

ABILITY OF *LACTOBACILLUS CASEI* AND *LACTOBACILLUS ACIDOPHILUS* TO PROTECT AGAINST THE TOXICITY IN BROILERS FED CONTAMINATED DIET AFLATOXIN B1

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ABSTRACT

The aim of this study was to evaluate the ability of *Lactobacillus casei* and *Lactobacillus acidophilus* in counteracting the deleterious effects of aflatoxin B1 (AFB1) in broiler chickens. One hundred and fifty Ross 308, one-day-old broiler chicks were assigned 10 treatments, 3 replicates with 5 birds cages each for 42 reared day. The experimental treatments were labeled as follows :-T1. BD no other addition (Control). T2. BD containing a 3 mg FB1/kg diet. T3. BD + *Lactobacillus casei* CFU (1.5×10^8 cell /ml live). T4. BD + *Lactobacillus acidophilus* CFU (1.5×10^8 cell /ml live). T5. BD containing a 3 mg AFB1/kg diet + *Lactobacillus casei* CFU (1.5×10^8 cell /ml live). T6. BD containing a 3 mg AFB1/kg diet+ *Lactobacillus acidophilus* CFU (1.5×10^8 cell /ml live). T7. BD + *Lactobacillus casei* CFU (1.5×10^8 cell /ml dead). T8. BD + *Lactobacillus acidophilus* CFU (1.5×10^8 cell /ml dead). T9. BD containing a 3 mg AFB1/kg diet + *Lactobacillus casei* CFU (1.5×10^8 cell /ml dead). T10. BD containing a 3 mg AFB1/kg diet+ *Lactobacillus acidophilus* CFU (1.5×10^8 cell /ml dead). Growth performance and serum biochemical parameters was measured from d21 and 42 organs weight were determined on d42 of age. Aflatoxin B1 significantly decreased BW-gain, feed intake and impaired feed conversion rate ($p < 0.05$) at both periods 21 and 42 days and increased relative organs weight (heart, liver, spleen and gizzard) and decreased fat and totha. The supplementation of *L. casei* or *L. acidophilus* to aflatoxin B1 treated birds significantly diminished the inhibitory effects of dietary AFB1 ($p < 0.05$) on the growth performance with no differences compared to the control diet. In conclusion our results showed that addition of *L. casei* and *L. acidophilus* may reduce the adverse effects produced by the presence of AFB1 in broiler chickens diet.

Key words: Aflatoxin, Broiler, *Lactobacillus casei*, *Lactobacillus acidophilus*, Growth performance.

*Part of M. Sc. thesis of the first author.

شليج وآخرون

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قابلية بكتريا *LACTOBACILLUS CASEI* و *LACTOBACILLUS ACIDOPHILUS* على الحماية ضد

التأثير السمي للأفلاتوكسين B1 الملوث لعلائق فروج اللحم

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المستخلص

هدفت هذه الدراسة لتقييم قابلية بكتريا *Lactobacillus casei* و *Lactobacillus acidophilus* في التقليل من التأثيرات الضارة للأفلاتوكسين على الصفات الانتاجية لفروج اللحم، وقد استخدم في التجربة 150 فرخاً للحم التجاري من نوع Ross 308 ويعمر يوم واحد وزعت الطيور الى عشرة معاملات تضمنت كل معاملة ثلاثة مكررات بواقع خمسة طيور للمكرر في القفص الواحد ولمدة 42 يوماً. وكانت معاملات التجربة على النحو الآتي:- المعاملة الاولى عليقة السيطرة من دون إضافات ، المعاملة الثانية عليقة السيطرة + 3 ملغم أفلاتوكسين /كغم علف ، المعاملة الثالثة عليقة السيطرة + 1.5×10^8 خلية حية/مل، المعاملة الرابعة عليقة السيطرة + 1.5×10^8 خلية حية/مل، المعاملة الخامسة عليقة السيطرة + 3 ملغم أفلاتوكسين /كغم علف + 1.5×10^8 خلية حية/مل، المعاملة السادسة عليقة السيطرة + 3 ملغم أفلاتوكسين /كغم علف + 1.5×10^8 خلية حية/مل، المعاملة السابعة عليقة السيطرة + 1.5×10^8 خلية ميتة/مل، المعاملة الثامنة عليقة السيطرة + 1.5×10^8 خلية ميتة/مل، المعاملة التاسعة عليقة السيطرة + 3 ملغم أفلاتوكسين /كغم علف + 1.5×10^8 خلية ميتة/مل، المعاملة العاشرة عليقة السيطرة + 3 ملغم أفلاتوكسين /كغم علف + 1.5×10^8 خلية ميتة/مل. تم قياس معدل وزن الجسم والزيادة الوزنية وكميات العلف المستهلك ومعامل التحويل الغذائي على مرحلتين وذلك في نهاية الاسبوع الثالث ونهاية الاسبوع السادس وكذلك أخذت الأوزان النسبية لبعض الأعضاء الحيوية للفروج قيست في 42 يوماً من عمر الطيور، تسبب تلوث العليقة بالأفلاتوكسين B1 أنخفاضا معنويا ($p \leq 0.05$) في قيم الزيادة الوزنية الاسبوعية، أستهلاك العلف، وتسبب في تدهور صفة كفاءة التحويل الغذائي وفي نهاية كلا الفترتين من التجربة. مع زيادة معنوية ($p \leq 0.05$) في الوزن النسبي (القلب والكبد والطحال والقانصة) وصاحب ذلك أنخفاض معنوي في وزن جراب فابريشيا وغدة النوتة. إن أعطاء كلا النوعين بكتريا *Lactobacillus casei* و *Lactobacillus acidophilus* الى الطيور المغذاة على العليقة الملوثة قلل من التأثيرات العكسية الناتجة من وجود الافلاتوكسين B1 في علائق فروج اللحم.

كلمات مفتاحية: أفلاتوكسين، فروج اللحم، بكتريا العصيات اللبنية، الاداء الانتاجي.

البحث مستل من أطروحة دكتوراة للباحث الاول.

Introduction

For several decades poultry production has intensified to meet the increased meat demand, changes in production practices had important effect on the evolution of health challenges. So, insofar as the production was intensified there was increase in animal health risk, some of the most important health challenges are related to feed quality (1). The cereals that are the main components in poultry diets are subject to contaminate by a diverse fungal .In addition under favorable environmental conditions, some specific strains of filamentous fungi produce toxic metabolites, called mycotoxins (2). These compounds are structurally diverse and are capable of causing a variety of well-characterized biological and toxicological effects (3). Aflatoxin B1 (AFB1) is the most toxic and carcinogenic among the known mycotoxins (4). (AFB1) are a group of heterocyclic secondary metabolites from the *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* fungi. Aflatoxins B1, B2, G1, and G2 are found in grains, and other foods or feeds, as natural contaminants. Other AF metabolites can be found in blood, urine, feces, muscles, and eggs (5, 6). It has been shown to be mutagenic, teratogenic, hepatotoxic and immunosuppressive properties, are of particular importance because of their adverse effects on animal and human health (7, 1). Practical methods to detoxify mycotoxins-contaminated grain on large scale and in cost-effective manner are not currently available. A variety of physical, chemical, and biological techniques have been employed, however, they have met with only limited success (8). The application of strategies to prevent their formation, as well as to eliminate, inactivate strategies or reduce their presence in food products, is desirable. Most of the approaches have not been adopted due to high costs, loss of nutritional and sensory properties of the products, or practical difficulties involved in detoxification process (9). Among natural biological antagonists, lactic acid bacteria (LAB) have several potential applications. These microorganisms are widely used for the production of fermented foods and are also part of intestinal microflora (10). Therefore, a promising alternative is the use of microorganisms as

FB1 sequestering agents. Inclusion of such microbes in the diet may reduce the toxic effects of mycotoxins on humans, as an AFB1–microorganism complex may decrease availability of the mycotoxin and consequently its absorption in the gastrointestinal tract (11). Despite several publications having reported in vitro binding by LAB and yeast strains of mycotoxins such as aflatoxin B1 (AFB1) (12, 13, 14) *L. casei* was reported to be the strongest binder of aflatoxin compared to other *Lb. plantarum* and fermentum strains (15).The objectives of this study were to assay the effects aflatoxins B1 with live or dead bacteria through determine the broiler performance parameters. To noticing the ability of microorganisms to bind AFB1 could decrease the bioavailability of these compounds and limit their toxic effects on broiler.

MATERIALS AND METHODS

This study was carried out at Poultry Farm of the Animal Resources Department College of Agriculture, University of Tikrit for the period (2/4/2013) to (14/5/2013) Bacterial strains, *Lactobacillus casei* and *Lactobacillus acidophilus* strains were obtained from Microbiological Resources Center. Ain Shams University (ASU), Faculty of Agriculture, Cairo MIRCEN. One hundred and fifty-one-day-old Ross 308 broiler chicks un-sexed that used in this study were purchased from commercial hatchery were divided into 10 treatments 3 replicate for each with 5 birds in each replicate. Birds were kept in identical wired cages, from day 1 to 42. The temperature was regulated at 36° C during the first week and gradually decreased to 22° C by the end of 3rd week. Except from day-1 a 23L: 1 D lighting program was applied during the experiment. The experimental birds received a corn-soybean basal diet which was formulated to meet the standard nutritional requirements (16) (table 1) and was provided *ad libitum* and did not contain antibiotics, coccidiostat or growth promoters, and depending on the addition were labeled as follows: Growth performance parameters including as body weight gain, feed intake (FI), and feed conversion ratio (FCR), defined as FI / weight gain (g:g) were determined every week. Overall BW gain, FI and FRC were calculated for the whole duration of the experiment, and

monitored daily for signs of morbidity and mortality. The parental and the transformed *Lactobacillus* feed supplements were prepared separately by inoculating MRS broth with the respective *Lactobacillus* strains at specific concentrations, under microaerophilic conditions for 24 h at 37 °C without shaking, using inoculum at 0.1% (v/v) from an overnight culture at 37°. The cells were harvested by centrifugation at 1800g for 10 min at 4°C, and stored at -20° C before they daily use. The LAB isolates suspensions was prepared by taking 10 ml from isolates suspensions and centerifugate at 2500 rpm for 10 mints. the cells precipitates were collected and adding the phosphates buffers gradually with shaking, until to obtained the turbidity equal the McFarland solution 0.5 concentration. Bacterial strains count was performed by the spectrophotometer instrument at the wave length of 600 nm so that an optical density of 1.5×10^8 CFU/mL from each bacterial strain was reached. The MRS medium was distributed at 5ml in test tubes after moderate to serious pH from 2 to 9 through adding 0.1 N HCl or 0.1 N NaOH, each tubes then inoculates with 0.1% from each LAB isolates, and incubated at 35° C for 24 hours. The aflatoxin was produced from *Aspergillus parasiticus* NRRL 2999 culture (Colleg of Agricultural Tikrit Uni., Food science department Lab) via fermentation of rice by the method of Shotwell *et al.* (17). Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. The aflatoxin content in rice powder was measured on an ELISA (ELX800; Bio-Tek Instruments, Winooski, VT). Aflatoxin extraction was performed according to Kawamura *et al.* (18). Aflatoxins were determined by a monoclonal antibody-based ic-ELISA. The rice powder was incorporated into the basal diet to provide the required 3 mg aflatoxin/kg feed. Separated organs lymphoma was weighted at the age of 3 weeks and at the end of the experiment (6 weeks) , which included all of the Thymus gland and from both sides of the neck and the Bursa of Fabricius gland and spleen (Spleen) of the sacrifices of 6 birds per treatment of experimental treatments and both sexes equally after cutting the connective tissue

around these organs , and weighed by a sensitive balance to four decimal places type Sartorius (Germany) , was calculated relative weight of these organs according to the following equation: Relative weight of a organ (%) = $\frac{\text{Organ weight(g)}}{\text{Body weight(g)}} \times 100$ Data were analyzed experiment using (19) design complete randomized (CRD) to study the effect of the transactions studied in different qualities and compared the differences morale among the averages using the test Duncan(20).

Table 1. Composition of Basal diet %

1Each kilogram contains calcium, 196 g; phosphorous, 64 g; sodium, 30 g; magnesium, 6 g; copper, 400 mg; zinc,

Ingredient %	Starter diet 1-14	Grower diet 15-28	Finisher diet 29-42 day
Corn	51.9	58.6	62
Soybean meal	36	30	27
Premix ¹	5	5	5
Dicalcium phosphate	0.4	0.4	0.5
Limestone	1.4	1.2	0.9
NaCl	0.3	0.3	0.3
Sunflower oil	5	4.5	4.3
Total	100	100	100
Approximate analysis(calculated)			
Crude protein %	22.25	20.18	19.15
Metabolizable energy(kcal/kg)	3098.95	3144.6	3173.06
Crude fiber %	3.761	3.489	3.354
Lysine %	1.295	1.151	1.08
Methionnine %	0.501	0.476	0.464
Calcium %	0.994	0.906	0.814
Available phosphate %	0.443	0.432	0.445

1,200 mg; iron, 2,000 mg; manganese, 1,200 mg; cobalt, 20mg; iodine, 40 mg; selenium, 8 mg; vitamin A, 200,000 IU; vitamin D3, 80,000 IU; vitamin E, 1,600 mg; vitamin K3, 34 mg; vitamin C, 1,300 mg; vitamin B1, 35 mg; vitamin B2, 135 mg; vitamin B6, 100 mg; vitamin B12, 670 µg; nicotinic acid, 1,340 mg; calcium pantothenic acid, 235 mg; choline chloride, 8,400 mg; folic acid, 34 mg; biotin, 3,350 µg; and methionine, 30 g.

Results and Discussion

The effects of *Lactobacillus ssp.* on body weight gain (BWG) , feed intake (FI) and feed conversion ratio(FCR)of broilers fed basal diets contaminated with aflatoxin B1 have been presented in Table 2 . From 1-21, 22-42 days and over the entire period (1-42 days) in BW-gain, FI and FCR of broilers fed live bacteria and dead bacteria (T3,T4 ,T7 and T8), did not showed significant differences between the live bacteria and dead bacteria groups and control group (T1). However, birds supplemented with live bacteria diet (T3 and

T4) had higher ($p \leq 0.05$) BW-gain compared with dead bacteria groups (T7 and T8) and control group (T1). It has been demonstrated that live bacteria and dead bacteria inhibit the *in vitro* growth of many enteric pathogens (21). Therefore, the ability of live bacteria and dead bacteria to prevent the colonization of the broiler gastrointestinal tract by pathogens and improvement of broiler performance was reported by Afsharmanesh *et al.* (22). Birds fed on ration contaminated with AFB1 (3mg/kg) showed significantly ($p \leq 0.05$) poorest BW-gain during 1-21, 21-42, and 1-42 days of experiment periods compared with other treatments (T3 to T10) in correspondence with previous studies (23, 24, 25, 26). Karaman *et al.* (27) reported that the minimum dietary level of aflatoxins that can significantly affect growth in broiler is 2mg AFB1/kg diet. The adverse effects of aflatoxins on birds growth performance may be a result of anorexia reluctance and inhabitation of protein synthesis (28) and lipogenesis (29). This delay of growth inhibition in broiler suggest that the length of exposure to aflatoxins as well as their level of concentration can influence an animal response in terms of performance (30). However, the BW-gain from 1 to 42 days did not show significant ($p \leq 0.05$) between live bacteria and dead bacteria treated groups. In the current study, supplementing a contaminated diet with aflatoxin B1 live bacteria and dead bacteria significantly ameliorated the toxic effects of aflatoxins on broilers growth performance. The basic mechanism seem to be that *Lactobacillus casei* and *Lactobacillus acidophilus* germinated in animal tract and secrete the active substance which degrades aflatoxin thus alleviating the effects of aflatoxicosis (31). Some studies have suggested that microbial enzyme are responsible for cleaving the lactone or difuran ring of the AFB1 molecule *in vitro* thus reducing its toxicity (32). It has been demonstrated that *Lactobacillus casei* strain is able to bind 49.2% of the available aflatoxin (4.6 mg/ml) after 4 h of incubation (33). Results from the present study demonstrated that the incorporation of live bacteria and dead bacteria (*L. casei* or *L. acidophilus*) in broiler diet was effective in alleviating of BW-gain caused by exposure to AFB1. In general the

positive effect of live bacteria and dead bacteria additive tested on BW-gain are in agreement with the results reported by several researchers (34, 35, 22) reported that live bacteria and dead bacteria can improve the body weight of birds. In contrast Yalcinkayal *et al.* (36) reported that using the live bacteria and dead bacteria in broiler rations had no significant effect on body weight gain.

Feed intake

Supplemented of live bacteria or dead bacteria to the broiler diet had no additive benefit on feed intake at 1-21 and 1-42 days Table 2. However, birds in control groups (T1) showed a significant ($p \leq 0.05$) lowest feed intake at 22-42 days. The results of current trail showed that addition of live and dead bacteria to the contaminated diet with (3mg AFB1/kg) resulted in significantly ($p \leq 0.05$) higher feed intake at different period of the experiment. When compared with positive control diet (T2) The lowest value of feed intake was recorded for broiler fed diet contaminated with aflatoxin B1 (T2). The results of this experiment clearly showed that the supplemented diet with live and dead bacteria stimulated the feed consumption during the whole of experimental period (37). In agreement with the results of this study and Afsharmanegh *et al.* (22) reported that birds supplemented with live and dead bacteria had greater feed intake than control bird (from day 1 to 42). Generally, it has been suggested that in animals efficacy for most live bacteria could be demonstrated with daily intake of 10^8 to 10^9 microorganisms (38). Mountzouris *et al.* (39) use 5-bacterial strain live bacteria product (10^9 CFU/kg diet) resulting in an average daily intake of 2×10^8 CFU microorganisms per broiler found a live bacteria efficacy in improving broiler growth and feed conversion ratio. Huang *et al.* (40) using inactivated Lactic acid bacteria (*L. acidophilus* and *L. casei*) and fungus (*Scytalidium acidiphilum*) to optimal concentration for administering live bacteria was strain-dependent and higher inclusion level did not always result in better performance. The lowest value of feed intake was recorded for broiler fed diet contaminated with aflatoxin B1 (T2). The results of this experiment clearly showed that the supplemented diet with live and dead bacteria

stimulated the feed consumption during the whole of experimental period (37). In agreement with the results of this study and Afsharmanegh *et al.* (22) reported that birds supplemented with live and dead bacteria had greater feed intake than control bird (from day 1 to 42). Generally, it has been suggested that in animals efficacy for most live bacteria could be demonstrated with daily intake of 10^8 to 10^9 microorganisms (38). Mountzouris *et al.* (39) use 5-bacterial strain live bacteria product (10^9 CFU/kg diet) resulting in an average daily intake of 2×10^8 CFU microorganisms per broiler found a live bacteria efficacy in improving broiler growth and feed conversion ratio. Huang *et al.* (40) using inactivated Lactic acid bacteria (*L. acidophilus* and *L. casei*) and fungus (*Scytalidium acidiphilum*) to optimal concentration for administering live bacteria was strain-dependent and higher inclusion level did not always result in better performance. Generally, it is very difficult to directly compare different studies using different live bacterias and different administration levels because the of live bacteria application will additionally depend on many other factors stated in the introduction section. In our study the result corroborate the hypothesis that protective effects of live and dead bacteria (*L. casei* and *L. acidophilus*) against aflatoxins might be due to its capability of effecting a specific biotransformation of aflatoxin in animals intestinal tract. Since the amount of aflatoxins absorbed by the intestinal tract is reduced, meat quality is consequently improved while the concentrations of aflatoxin residue in tissues are decreased (41). Pizzolitto *et al.* (31) reported that the micrographs obtained in vivo assessment of the potential protective effect of *Lactobacillus casei* against aflatoxin B1 showed for the first time a clear visual image in age of the ability of *L. casei* to bind AFB1 into bacteria cell envelope. The image also revealed that aflatoxin binding produces structural changes that modify the bacterial cell surface. Also results from the present study demonstrated that the incorporation of live and dead bacteria significantly increased ($p \leq 0.05$) the feed intake of broiler (Table 2).

Feed conversion ratio (FCR)

Mean of FCR values given in Table 2. FCR for live bacteria (1.68 and 1.77) and dead bacteria (1.68) was significantly higher ($p \leq 0.05$) than group (1.60) and control (1.61) during 1-22 days of experiment period, which are not significantly different. However, there were no significant differences in FCR between treatments during 22-42, as well as for the whole experiment period (1-42 days). The results of this experiment showed that the supplemented diet with live and dead bacteria singly did not had any significant ($p \leq 0.05$) difference in treated group for FCR compared with control group (T1). Current study showed that the addition of live bacteria or dead bacteria, did not differ from those on control group (T1). A similar effect due to live and dead bacteria supplementation in broiler chickens was observed by (42: 22). In contrast Awad *et al.* (43) reported that the use of probiotic in the ration of broiler chickens reduced feed conversion ratio. However, the beneficial effects of live bacteria products on broiler performance including FCR have been reported by (44;45;39;46). Although FCR was reduced ($p \leq 0.05$) in the birds fed 3mgAFB1/kg contaminated diet (T2) compared with control and other groups (T3 to T10) during 1-21, 22-42 and 1-42 days. In this study diet contaminated with aflatoxins at level 3mg/kg marked increase ($p \leq 0.05$) FCR values between 1-21, 22-42 and 1-42 days as compared with the control group (T1). The toxicities of aflatoxin have been widely investigated for their toxic effects, consumption of aflatoxin contaminated feed by poultry may lead to other consequences such a decreased in growth performance, poor feed utilization and an increase in the incidence of disease in poultry (47; 48;49). A large number of studies have been performed that have demonstrated the negative effects of high level (4-5 mg/kg) of aflatoxins on broilers (50;26). In the current study supplementing a contaminating diet with live and dead bacteria (*L. casei* or *L. acidophilus* 1.5×10^8 cell/ml) significantly decrease FCR ratio values as compared with +ve control group during the experimental period. Some studies have suggested that many kinds of bacteria can reduce the amount of aflatoxins in feed and food (51;50;41).

Table 2. Effects of contaminated diet and detoxification ability of *Lactobacillus Casei* and *Lactobacillus Acidophilus* on production performance of broiler chickens.

Age Day	Control	AFB	Live bacteria		AFB1+ Live bacteria		Dead bacteria		AFB1 + Dead bacteria	
	T 1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Body weightgain (g/bird)										
d 1-21	557±6.46 ^a	437±9.29 ^c	566±6.21 ^a	576±8.36 ^a	523±13.16 ^b	502±13.2 ^b	580±11.14 ^a	577±8.71 ^a	509±8.53 ^b	509±7.9 ^b
d 22-42	1708±14.5 ^b	1123±16.56 ^f	1803±10.3 ^a	1803±12.9 ^a	1431±7.95 ^d	1376±9.95 ^e	1756±13.7 ^b	1732±21.2 ^b	1600±18.8 ^c	1596±22.7 ^c
d 1-42	2265.25±20.9 ^a	1560.75±32 ^d	2369±37.19 ^a	2379±28.36 ^a	1954.25±76.9 ^c	1878.75±67.5 ^c	2327.75±25.8 ^a	2309±31.88 ^a	2112±48.54 ^b	2105.75±15.5 ^b
Feed intake (g/bird)										
d 1-21	912±12.5 ^a	880±6.7 ^b	951±7.8 ^a	962±5.6 ^a	905±7.5 ^a	875±14 ^b	920±8.3 ^a	957±8.9 ^a	919±18.2 ^a	954±11.3 ^a
d 22-42	2996±25.1 ^b	2661±8.4 ^d	3195±6.4 ^a	3181±10.5 ^a	2738±24.2 ^c	2786±27.5 ^c	3180±17.9 ^a	3145±21.7 ^a	3220±22.4 ^a	3209±16.3 ^a
d 1-42	3908.5±78 ^a	3542±10.33 ^c	4151.8±8.6 ^a	4143.8±17 ^a	3644±50.9 ^b	3663±51 ^b	4108.5±b29 ^a	4104.8±27.3 ^a	4140.8±23.6 ^a	4164.8±7.8 ^a
Feed conversion										
d 1-21	1.61±.036 ^c	2.01±.1 ^a	1.68±.03 ^b	1.77±.027 ^b	1.70±.05 ^b	1.70±.07 ^b	1.60±.02 ^c	1.68±.02 ^b	1.80±.12 ^b	1.87±.04 ^{ab}
d 22-42	1.76±.047 ^c	2.37±.11 ^a	1.77±.024 ^c	1.78±.022 ^c	1.90±.06 ^b	2.02±.08 ^b	1.80±.01 ^c	1.80±.03 ^c	2.0±.06 ^b	1.97±.07 ^b
d 1-42	1.677±.014 ^d	2.277±.03 ^a	1.77±.02 ^d	1.691±.01 ^d	1.88±.06 ^c	1.967±.05 ^{bc}	1.782±.01 ^d	1.78±.01 ^d	1.95±.05 ^{bc}	1.97±.01 ^b

T1. BD no other addition (Control). T2. BD containing a 3 mg AFB1/kg diet. T3. BD + *Lactobacillus Casei* CFU(1.5×10^8 cell/ml). T4. BD + *Lactobacillus Acidophilus* CFU(1.5×10^8 cell/ml). T5. BD containing a 3 mg AFB1/kg diet + *Lactobacillus Casei* CFU(1.5×10^8 cell/ml). T6. BD containing a 3 mg AFB1/kg diet+ *Lactobacillus Acidophilus* CFU(1.5×10^8 cell/ml). T7. BD + *Lactobacillus Casei* CFU(1.5×10^8 cell/ml). T8. BD + *Lactobacillus Acidophilus* CFU(1.5×10^8 cell/ml). T9. BD containing a 3 mg AFB1/kg diet + *Lactobacillus Casei* CFU(1.5×10^8 cell/ml). T10. BD containing a 3 mg AFB1/kg diet+ *Lactobacillus Acidophilus* CFU(1.5×10^8 cell/ml). Within the same row means with different letter are significantly different $p \leq 0.05$. Results are reported as means \pm SEM.

Hernandez-Mendoza *et al* (33) reported that *L. casei* strain is able to bind 49.2% of available aflatoxin (4.6 mg/ml) after 4h incubation. Therefore as a feed additive for biodegradation of aflatoxin, the addition of *L. casei* or *L. acidophilus* to broiler diets is believed to be a viable potential approach to the biodegradation of aflatoxins naturally occurring in moldy feedstuffs.

Relative organ weights

Relative weights of organs of birds used in this experiment are presented in Table 3. An increase was observed ($p \leq 0.05$) in the relative weights of the heart, liver, spleen, abdominal fat and gizzard relative weight of group receiving diet with AFB1 when compared to control group (T1), dead bacteria and live bacteria plus AFB1 group. The relative of bursa of fabricius and totha decreased significantly ($p \leq 0.05$) only in birds fed diet containing with AFB1. There was no significant difference ($p > 0.05$) in the relative weight of heart, liver, spleen, abdominal fat, bursa of fabricius, totha and gizzard between treatment groups. These results are in agreement with results in previous reports on the effect of aflatoxins on organ relative weight (28, 25) They found an increased in the relative weight of spleen, heart, liver, abdominal fat and gizzard relative weight decreased in bursa fabricius and thymus, however no significant were observed on birds fed on ration containing aflatoxin. Indicating the hepato and nephrotoxicity of aflatoxin the liver is the target organ for aflatoxin B1 because it is the organ where most aflatoxin are bioactivated to the reactive bind DNA and protein damaging the liver structures and increasing liver weight (52; 53; 26). Tessari *et al* (48) found that the relative weight other heart increased in all groups receiving diet with AFB1 and/or AFB1 singly or in combination, and the possible specific mechanism involved in this effect are difficult to assess at this time. Verma *et al* (28) who observed AFB1 at 2 mg/kg in the diet caused a significant decrease in the relative weight of the bursa of fabricius was noted. Moreover, AFB1 at dietary concentration of 1 mg AFB1/kg or more caused severe reduction in growth and immune response. Sur and Celk (54) reported that chicks in AFB1 treated

groups hatched with poorly developed bursa compared with those of control group. The severing decreased in treated group with AFB1 was seen in turkeys that treated with 2mg AFB1/kg feed during 21d (55). However, FB1 alone caused an increase in relative liver weight of turkeys, as observed in our study. These data indicate that, at the concentrations reported, FB1 has the predominant effect on liver weight. In our experiment, no interaction was also observed on the weight of the bursa of Fabricius. No difference ($P > 0.05$) was observed between the treatments in carcass yield or the relative weights of the heart and parts of the digestive organs (Table 3). Relative abdominal fat weight was significantly ($P < 0.01$) greater for the broilers fed diets containing antibiotic than control diets, and dead bacteria and live bacteria were not different from either control or antibiotic treatments.

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