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Research Article

Effects of Dietary Sodium Bentonite and Mannan Oligosaccharide Supplementation on Performance, Egg Quality, Blood and Digestion Characteristics of Laying Hens Fed Aflatoxin Contaminated Diet

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Abstract

In this experiment, sodium bentonite (SB) (0.5% and 1%) and mannan oligosaccharide (MOS, 0.1%) were fed to laying hens each receiving approximately 120 ppb total aflatoxin (AF), and were compared to AF contaminated negative control (NC) and control without AF (C) groups. A total of 180 hens at 26 weeks of age from Barred Rock were tested for 12 weeks. No significant differences in liveability, feed intake, feed conversion ratio, egg quality characteristics except for egg yolk redness (a*) and blood parameters were observed among the groups. When compared NC with C, egg weight, egg mass and body weight gain were decreased. The addition of 0.5% SB (SB-1) increased egg production and egg mass compared to NC and MOS. The addition of 1% SB (SB-2) increased egg mass compared to NC. The AF contaminated diet (NC) caused a significant decrease in a* compared to C. Aflatoxin was not detected in eggs obtained from any of the treatments. Faeces pH was higher in NC than in C, SB-1 and SB-2 and similar to that of MOS. The proportion of dry matter of the faeces in C was higher than that of NC. As a result, SB appears to be more effective than MOS as a toxin-binding agent in counteracting the adverse effects of AF in laying hens.

Keywords: *Aflatoxin, Bentonite, Laying hen, Mannan oligosaccharide, Performance and egg quality, Blood and digestion parameters*

Aflatoksin İçeren Yumurta Tavuğu Yemlerine Sodyum Bentonit ve Mannan Oligosakkarit İlavesinin Performans, Yumurta Kalitesi, Kan ve Sindirim Özelliklerine Etkileri

Özet

Bu araştırmada, yaklaşık 120 ppb toplam aflatoksin (AF) içeren yumurta tavuğu yemlerine sodyum bentonit (SB) (%0.5 ve %1) ve mannan oligosakkarit (MOS, %0.1) ilavesi yapılmış, bu gruplar AF bulaşık negatif kontrol (NC) ve AF içermeyen kontrol (C) grupları ile karşılaştırılmıştır. Barred Rock hattından 26 haftalık yaşlı toplam 180 adet tavuk 12 hafta süresince denenmiştir. Gruplar arasında yaşama gücü, yem tüketimi, yem değerlendirme oranı, yumurta sarısı kırmızılık değeri (a*) haricindeki yumurta kalite kriterleri ve kan parametreleri bakımından önemli farklılıklar gözlenmemiştir. Yumurta ağırlığı, yumurta kütlesi ve canlı ağırlık kazancı NC de, C ile karşılaştırıldığında azalmıştır. İlave edilen %0.5 SB (SB-1) yumurta verimi ve kütlesini NC ve MOS gruplarına göre artırmıştır. İlave edilen %1 SB (SB-2) yumurta kütlesini NC grubuna göre artırmıştır. Aflatoksin bulaşık yem (NC) C ile karşılaştırıldığında a* değerinde önemli bir azalmaya sebep olmuştur. Hiçbir grubun yumurtasında aflatoksin tespit edilmemiştir. Dışkı pH'ı NC grubunda, C, SB-1 ve SB-2 gruplarından daha yüksek ve MOS grubu ile benzerdir. Dışkı kuru madde oranı C grubunda, NC grubundan daha yüksektir. Sonuç olarak, bir toksin bağlayıcı ajan olarak SB yumurta tavuklarında AF'nin zararlı etkilerini önlemede MOS'dan daha etkili görünmektedir.

Anahtar sözcükler: *Aflatoksin, Bentonit, Yumurta tavuğu, Mannan oligosakkarit, Performans ve yumurta kalitesi, Kan ve sindirim parametreleri*

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INTRODUCTION

The occurrence of mycotoxins in foods and feeds is a problem of major concern all over the world. It has been estimated that 25% of the world's crop production is contaminated with mycotoxins. The process of pre or post-harvest, storage and the steps of feed production are all potential sources of microbial contamination [1-3]. Acute mycotoxicosis outbreaks are rare events in modern poultry production; however, low mycotoxin doses which very often are not detected are responsible for reduced efficiency of production and increased susceptibility to infectious disease [2]. Aflatoxins (AF), a group of closely related and biologically active mycotoxins, are produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus* [3]. Aflatoxicosis in poultry also cause listlessness, hepato-toxicosis, haemorrhage, anorexia with lowered growth rate, poor feed utilization and decreased egg production and fertility [4-8].

Practical and cost-effective methods for detoxifying AF contaminated feed and feedstuffs are in high demand. In recent years, in particular, effective results were seen with the adding of organic and inorganic additives to feeds containing toxins. Detoxifying agents bind aflatoxins from the digestive-tract and thus reduce their absorption into the organism [9]. One of the inorganic materials used for such purpose is the natural bentonite. Bentonite is an aluminosilicate compound, used as adsorbent, and can be added to poultry feed without harmful effects to the animals [10]. *In vitro* and *in vivo* studies have indicated that natural sodium bentonite (SB) has a strong ability to absorb AF [11,12]. Previous works have shown that the adverse effects of AF on broiler performance can be minimized by supplementing their feed rations with the various levels of SB (0.3 to 1.5%) [6,13,14]. Incorporation of SB (0.25 to 0.5%) reduces the incidence and severity of the hepatic histopathology changes associated with aflatoxicosis in broilers [14,15]. Mannan oligosaccharides (MOS) used as organic additives, which are derived from the cell wall of yeast (*Saccharomyces cerevisiae*), also have considerably high AF binding capability [11,16]. Studies using MOS (0.1%) in broiler [17,18] and laying hens [5,19] indicate that MOS partially or completely reversed the effects of AF on performance and blood biochemistry.

Reports on the effects of MOS and SB as AF binder on the performance, egg quality, blood and digestion characteristics in laying hens are lacking. The studies so far have focused on broilers. Therefore the present study was designed to observe the possible adverse effects of AF on laying hens, and to evaluate the possible beneficial effects of dietary SB and MOS as a toxin-binder.

MATERIAL and METHODS

Experimental Design and Diets

Sodium bentonite (SB) (0.5% and 1%) and mannan oligosaccharide (MOS, 0.1%) were supplemented to the feed of laying hens receiving approximately 120 ppb total aflatoxin (AF), and were compared to control (C) and negative control (NC) groups *(Table 1)*. Prior to the experiment, hens were given commercial layers' diet for one week. During this period, egg production and egg weight was monitored and hens of similar body weight and egg production were selected. A total of 180 Barred Rock laying hens were randomly distributed to the individual cages (25 × 47 × 55 cm) in the form of 5 groups having 6 replicates each (6 hens in each replicate). The experiment began at 26 weeks of hen age and continued for 12 weeks. Feed and water was supplied to the hens *ad libitum*. The environment of the hen house was fully controlled with a 14 h light period. Yellow corn contaminated with AF was obtained from a private company (Adana, Turkey) and mixed to the trial diets in a proportion of 60% in order to increase the amount of AF. Diets were analysed [20] for AFB_1 and total AF (B_1+B_2+G_1+G_2) composition *(Table 1)*. Sodium bentonite with Turkey origin was obtained from Çanbensan A.Ş. (Ankara, Turkey). The composition of the fine-grained powder was nearly 61.3% SiO_2, 17.8% Al_2O_3, 3.0% Fe_2O_3, 2.7% Na_2O, 2.1% MgO, 1.3% K_2O and 4.5% CaO. The loss of ignition at 1050°C was 8%. Specific gravity was 2.5 g/cm^3, humidity was 8%, and the swelling index was 18-25. Mannan oligosaccharide (Bio-Mos; Alltech Inc, Nicholasville, Kentucky) is a product derived from

Table 1. *Experimental design and aflatoxin composition of diets*
Tablo 1. *Deneme deseni ve karma yemlerin aflatoksin içerikleri*

Experimental Groups	Sodium Bentonite (%)	Mannan Oligosaccharide (%)	Aflatoxin B_1 (ppb)	Total Aflatoxin (B_1+B_2+G_1+G_2) (ppb)
C	0	0	2.0	2.0
NC	0	0	107.3	121.9
SB-1	0.5	0	106.2	118.9
SB-2	1	0	106.1	120.6
MOS	0	0.1	103.3	122.3

C: *Control, without aflatoxin;* ***NC:*** *Negative control, aflatoxin contaminated diet;* ***SB-1:*** *Contaminated diet plus 0.5% sodium bentonite;* ***SB-2:*** *Contaminated diet plus 1% sodium bentonite;* ***MOS:*** *Contaminated diet plus 0.1% mannan oligosaccharide*

the outer cell wall of a selected strain of *Saccharomyces cerevisiae* yeast.

The experimental diets were formulated according to NRC requirements [21] *(Table 2)*. Feeds were prepared as mash using a cracker-mixer machine with 300 kg/h capacity. Crude nutrient, starch, and sugar analyses of feed ingredients were performed according to AOAC [22] procedures and their metabolisable energy were calculated using the equations of Janssen [23]. The resulting values were used in the calculation of diet composition and chemical components.

Production and Egg Quality Parameters

Hen body weight was measured individually at the beginning and at the end of the experiment, and was used in the calculation of body weight gain. Egg production, broken-cracked eggs and hen deaths were recorded daily. Feed intake and egg weight were determined every 2 weeks. Egg mass was calculated from laying rate and egg weight [egg production (hen-day, %) × egg weight (g)/100], and feed conversion ratio was determined from feed intake and egg mass [feed intake (g/hen/day)/egg mass (g/hen/day)]. Twenty four randomly selected eggs from each group were collected every 4 weeks, and egg quality characteristics were determined 24 h after collection of the eggs. Eggshell thickness was measured after peeling off the membrane under the shell with Mitutoyo digital micrometer (digital 395 series with 0.001 mm sensitivity, Kawasaki, Japan) on three points at the equatorial region

Table 2. *Compositions of the experimental diets*
Tablo 2. *Deneme yemlerinin kompozisyonu*

Feed Ingredients	**Experimental Diets (%)**				
	C	**NC**	**SB-1**	**SB-2**	**MOS**
Yellow corn	60	60	60	60	60
Wheat	2.64	2.64	1.34	0.84	2.34
Soybean meal (42% CP)	19.8	19.8	19.3	18.4	19.7
Full fat soya (32% CP)	5.7	5.7	7.0	7.9	6.0
Vegetable oil	0.5	0.5	0.5	0.5	0.5
Ground limestone	8.6	8.6	8.6	8.6	8.6
Di calcium phosphate	1.7	1.7	1.7	1.7	1.7
Salt (NaCl)	0.35	0.35	0.35	0.35	0.35
DL-Methionine	0.26	0.26	0.26	0.26	0.26
Vitamin-mineral premix[1]	0.2	0.2	0.2	0.2	0.2
Antioxidant	0.05	0.05	0.05	0.05	0.05
Salmonella inhibitor	0.2	0.2	0.2	0.2	0.2
Sodium bentonite	0	0	0.5	1.0	0
Mannan oligosaccharide	0	0	0	0	0.1
Nutrient Content					
Crude protein (%)	16.56	16.52	16.49	16.45	16.53
Metabolizable energy (kcal/kg)	2780	2773	2770	2770	2774
Dry matter (%)	88.41	88.40	88.45	88.50	88.42
Crude ash (%)	12.46	12.46	12.49	12.51	12.46
Ether extract (%)	3.58	3.58	3.79	3.96	3.62
Calcium (%)[2]	3.70	3.70	3.70	3.70	3.70
Available phosphorus (%)[2]	0.40	0.40	0.40	0.40	0.40
Methionine (%)[2]	0.50	0.50	0.50	0.50	0.50
Methionine+cystine (%)[2]	0.78	0.78	0.78	0.78	0.78
Lysine (%)[2]	0.85	0.83	0.83	0.83	0.83

[1] Vitamin-mineral premix provided per kilogram of diet; vitamin A, 15.000 IU; vitamin D_3, 5.000 IU; vitamin E, 50 mg; vitamin K_3, 10 mg; thiamine, 4 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vitamin B_{12}, 0.025 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.25 mg; ascorbic acid, 75 mg; choline, 175 mg; Mg, 35 mg; Mn, 56 mg; Zn, 140 mg; Fe, 56 mg; Cu, 10.5 mg; I, 1 mg; Co, 0.28 mg; Se, 0.28 mg; Mo, 0.7 mg
[2] Calculated values from NRC [21] tables
C: *Control, without aflatoxin;* ***NC:*** *Negative control, aflatoxin contaminated diet;* ***SB-1:*** *Contaminated diet plus 0.5% sodium bentonite;* ***SB-2:*** *Contaminated diet plus 1% sodium bentonite;* ***MOS:*** *Contaminated diet plus 0.1% mannan oligosaccharide;* ***CP:*** *Crude protein*

of the egg, and expressed as an average value. Eggshell breaking strength and haugh unit were measured by using Futura 3/A egg quality measuring system (Futura, Lohne, Germany). Redness (a*) and yellowness (b*) of egg yolk was determined by CR-10 Konica Minolta Color Reader (Osaka, Japan).

Collection and Analysis of Samples

Blood samples of 10 hens from each group were taken individually from brachial vein using injector at the end of the experiment. Serum was isolated by centrifuging the blood at 4500 × g for 10 min. Serum samples were analyzed for total protein, albumin, bilirubin, total cholesterol, calcium, phosphorus, aspartate amino transferase (AST) and alanine amino transferase (ALT) using Roche Cobas Integra original kits by a Roche Cobas Integra 800 automatic analyzer (Roche, Switzerland) [24]. The methodology and reagents were those recommended by the manufacturer of the system [25]. In addition, 6 randomly selected eggs from each group were analyzed for AFB_1 and total AF (B_1+B_2+G_1+G_2) by Agilent 1100 HPLC system [20]. At the end of the experiment, faeces pH and dry matter content of 6 hens from each group was measured. Faeces were collected in plates, which were placed under the cages for 24 h. Feed and feathers were carefully removed from fecal samples. Dry matter was determined according to AOAC [22], pH was measured by a digital pH meter (Hanna pH 211, Italy) calibrated at 22°C. Absorption rate of AFB_1 and total AF were determined, by mixing chromium oxide in 0.3% ratio to the feed of six hens from each treatment, and for 3 days. Faeces from the last 2 days were collected and analyzed for chromium oxide and AF content [20]. Digestibility of AF was calculated on the basis of the equation by Maynard and Loosli [26]: Nutrient digestibility (%) = (Indicator in feed/Indicator in faeces) × (Nutrient in faeces/Nutrient in feed). The animal care protocol used in this study was reviewed and approved by the Ethics Committee of the Poultry Research Station, Ankara, Turkey (22.01.09-2009/06).

Statistical Analysis

The results of all experiments were analysed using the analysis of variance procedures of the statistical program MINITAB Release 14 and the means were compared for differences using Duncan's multiple range test [27] at P<0.05.

RESULTS

The effects of SB and MOS supplementation on the performance of laying hens fed an AF contaminated diet are shown in *Table 3*. No difference in liveability, feed intake and feed conversion ratio were observed among the experimental groups (P>0.05). The AF contaminated diet (NC) caused significant decreases in egg weight, egg mass and body weight gain compared to the control (C) (P<0.05). The addition of 0.5% bentonite (SB-1) resulted in increased egg production (7.6% and 6.3%) and egg mass (10.9% and 7.7%) compared to NC and MOS (P<0.05). However, the addition of 1.0% bentonite (SB-2) increased egg mass (7.3%) compared to NC (P<0.05).

Egg quality characteristics are presented in *Table 4*. There were no significant differences in broken-cracked egg rate, eggshell breaking strength, eggshell thickness, haugh unit and egg yolk yellowness (b*) observed among groups (P>0.05). NC caused a significant decrease in egg yolk redness (a*) compared to C (P<0.05). The eggs collected on the last day of the experiment, when subjected to HPLC analysis, indicated no detectable levels of AFB_1 or total AF residues in any of the treatments.

No significant differences were observed in serum total protein, albumin, bilirubin, total cholesterol, calcium, phosphorus, AST and ALT levels (P>0.05) *(Table 5)*. The measured digestion parameters are given in *Table 6*. Faeces pH of NC was higher than those of C, SB-1 and SB-2 (P<0.05) and similar to that of MOS (P>0.05). The proportion of dry matter in the faeces of C was higher than that of NC (P<0.05).

Table 3. *Effects of dietary sodium bentonite and mannan oligosaccharide supplementation on performance traits of laying hens fed aflatoxin contaminated diet*
Tablo 3. *Aflatoksin içeren yumurta tavuğu yemlerine sodyum bentonit ve mannan oligosakkarit ilavesinin performans ölçütleri üzerine etkileri*

Treatment	Liveability (%)	Egg Production (%/hen/day)	Egg Weight (g/egg)	Egg Mass (g/hen/day)	Feed Intake (g/hen/day)	Feed Conversion Ratio (g feed/g egg)	Body Weight Gain (g/hen)
C	94.4	85.2 ^{ab}	61.9 ^{a}	52.7 ^{a}	119.6	2.27	134.4 ^{a}
NC	94.4	80.2 ^{b}	59.4 ^{b}	47.7 ^{c}	113.8	2.39	46.7 ^{b}
SB-1	94.4	86.3 ^{a}	61.2 ^{ab}	52.9 ^{a}	118.6	2.24	115.6 ^{ab}
SB-2	100	83.7 ^{ab}	61.2 ^{ab}	51.2 ^{ab}	116.1	2.27	116.3 ^{ab}
MOS	94.4	81.2 ^{b}	60.5 ^{ab}	49.1 ^{bc}	113.8	2.32	53.3 ^{b}
SEM	5.56	1.22	0.48	0.72	2.4	0.05	19.4
P	0.903	0.039	0.006	0.003	0.433	0.417	0.006

^{a,b,c} Means within columns with no common superscripts are significantly different (P<0.05); ***C:*** *Control, without aflatoxin;* ***NC:*** *Negative control, aflatoxin contaminated diet;* ***SB-1:*** *Contaminated diet plus 0.5% sodium bentonite;* ***SB-2:*** *Contaminated diet plus 1% sodium bentonite;* ***MOS:*** *Contaminated diet plus 0.1% mannan oligosaccharide;* ***SEM:*** *Standard error of means*

Table 4. *Effects of dietary sodium bentonite and mannan oligosaccharide supplementation on egg quality characteristics of laying hens fed aflatoxin contaminated diet*

Tablo 4. *Aflatoksin içeren yumurta tavuğu yemlerine sodyum bentonit ve mannan oligosakkarit ilavesinin yumurta kalite özelliklerine etkileri*

Treatment	Broken-Cracked Egg (%)	Eggshell Breaking Strength (Newton)	Eggshell Thickness (10^{-2} mm)	Haugh Unit	Egg Yolk Redness (a*)	Egg Yolk Yellowness (b*)
C	0.91	38.0	32.2	75.0	5.91 ^{a}	13.96
NC	1.38	37.5	31.7	74.6	5.54 ^{b}	13.94
SB-1	0.85	38.0	31.9	76.8	5.71 ^{ab}	13.67
SB-2	1.14	37.8	32.2	76.8	5.68 ^{ab}	13.70
MOS	1.35	37.6	31.7	75.1	5.70 ^{ab}	13.28
SEM	0.39	0.95	0.31	1.13	0.07	0.18
P	0.862	0.996	0.634	0.518	0.006	0.069

^{a,b} Means within columns with no common superscripts are significantly different (P<0.05); ***C:*** *Control, without aflatoxin;* ***NC:*** *Negative control, aflatoxin contaminated diet;* ***SB-1:*** *Contaminated diet plus 0.5% sodium bentonite;* ***SB-2:*** *Contaminated diet plus 1% sodium bentonite;* ***MOS:*** *Contaminated diet plus 0.1% mannan oligosaccharide;* ***SEM:*** *Standard error of means*

Table 5. *Effects of dietary sodium bentonite and mannan oligosaccharide supplementation on some blood parameters of laying hens fed aflatoxin contaminated diet*

Tablo 5. *Aflatoksin içeren yumurta tavuğu yemlerine sodyum bentonit ve mannan oligosakkarit ilavesinin bazı kan parametrelerine etkileri*

Treatment	Total Protein (g/dL)	Albumin (g/dL)	Bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	Total Cholesterol (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)
C	5.50	2.25	0.02	163	1.67	194	36.1	6.60
NC	5.70	2.23	0.03	164	1.50	164	33.0	6.60
SB-1	5.80	2.29	0.02	159	1.67	162	34.8	6.60
SB-2	5.80	2.25	0.02	161	1.70	167	35.4	7.30
MOS	5.70	2.22	0.03	160	1.63	166	33.9	6.50
SEM	0.18	0.05	0.006	6.98	0.19	18.12	1.84	0.38
P	0.674	0.889	0.511	0.978	0.952	0.696	0.792	0.572

C: *Control, without aflatoxin;* ***NC:*** *Negative control, aflatoxin contaminated diet;* ***SB-1:*** *Contaminated diet plus 0.5% sodium bentonite;* ***SB-2:*** *Contaminated diet plus 1% sodium bentonite;* ***MOS:*** *Contaminated diet plus 0.1% mannan oligosaccharide;* ***SEM:*** *Standard error of means;* ***AST:*** *Aspartate amino transferase;* ***ALT:*** *Alanine amino transferase*

Table 6. *Effects of dietary sodium bentonite and mannan oligosaccharide supplementation on some digestion parameters of laying hens fed aflatoxin contaminated diet*

Tablo 6. *Aflatoksin içeren yumurta tavuğu yemlerine sodyum bentonit ve mannan oligosakkarit ilavesinin bazı sindirim parametrelerine etkileri*

Treatment	Faeces pH	Faeces Dry Matter (%)	Digestibility of Aflatoxin B_1* (%)	Digestibility of Total Aflatoxin * (B_1+B_2+G_1+G_2) (%)
C	7.80 ^{bc}	26.0^{a}	95.4	95.3
NC	8.56 ^{a}	22.2^{b}	71.8	78.5
SB-1	7.30 ^{c}	23.9^{ab}	66.0	64.2
SB-2	7.87 ^{b}	23.2^{ab}	58.5	55.1
MOS	8.05 ^{ab}	22.7^{ab}	65.0	66.3
SEM	0.12	1.25		
P	0.0001	0.047		

** In the determination of the digestive rate of total AF and AFB_1, samples taken from six hens were mixed for analysis without repetition, and statistical evaluation was not carried out;* *^{a,b,c} Means within columns with no common superscripts are significantly different (P<0.05)* ***C:*** *Control, without aflatoxin;* ***NC:*** *Negative control, aflatoxin contaminated diet;* ***SB-1:*** *Contaminated diet plus 0.5% sodium bentonite;* ***SB-2:*** *Contaminated diet plus 1% sodium bentonite;* ***MOS:*** *Contaminated diet plus 0.1% mannan oligosaccharide;* ***SEM:*** *Standard error of means;*

DISCUSSION

The results of present study indicate that feed contaminated with AF (120 ppb total AF; 106 ppb AFB_1) caused adverse effects on egg weight, egg mass and body weight gain of laying hens. However, liveability, egg production, feed intake and feed conversion ratio were not affected by AF contamination. Similarly, Pandey and Chauhan [8] reported that body weight gain of laying hens were significantly lower in the contaminated groups with AF compared to control group. In another study, the inclusion of dietary AFB_1 from 0 to 2.0 ppm resulted in lowered egg weight and nitrogen retention [28]. However, in 2-week a short feeding study, AF contamination did not have any detrimental effect on body weight gain [29]. Aflatoxin appears to exert its negative effect on animal performance chiefly by depressing the DNA and RNA synthesis and eventually protein synthesis [28,30]. Ali et al.[13] reported that the toxicity of aflatoxin was characterized by reduction in body weight gain as aflatoxins interfere with normal metabolic pathway through the inhibition of protein synthesis and enzyme system that is involved in carbohydrate metabolism and energy release. The results of present study agree with data showing that AF contamination did not affect the liveability, egg production and feed intake of laying hens [5,19,28,29,31]. However, some researchers did report that dietary AF decreased egg production and feed intake [8,32,33]. The different results in the studies may be due to causes as the AF concentration, its form, the length of trial period and poultry genotype and age.

In the present study, dietary SB supplementation ameliorated the effects of AF on performance of laying hens. Previous studies reported that SB supplementation to AF contaminated broiler diets significantly improved their performance [6,13]. However, there are currently no studies on the effect of dietary SB supplementation on laying hen performance with which to compare our results. Our results suggest that MOS supplementation to the AF contaminated diet had no a positive effect on performance of laying hens, which agrees with the findings of previous reports [5,33]. However, in other studies, the addition of MOS allowed significant recovery from the adverse effects of AF on the performance of broilers [17,18]. Detoxifying agents as bentonite and MOS form a complex with the toxin thus preventing the absorption of aflatoxin across the intestinal epithelium [1]. Therefore, these agents may ameliorate the negative effects of AF on performance of poultry.

The present study showed that an AF contaminated diet did not have adverse effects on egg quality characteristics except for egg yolk redness (a*). Similarly, previous studies reported that different AF doses did not affect egg quality [8,28,33], but had a negative effect on egg yolk colour parameters [5,8]. The decrease in the value of colour parameters may be connected to AF interference with lipid metabolism [34], carotenoid absorption, or deposition in yolk [35]. The supplementation of SB and MOS numerically increased a* according to NC in the present study. Similarly, Zaghini et al.[5] reported that MOS supplementation to diets contaminated with AF did not affect eggshell rate and eggshell thickness, but improved a*.

No AFB_1 or total AF residues were detected in the eggs of any of the treatments in the present study. Similarly, in other trials, no measurable residual AF or metabolites were found in eggs despite the consumption of different doses of AF (100 ppb to 2.5 ppm) [1,5,19,33]. Conversely, the residues of AFB_1 and total AF were detected in the eggs of hens given 500 ppb AFB_1 [31] and the inclusion of dietary total AF from 190 to 900 ppb [32]. These contrasting results may be ascribed to the administration of naturally contaminated feeds or diets containing different AF with different levels of toxicity [31].

The blood parameters of laying hens were not effect by the experimental treatments in the present study. Similarly, AF contaminated diet and the addition of MOS to this diet did not affect the serum total protein, albumin, bilirubin, total cholesterol, calcium, phosphorus, AST and ALT levels of laying hens [33]. SB supplementation (0.5-1%) to a contaminated broiler diet in another study did not affect serum albumin and ALT levels [6]. However, some studies demonstrated a decrease in serum total protein, albumin, total cholesterol, triglyceride, calcium and phosphorus levels, and an increase in bilirubin, ALT and AST levels of broilers given AF contaminated diets [14,15,17,36-38]. In these studies, total protein, albumin, cholesterol, enzymes as ALT and AST are consistent indicators of the hepatocellular damage [15,37]. AF may cause alteration of calcium and inorganic phosphorus metabolism. It may directly alter the renal, intestine and parathyroid regulation of calcium and inorganic phosphorus [36]. In many studies, dietary SB and MOS supplementations resulted in significant improvements in blood biochemical parameters adversely affected by AF ingestion [14,15,17,36,37]. However, Ghahri et al.[38] reported that the biochemical parameters for broilers fed diets containing SB+AF and MOS+AF did not completely return to normal values. But, MOS supplementation counteracted the observed increase in liver enzymes.

Our results indicate that the AF contaminated diet negatively affected the pH and dry matter of laying hen faeces. Dietary SB and MOS supplementations were partially effective in counteracting the adverse effects of AF on these digestive parameters. Some studies reported that the differences in the digestive parameters of hens receiving AF may be due to the alteration in intestinal morphology [29] or organic matter digestibility [8].

The digestibility of AFB_1 and total AF in C, NC, MOS, SB-1 and SB-2 were 95.4, 71.8, 65.0, 66.0, 58.5 and 95.3, 78.5, 66.3, 64.2, 55.1%, respectively. A portion of the received AF became bound by MOS or SB in the digestive tract and was

discarded in the faeces. *In vitro* studies have indicated that SB and MOS have a strong ability to absorb AF (95-98 and 80-97%, respectively) [11,16]. However, the measurements taken under laboratory conditions had been difficult to reproduce in experiments with animals *(in vivo)* [39].

Results of this study demonstrated that approximately 120 ppb dietary AF contamination resulted in adverse effects on some performance, egg yolk colour and digestion parameters of laying hens. SB and MOS were inert and non-toxic and SB appears to be more effective than MOS in counteracting the adverse effects of AF for layers. These findings suggest that dietary supplementation with detoxifying agents, such as SB, may be a solution to the problem of dietary AF contamination and toxicity in laying hens.

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