
Limestone	0.3	0.2	0.2
L-Lysine%	0.3	0.2	0.12
DL-Methionine%	0.1	0.1	0.1
NaCl	0.3	0.2	0.2
Total	100	100	100
Crude protein%	23,02	21,52	20,02
Lysine (%)	1,48	1.28	1.12
Methionine (%)	0.57	0.56	0.54
Cysteine+ Methionine%	0.92	0.89	0.85
Calcium%	0.97	0.84	0.83
Available phosphate%	0.61	0.52	0.45

Experimental methods and housing arrangement

In the experiment, 180 unsexed Ross 308 chicks were used(Shlej et al ,2015: Jahanian and Ashnagar,2015) and no grouping was done until the chicks were 7 days old. Then the chicks were divided into six groups. Each group contains 30 chicks with an average weight of 120 g. Since each procedure was performed in triplicate, the groups were arranged with 10 chicks in each cage. The chicks were raised on the floor on sawdust litter in a closed-system hall consisting of 18 cages. The feed and water were provided ad libitum to the birds. The weekly body weight and the feed intake rates of broiler were measured from 7 days to 5 weeks. The weekly average live weight (LW), weekly weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were calculated. At the end of the experiment, 6 individuals from each group (1 male + 1 female from each replicate) were randomly taken and weighed. After the broilers were slaughtered, abdominal fat and internal organs were removed from the carcasses and relative organ weight was calculated.

Statistical Analysis

Data were statistically analyzed using a completely randomized design (CRD) to examine the effect of coefficients on the studied characteristics. Significant differences between means were compared using Duncan's (1955) polynomial test. SAS (2001) statistical program was used in statistical analysis.

RESULTS AND DISCUSSION

The results shown in Table 2 illustrate the effect of diets contaminated with AFB1 (3 mg/kg diet) on the weekly live weight ratio of broilers and the role of oligosaccharides in this interaction. Weekly live weight ratios were recorded for six groups from 7 days to 5 weeks of age. According to the results at the end of the fifth week, the G1 group (2123.18 g) had the highest live weight, while the G2 group (1108.48 g) had the lowest live weight. Significant differences were observed between control and other groups. The weight gains of G3, G4, G5 and G6 showed a statistically significant superiority compared to the G2 (P<0.05). These results clearly indicate a significant improvement resulting from the addition of xylo-oligosaccharides to AFB1-contaminated diets. G2 had a significantly lower feed intake than the control group (P < 0.05). However, no statistically significant differences were observed between G3, G4, G5 and G6 and G1 (control group). G2 group had the highest feed conversion ratio, while the G1 group had the lowest ratio Table 2).

Table 2. Growth performance of Broilers fed diets supplemented with or without xylo-oligosaccharide.

Variables	Group					
	G1	G2	G3	G4	G5	G6
LW (g)						
7-14 days	306,52 a	191,62 b	303,75 a	293,75 ab	276,15 a	283,37 a
15-21days	621,62 b	391,00 c	602,500 b	667,87 c	590,15 b	611,12 b
22-28 days	1232,90 a	747,97 c	1177,75 a	1222,70 ab	1165,88 b	1165,88 b
29-35 days	2123,18 a	1108,48 c	1962,63 b	1962,85 b	1962,25 b	1967,63 b
WG (g)						
7-14 days	186,50 a	71,62 c	183,75 ab	173,72 ab	156,17 b	163,32 b
15-21days	315,10 b	199,38 c	298,75 b	374,12 b	314,00 b	327,75 b
22-28 days	611,28 a	356,97 b	575,25 a	554,82 a	575,72 a	554,77 a
29-35 days	890,28 a	360,51 c	784,88 ab	740,15 b	796,37 ab	801,72 b
FI (g)						
7-14 days	261,42 b	189,97 c	269,87 ab	277,12 a	275,12 ab	275,25 ab
15-21days	531,87 a	376,37 b	543,00 a	535,00 a	513,62 a	522,75 a
22-28 days	894,50 ab	658,75 c	856,00 b	846,75 b	891,25 ab	945,25 a
29-35 days	1188,75 a	893,62 b	1127,75 a	1204,35 a	1159,63 a	1212,50 a
FCR (g)						
7-14 days	1,40 c	2,65 a	1,47 bc	1,59 bc	1,76 b	1,68 b
15-21days	1,69 ab	1,89 a	1,82 ab	1,43 c	1,64 bc	1,59 bc
22-28 days	1,46 b	1,85 a	1,49 b	1,40 b	1,55 b	1,70 b
29-35 days	1,34 c	2,48 a	1,44 bc	1,63 bc	1,46 bc	1,51 b

These results indicate that AFB1 contamination has detrimental effects on the growth performance of broilers and that the addition of xylo-oligosaccharides can mitigate some of these adverse effects. Particularly, the G2 group, compared to other groups, showed lower live weight, lower weight gain, lower feed intake and higher feed conversion ratio, demonstrating the harmful effects of AFB1. The addition of xylo-oligosaccharides partially alleviated these negative effects. Aflatoxin toxicity causes some changes in the internal organs of broilers. While the size of the liver, spleen, and kidney increases (Osweiler, 1990), the bursa of fabricius and thymus shrink (Sur, 2003).The results shown in Table 3 clearly demonstrate the effect of adding xylo-

oligosaccharides to AFB1-contaminated chicken feed on the relative weights of internal organs (liver, gizzard, heart, bursa of Fabricius, glandular stomach, abdominal fat and intestinal length). Liver weight in the G2 group was significantly higher than in the other groups ($p < 0.05$). There was no significant difference in liver weight among the G1, G3, G4, G5, and G6 groups. Gizzard weight in the G2 group was the highest, while there was no significant difference between the other groups. Gizzard weights in groups G1, G3, G4, G5 and G6 were similar. The heart weight in the G2 group was significantly higher than in the other groups. There was no significant difference in heart weight among the G1, G3, G4, and G6 groups, while the G5 group had a lower heart weight. There was no significant difference in the weight of the bursa of fabricius between the G1, G3, and G4 groups. However, the G2 group had a significantly lower weight compared to the other groups. Glandular stomach weights were highest in the G2 and G5 groups. There was no significant difference among the G1, G3, G4 and G6 groups. The abdominal fat weight in the G2 was the highest. The G1 had the lowest abdominal fat weight, with significant differences observed among the other groups. Intestinal length was significantly shorter in the G2 than in the other groups. There was no significant difference in intestinal length among the G1, G3, G4, G5, and G6 groups (Table 3).

Table 3. Broilers’ liver, gizzard, heart, bursa fabricius, glandular stomach, abdominal fat weight (g) and intestinal length (cm) of Broilers fed diets supplemented with or without xylo-oligosaccharide.

Group	Liver	Gizzard	Heart	Bursa Fabricius	Glandular Stomach	Abdominal Fat	Intestinal Length
G1	4,06 b	2,47 b	0,85 c	0,32 a	0,56 c	0,78 c	180,50 a
G2	6,03 a	4,15 a	1,15 a	0,24 b	0,64 a	1,28 a	151,25 b
G3	4,04 b	2,37 b	0,87 c	0,31 a	0,58 c	0,85 bc	182,00 a
G4	4,28 b	2,82 b	0,84 c	0,30 a	0,54 c	0,82 bc	186,25 a
G5	4,00 b	3,05 b	0,64 b	0,27 ab	0,64 ab	0,94 b	185,75 a
G6	4,03 b	2,87 b	0,83 c	0,29 ab	0,58 abc	0,94 b	180,50 a

Studies have shown that high concentrations of AFB1 in feed can lead to a reduction in broiler growth rate by 5% to 40% (Celyk et al , 2003: Shlij et al , 2015).In our study, this reduction was approximately 37%, which is consistent with previous findings. In our study, analyses conducted after adding aflatoxin and xylo-oligosaccharides to the broiler diets revealed statistically significant differences in live weight and weight gain between the control group and the group fed with aflatoxin-contaminated feed. However, no significant difference was observed between the control group and the group fed with aflatoxin + xylo-oligosaccharide supplemented diet.

Our results are consistent with studies reporting the adverse effects of aflatoxin on weight gain in broilers (Allameh et al , 2005: Miazzo et al, 2005 : Sacakli et al , 2007). Our results suggest that the addition of xylo-oligosaccharides to aflatoxin-contaminated diets has a protective effect on broilers. Groups with aflatoxin in their diets had significantly lower total feed intake compared to the control group ($P < 0.05$). There were statistically significant differences in feed conversion ratio between the control group and the group fed with aflatoxin + xylo-oligosaccharide

supplemented group. These results can be supported by studies indicating that xylo-oligosaccharides regulate gut microbiota in broilers (Pourabedin et al ,2015).

The presence of fungal toxins such as AFB1 leads to decreased protein synthesis and in particular, a deficiency of proteins that function to transport fat from the liver to tissues. This condition causes fat accumulation around and inside the internal organs. Accumulation of fat in the liver disrupts the water balance, puts pressure on the cells, and causes the liver to have a fatty appearance. Additionally, these toxins cause fat accumulation around the heart, causing damage and lubrication of the heart muscles, thereby increasing the relative weight and size of the heart (Kubena et al ,1998).

The addition of xylo-oligosaccharides to AFB1 contaminated diets can have significant effects on the relative weights of certain organs. The significant increases observed in the G2 group highlight how the addition of xylo-oligosaccharides modifies these effects beyond the effects of AFB1 alone. These results support the study by Al-Jubouri (2002) and suggest that oligosaccharides can influence the relative weights of some organs in chickens exposed to AFB1 contamination.

CONCLUSION

These results suggest that the oligosaccharide has promising potential to reduce or limit the effects of AFB1 and may provide a positive impact on the health and production of broilers.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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