

## The effects of novel commercial toxin binders on growth performance, immunity and intestinal morphology of broiler chicks infected with aflatoxin B1

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### ABSTRACT

This study was conducted to evaluate the effects of commercial toxin binders on growth performance, immunity and intestinal morphology of broiler chicks fed with diets contaminated with aflatoxin B1 (AFB1). One-day-old male Cobb 500 broiler chicks (n=350) with an initial weight of 42±3 g was assigned to one of 7 treatments with 5 replications and 10 broiler chicks per group. Experimental treatments were: 1) Basal diet without aflatoxin and additive (NC); 2) Basal diet containing aflatoxin (PC); 3) PC diet containing test toxin binder-1 (ASRI1) 4) PC diet containing test toxin binder-2 (ASRI2); 5) PC diet containing test toxin binder-3 (STB1); 6) PC diet containing test toxin binder-4 (STB2) and 7) PC diet containing commercial toxin binder (Mycofix). Growth performance, cellular and humoral immune responses, carcass traits and intestinal morphology were assessed. Average daily weight gain (ADG), and cellular and humoral immunities were significantly lower in broiler chicks in the PC group compared to broiler chicks in the NC group in the different growth periods ( $P<0.05$ ), but dietary supplementation with none of the test toxin binders improved ADG compared to the PC group ( $P>0.05$ ). Dietary inclusion of ASRI1 and ASRI2 significantly decreased adverse the effects of aflatoxin on immune responses ( $P<0.05$ ). The results showed that villus length in the different parts of intestine, ileal villus width and crypt depth and duodenal crypt depth were significantly decreased in broiler chicks fed with PC diet in comparison to broiler chicks fed the NC diet ( $P<0.05$ ). Dietary inclusion of different toxin binders also alleviated adverse effects of aflatoxin on intestinal morphology ( $P<0.05$ ). In conclusion, dietary inclusion of ASRI1 and ASRI2 toxin binders is recommended for alleviation of the negative effects of AFB1 on immune responses and intestinal morphology.

**Keywords:** Aflatoxin B1, Broiler chicks, Cellular immunity, Crypt depth, Feed intake

### INTRODUCTION

Mycotoxins are toxic metabolites that are produced by some fungi. These are major issues for human beings and domestic animals due to their negative effects such as teratogenic, carcinogenic, mutagenic, and immunosuppressive

effects (Yunus *et al.*, 2011; El-Katcha *et al.*, 2017; Zahedi *et al.*, 2023).

Aflatoxins cause significant economic impacts on animal production due to their effects on growth and decreased meat production (Fan *et al.*, 2013; Do and Choi, 2007). Devegowda (2005) reported decreased 21% in weight gain of

broiler chicks fed with 0.3 mg Aflatoxin B1 (AFB1)/kg diet. It was also reported that there was a 10% decrease in weight gain of broiler chicks following 28 days of exposure to 0.8 mg AFB1/kg diet (Tedesco *et al.*, 2004).

Aflatoxins found in animal feed increase morbidity and mortality in commercial poultry (Nabi *et al.*, 2018). The presence of aflatoxin contamination in poultry diet influences animal health, production, mortality and immunosuppression that may increase susceptibility to infectious diseases (He *et al.*, 2013). Immune function in poultry is affected by mycotoxins. It was reported that ingestion of aflatoxins suppressed the immune system in terms of the bursa of Fabricius, thymus, and spleen (El-Katcha *et al.*, 2017).

The Food and Agriculture Organization have previously estimated at least 25% of production cereal is contaminated by mycotoxins (Poorghasemi *et al.*, 2024). Negative effects of aflatoxins have pushed researchers to find the ways to detoxify feedstuffs. Detoxification methods not only decrease the concentration of toxins to “safe” levels but also inhibit production of new toxic products obtained from aflatoxin degradation, and prevent a decrease in the nutritional value of the treated commodities thus maintaining the nutritional value of aflatoxin contaminated feeds (El-Katcha *et al.*, 2017).

The different methods used for alleviation of the adverse effects of aflatoxins on economic parameters of broiler chicks are divided into physical, biological and chemical methods. Natural compounds such as medicinal plants are used as toxin binders. Medicinal herbs are used in diets for improving growth and immunity parameters in broiler chicks (Poorghasemi *et al.*, 2017; Rafeeq *et al.*, 2017). Medicinal plants have antifungal activity (Abayhne and Chauhan, 2016).

Seemingly, the fabrication of herbal-based toxin binders could alleviate adverse effects of AFB1 on growth performance and immunity of broiler chicks. This study was thus conducted to evaluate the effects of novel commercial toxin binders for decreasing the negative effects of AFB1 on growth performance and immunity of broiler chicks.

## MATERIALS AND METHODS

### Broiler Chicks and Diets

All the used methods were approved by the Islamic Azad University (Arak-Iran) Care and Use Committee (IAAU 10155. 15/December 2017). A total of 350 one-day-old male Cobb 500 broiler chicks with initial weight of  $42 \pm 3$  g was studied. The chicks were reared in pens up to 42 days of age. The chicks received similar diets with no toxin until day 10 of age. A lighting regime of 23L:1D was used during the experimental period and broiler chicks had free access to feed and water. From day 10 until the end of the experiment, birds were assigned to one of 7 treatments with 5 replications and 10 birds per replicate. The experimental treatments were prepared in three stages: starter (1-10 days), grower (11-24 days) and finisher (25-42 days). Trial treatments included: 1) Basal diets lack of aflatoxin and additive (NC); 2) Basal diet containing aflatoxin (PC); 3) PC diet + test toxin binder-1 (ARSI1); 4) PC diet + test toxin binder-2 (ARSI2); 5) PC diet + test toxin binder-3 (STB1) 6) PC diet + test toxin binder-4 (STB2) and 7) PC diet + commercial toxin binder (Mycofix). Test toxin binders (1 and 2) were multiple function toxin binders produced by the Animal Science Research Institute and included the herbal supplement, bentonite, yeast wall, organic acid, and vitamins. The diets had differences in the herbal supplement compounds. All the diets were formulated in mash form and formulated as recommended by NRC (NRC, 1994) (Table 1). Test toxin binders (3 and 4) were multiple function toxin binders produced by the Soroush Sabz Company and contained the herbal supplement, bentonite, yeast wall, organic acid, and vitamins. Each of the toxin binders was included to the diet at a rate of 3 kg/ton. Crude protein was analyzed based on AOAC (2005) recommendations.

### Infection with AFB1

On day 10, feeds in PC groups were contaminated with AFB1 as reported by Khan *et al.* (2017). To increase the absorption of the aflatoxin, the feed was moistened with water and mixed with 15-day-old cultures of *Aspergillus parasiticus* PTCC-5286 grown on potato dextrose agar slants. To increase the production of mycotoxins, it was stored in bags for 18 days at ambient temperature. The quantification of AFB1 was assessed by using the ELISA test as reported by

Peltonen *et al.* (2000). A level of 1.5 mg AFB1/kg feed was added into basal diet. Average daily body weight gain (ADG) and average daily feed intake (ADFI) per experimental unit were recorded periodically and the feed conversion ratio (FCR) was calculated. Mortality was recorded daily, and birds that had died were weighed and the FCR calculated by dividing ADFI by ADG of live plus dead birds.

### Cellular Immunity

On the 28<sup>th</sup> day of the trial, 0.1 mL of DNCB and PHA was injected into the wing of two broiler chicks per pen. One area, 10 cm<sup>2</sup>, was determined for injection of DNCB. Skin thickness was measured before sensitization. Skin thickness was measured at three places in this area, after 24 and 48 hours. Also, 0.1 mL PHA (10 mg/mL acetone and olive oil at a ratio of 4:1) was administered using intradermal injection between the third and fourth digits of the right

foot. The area thickness was assessed by means of a constant tension micrometer 24 and 48 hours after administration (Global Sources, Shanghai, China).

### Humoral Immunity

On the 28<sup>th</sup> day of the trial, 3 mL of 10% suspension of SRBCs was intravenously administered into the wing of two birds per replicate. Blood samples were collected 7 days after injection. The blood samples were centrifuged at 2200 g for 12 minutes and the serum collected. The samples were stored at -20°C until analysis. Serum samples were analyzed for total anti-SRBC antibodies as explained by Delhanty and Solomon (1996). In summary, each inactivated serum sample was titrated for total and mercaptoethanol (ME)-resistant (IgG) anti-SRBC antibody titers. ME-sensitive (IgM) antibody titers were achieved by subtracting the level (titer) of IgG antibodies from total antibodies. All the titer data

Table1. The experimental diets used in the different periods

Ingredients (%)	Starter (1-10 days old)	Grower (11-24 days old)	Finisher (25-42 days old)
Corn	51.83	58.23	62.24
Vegetable oil	3.53	4.26	3.22
Soybean meal	38.35	29.10	39.10
DL-Methionine	0.35	0.31	0.25
Lysine	0.25	0.15	0.14
Threonine	0.10	0.00	0.14
CaCO <sub>3</sub>	1.80	0.97	1.43
Fish powder	2.11	5.00	0.00
NaCl	0.25	0.25	0.30
Mineral premix <sup>1</sup>	0.25	0.25	0.25
Vitamin premix <sup>2</sup>	0.25	0.25	0.25
Dicalcium phosphate	0.90	1.23	0.90
Chemical analyses			
Metabolizable energy (kcal/kg)	3025	3100	3200
Crude protein (%)	23.12	21.30	19.30
Lysine (%)	1.44	1.24	1.09
Methionine + Cysteine (%)	1.07	0.95	0.86
Ca (%)	1.05	0.90	0.85
Available Phosphorus (%)	0.50	0.45	0.42

<sup>a</sup> Vitamin & mineral premix supplied (content per kg): vitamin A, 1 800000 IU; vitamin D3, 400000 IU; vitamin E, 3600 IU; vitamin K3, 400 mg; thiamine, 360 mg; riboflavin, 1320 mg; niacin, 6000 mg; vitamin B6, 600 mg; vitamin B5, 2000; vitamin B12, 3 mg; folic acid, 200 mg; biotin, 20 mg; choline, 80 g; zinc, 17 g; iron, 10 g; copper, 2 g; manganese, 20 g; selenium, 40 mg; iodine, 200 mg.

were reported in terms of log<sub>2</sub> (Poorghasemi *et al.*, 2015).

### Carcass Traits and Intestinal Morphology

On day 42, two birds/replicate were killed and carcass traits were estimated as a percentage of live body weight. Intestinal segments were separated, 2 cm of duodenal, jejunal and ileal samples were preserved in a 10% formaldehyde phosphate buffer for 48 hours, subsequently embedded in paraffin, fixed on Microtome, sliced to a thickness of 3 µm, and dehydrated on a hot-plate. The sample was put on a glass slide, stained with hematoxylin and eosin, and examined under a microscope. From the prepared slides (n=5) from the jejunal segments of each broiler chick, five well-oriented villi were measured, and the average of the villi measurements was reported as a mean for each bird. Villus width (VW) was assessed at the base of each villus, villus length (VL) was evaluated from the top of the villus to the villus-crypt junction, and crypt depth (CD) was evaluated from the base of the villus to the sub-mucosa (Poorghasemi *et al.*, 2017).

### Statistical Analyses

The data obtained from the trial were analyzed by ANOVA (SAS software) (SAS, 2001). Different group means were assessed using Duncan's multiple range tests (DMRT). The level of significance was taken as  $P < 0.05$  or lower. For antibody titer, log<sub>2</sub> transformations were performed on antibody titers before statistical analysis.

## RESULTS AND DISCUSSION

The effects of experimental treatments on growth performance of broiler chicks are shown in Table 2. ADFI between 10-22 days and 10-42 days and FCR between 10-22 days were not influenced by the experimental treatments ( $P > 0.05$ ). ADG was significantly lower in broiler chicks in PC group compared to broiler chicks in NC group during the different periods ( $P < 0.05$ ). There was no significant difference between NC group and those fed ASRI1 and ASRI2 in 10-22 days ( $P > 0.05$ ). Dietary supplementation of toxin binders could not improve ADG compared with the PC group ( $P > 0.05$ ). Aflatoxin increased the

FCR and inclusion of toxin binders could not improve the FCR ( $P > 0.05$ ).

Contamination with aflatoxin had adverse effects on ADG and FCR and dietary inclusion of different toxin binders could not decrease the adverse effects of aflatoxin on growth performance. Growth performance is an index for growth performance, because it shows feed utilization and total efficacy in the poultry industry (Ajuwon, 2015). Parallel to our findings, Raju and Devegowda (2002) and Tedesco *et al.* (2004) have shown that dietary supplementation of 300 ng/kg AFB1 had negative effects on body weight. Suppression of performance in PC groups was due to anorexia, listlessness, compromised health and preventory effects of AFB1 on protein production and lipogenesis (Xu *et al.*, 2022) and/or disorders in macromolecules metabolism (Mesgar *et al.*, 2022). Mahmood *et al.* (2017) have reported that aflatoxins increase toxic metabolites in the liver and inhibit protein formation. It was expected that herbal-based toxin binders such as ASRI1 and ASRI2 could alleviate adverse effects of AFB1 on growth performance. Simitzis (2017) have reported the beneficial effects of medicinal herbs on animals. Ragga *et al.* (2016) also showed that dietary supplementation of thyme essential oil could improve ADG in broiler chicks. It could be stated that herbal-based toxin binders did not improve growth in broiler chicks fed with aflatoxin-infected diets in the present study. Other studies stated that the effect of toxin binders on reducing the side effects of AFB1 on growth performance is directly related to the amount of AFB1 in the diet (Liu *et al.*, 2019). By increasing the amount of AFB1, due to the deterioration of digestive and metabolic efficiency, toxin binders will not have the necessary efficiency (Liu *et al.*, 2019). Also, the effects of toxin binders on the yield of feed contaminated with AFB1 are variable due to the different components and the amount used in the diet, as well as the type of breed and the different rearing conditions of broiler chickens. Therefore, the mentioned reasons can probably be the cause of the non-significance of the toxin binders used in the present experiment (Rajput *et al.*, 2017).

The results for cellular and humoral immune responses are presented in Table 3. Broiler chicks in PC group showed lower cellular and humoral responses in comparison to those fed the

Table 2. The Effects of Dietary Inclusion of Test and Commercial toxin binders' chicks on average daily feed intake (ADFI, g), body weight gain (BWG, g) and the feed conversion ratio (FCR) of broiler chicks fed with aflatoxin-contaminated diets

Treatments	ADFI			BWG			FCR			
	day	10-22	22-42	10-42	10-22	22-42	10-42	10-22	22-42	10-42
NC		77.29	105.49 <sup>c</sup>	95.16	47.36 <sup>a</sup>	75.58 <sup>a</sup>	65.27 <sup>a</sup>	1.646	1.397 <sup>a</sup>	1.462 <sup>a</sup>
PC		65.10	114.65 <sup>abc</sup>	95.84	41.70 <sup>b</sup>	49.16 <sup>bc</sup>	46.32 <sup>bc</sup>	1.567	2.353 <sup>b</sup>	2.074 <sup>b</sup>
ASRI1		67.64	120.53 <sup>ab</sup>	100.85	44.46 <sup>ab</sup>	49.70 <sup>bc</sup>	47.78 <sup>bc</sup>	1.527	2.432 <sup>b</sup>	2.109 <sup>b</sup>
ASRI2		71.22	125.83 <sup>a</sup>	105.79	43.61 <sup>ab</sup>	54.29 <sup>b</sup>	50.38 <sup>b</sup>	1.633	2.320 <sup>b</sup>	2.101 <sup>b</sup>
STB1		71.97	115.37 <sup>abc</sup>	98.75	43.87 <sup>ab</sup>	45.87 <sup>bc</sup>	45.13 <sup>bc</sup>	1.639	2.537 <sup>b</sup>	2.197 <sup>b</sup>
STB2		66.80	110.96 <sup>bc</sup>	94.08	42.20 <sup>b</sup>	43.53 <sup>c</sup>	42.98 <sup>c</sup>	1.586	2.575 <sup>b</sup>	2.197 <sup>b</sup>
Mycofix		77.70	118.81 <sup>abc</sup>	103.20	44.85 <sup>ab</sup>	48.22 <sup>bc</sup>	46.92 <sup>bc</sup>	1.749	2.486 <sup>b</sup>	2.205 <sup>b</sup>
P-value		0.201	0.055	0.173	0.063	0.000	0.000	0.859	0.000	0.000
SEM		4.012	4.288	3.436	1.232	2.768	1.80	0.110	0.083	0.065

NC diet ( $P < 0.05$ ). Dietary inclusion of ASRI1 and ASRI2 toxin binders significantly decreased the adverse effects of aflatoxin on immune responses ( $P < 0.05$ ). Previous studies have shown that broiler chicks fed with mycotoxins showed lower antibody titers (Khan *et al.*, 2014). Decreased immune responses in response to AFB1 could be attributed to faulty protein formation that reduces IgG and IgA synthesis (Sur and Celik, 2004). Herbal-based toxin binders could improve immune responses. Talazadeh and Mayahi (2017) have reported that dietary supplementa-

tion of medicinal plants improved immune response in broiler chickens. Other studies have reported that herbal-based diets did not have any significant effect on antibody titers in poultry (Ozek *et al.*, 2011; Hosseini *et al.*, 2013). Plant compounds help to improve the immune system by their antioxidant activity (Amresh *et al.*, 2007) and thus alleviate adverse effects of AFB1 on immunity.

AFB1 decreased some carcass traits and herbal-based toxin binders could not reverse this. Ortatatli *et al.* (2005) showed an increase in the abso-

Table 3. Effects of Dietary Inclusion of Test and Commercial Toxin Binders on Antibody Titers Against Sheep Red Blood Cell (SRBC) and the Response to Phytohemagglutinin (PHA) and 2, 4-Dinitrochlorobenzene (DNCB) after 24h And 48h in Broiler Chicks Fed with Aflatoxin-Contaminated Diets

Treatments	IgG	IgM	SRBC	PHA-24	PHA-48	DNCB-24	DNCB-48
NC	4.40 <sup>ab</sup>	1.60 <sup>ab</sup>	6.00 <sup>a</sup>	1.51 <sup>a</sup>	1.11 <sup>a</sup>	1.34 <sup>a</sup>	0.93 <sup>a</sup>
PC	3.00 <sup>c</sup>	0.80 <sup>b</sup>	3.80 <sup>d</sup>	0.71 <sup>d</sup>	0.57 <sup>c</sup>	0.58 <sup>d</sup>	0.45 <sup>c</sup>
ASRI1	3.60 <sup>b</sup>	1.80 <sup>a</sup>	5.40 <sup>ab</sup>	1.05 <sup>b</sup>	0.80 <sup>b</sup>	1.03 <sup>b</sup>	0.70 <sup>b</sup>
ASRI2	4.60 <sup>a</sup>	1.30 <sup>a</sup>	5.60 <sup>ab</sup>	0.98 <sup>bc</sup>	0.72 <sup>b</sup>	0.88 <sup>b</sup>	0.62 <sup>b</sup>
STB1	3.60 <sup>b</sup>	0.80 <sup>b</sup>	4.40 <sup>cd</sup>	0.84 <sup>cd</sup>	0.64 <sup>bc</sup>	0.79 <sup>c</sup>	0.61 <sup>b</sup>
STB2	3.00 <sup>c</sup>	1.00 <sup>ab</sup>	4.00 <sup>d</sup>	0.87 <sup>bcd</sup>	0.70 <sup>bc</sup>	0.76 <sup>cd</sup>	0.58 <sup>b</sup>
Mycofix	3.40 <sup>bc</sup>	1.60 <sup>ab</sup>	5.00 <sup>bc</sup>	0.96 <sup>bc</sup>	0.73 <sup>bc</sup>	0.82 <sup>bc</sup>	0.60 <sup>b</sup>
P-value	0.001	0.061	0.000	0.000	0.000	0.000	0.001
SEM	0.267	0.278	0.273	0.061	0.065	0.068	0.061

NC= Non aflatoxin contaminated diet; PC= aflatoxin-contaminated diet.

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.05$ ).

SEM: standard error of the means.

Table 4. The Effects of Dietary Inclusion of Test and Commercial Toxin Binders on Carcass Traits Of Broiler Chicks Receiving Aflatoxin-Contaminated Diets

Treatments	Carcass	Breast	Thigh	Wings	Back and neck	Gizzard	Abdominal Fat	Heart	Liver	Spleen	Bursa
NC	77.18 <sup>a</sup>	23.49 <sup>a</sup>	18.92 <sup>a</sup>	6.48 <sup>a</sup>	17.88	2.42	1.32	0.43	1.68 <sup>d</sup>	0.104	0.082
PC	70.74 <sup>bc</sup>	21.24 <sup>bc</sup>	17.66 <sup>ab</sup>	5.61 <sup>c</sup>	17.30	2.98	1.38	0.45	3.72 <sup>ab</sup>	0.131	0.107
ASRI1	70.74 <sup>bc</sup>	20.16 <sup>bc</sup>	17.63 <sup>ab</sup>	5.92 <sup>bc</sup>	18.66	3.09	1.25	0.50	4.20 <sup>a</sup>	0.159	0.120
ASRI2	72.00 <sup>abc</sup>	20.11 <sup>bc</sup>	18.40 <sup>ab</sup>	5.89 <sup>bc</sup>	18.41	3.05	1.26	0.47	2.84 <sup>c</sup>	0.094	0.119
STB1	67.23 <sup>c</sup>	19.24 <sup>c</sup>	17.27 <sup>b</sup>	5.65 <sup>c</sup>	16.48	2.78	1.43	0.46	3.90 <sup>ab</sup>	0.115	0.074
STB2	67.50 <sup>c</sup>	21.23 <sup>bc</sup>	17.14 <sup>b</sup>	5.92 <sup>bc</sup>	17.17	2.93	1.36	0.52	3.81 <sup>ab</sup>	0.149	0.118
Mycofix	76.28 <sup>ab</sup>	22.10 <sup>ab</sup>	19.10 <sup>a</sup>	6.31 <sup>ab</sup>	18.83	2.92	1.17	0.41	3.30 <sup>bc</sup>	0.103	0.108
<i>P</i> -value	0.003	0.001	0.042	0.009	0.184	0.153	0.92	0.545	0.000	0.150	0.096
SEM	2.031	0.697	0.521	0.18	0.706	0.178	0.152	0.04	0.284	0.019	0.013

NC= Non aflatoxin contaminated diet; PC= aflatoxin-contaminated diet.  
The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).  
SEM: standard error of the means.

Table 5. The Effects of Dietary Inclusion of Test And Commercial Toxin Binders on Villus Length (VL), Villus Width (VW) and Crypt Depth (CD) of Different Intestinal Parts of Broiler Chicks Fed the Contaminated Diets

Treatments	Duodenum			Jejunum			Ileum		
	VL	VW	CD	VL	VW	CD	VL	VW	CD
day	( $\mu\text{m}$ )	( $\mu\text{m}$ )	( $\mu\text{m}$ )	( $\mu\text{m}$ )	( $\mu\text{m}$ )	( $\mu\text{m}$ )	( $\mu\text{m}$ )	( $\mu\text{m}$ )	( $\mu\text{m}$ )
NC	0.922 <sup>ab</sup>	0.270	0.230 <sup>a</sup>	1.34 <sup>a</sup>	1.88	0.190	1.296 <sup>a</sup>	0.188 <sup>a</sup>	0.214 <sup>a</sup>
PC	0.510 <sup>c</sup>	0.370	0.128 <sup>d</sup>	1.09 <sup>c</sup>	0.292	0.271	0.946 <sup>b</sup>	0.088 <sup>c</sup>	0.110 <sup>b</sup>
ASRI1	0.880 <sup>ab</sup>	0.240	0.186 <sup>b</sup>	1.26 <sup>bc</sup>	0.156	0.180	1.288 <sup>a</sup>	0.162 <sup>ab</sup>	0.220 <sup>a</sup>
ASRI2	0.816 <sup>ab</sup>	0.230	0.188 <sup>b</sup>	1.30 <sup>ab</sup>	0.154	0.186	1.294 <sup>a</sup>	0.166 <sup>ab</sup>	0.196 <sup>a</sup>
STB1	0.690 <sup>bc</sup>	0.218	0.162 <sup>bc</sup>	1.20 <sup>d</sup>	0.132	0.164	1.290 <sup>a</sup>	0.144 <sup>b</sup>	0.202 <sup>a</sup>
STB2	0.682 <sup>bc</sup>	0.208	0.156 <sup>c</sup>	1.21 <sup>d</sup>	0.126	0.150	1.256 <sup>a</sup>	0.140 <sup>b</sup>	0.188 <sup>a</sup>
Mycofix	1.010 <sup>a</sup>	0.226	0.180 <sup>bc</sup>	1.24 <sup>cd</sup>	0.146	0.178	1.268 <sup>a</sup>	0.138 <sup>b</sup>	0.200 <sup>a</sup>
<i>P</i> -value	0.002	0.782	0.000	0.000	0.760	0.925	0.000	0.000	0.000
SEM	0.078	0.077	0.009	0.014	0.077	0.070	0.023	0.011	0.012

NC= Non aflatoxin contaminated diet; PC= aflatoxin-contaminated diet.

The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).

SEM: standard error of the means.

lute and relative weights of liver, kidney and gizzard of broiler chicks fed with aflatoxin diets. Liver is a target organ for AFB1 since it is responsible for aflatoxin detoxification where aflatoxins are bioactivated to the reactive 8, 9-epoxide form that is bond DNA and proteins and destroy liver compounds and increase liver weight (Miazzo *et al.*, 2005; Bailey *et al.*, 2006 and Pasha *et al.*, 2007). Increased liver weight could be attributed to increased lipid deposits which could be attributed to fat metabolism (Denli and Okan, 2006).

The effects of dietary inclusion of commercial toxin binders on carcass traits are presented in Table 4. Broiler chicks fed the PC diet showed lower percentages of carcass, breast, thigh, wing and liver. Dietary inclusion of Mycofix toxin binder could significantly alleviate the effects of aflatoxin on wings ( $P<0.05$ ). Herbal-based toxin binders did not improve carcass traits. The investigations made from the results of similar researches have determined that with the increase of breeding days and at the end of the period, the amount of feed consumed by the birds increases compared to the beginning and growth periods, and as a result, the poison entered into the body also increases. With the increase in the amount of AFB1 in the body at the end of the period, probably the toxin binders do not have the necessary ability to neutralize the effect of AFB1, which can be one of the reasons for the lack of

improvement in carcass traits in the present experiment (Fouad *et al.*, 2019).

The data for intestinal morphology are shown in Table 5. The results showed that VL in the different parts of intestine, ileal VW and CD and duodenal CD were significantly decreased in broiler chicks fed with PC diet in comparison to broiler chicks fed with NC diet ( $P<0.05$ ). Dietary inclusion of different toxin binders generally alleviated adverse effects of toxin binders on intestinal morphology ( $P<0.05$ ). Aflatoxins produced adverse effects on intestinal morphology and adding different toxin binders did alleviate many effects AFB1 on intestinal morphology. Pelicano *et al.* (2005) showed that pathogens could injure normal microflora and intestinal epithelium and destroy the ability to digest and to absorb nutrients through a decrease in villus height. Pathogens could decrease CD, VW and VL. The deeper CD is considered a result of fast tissue turnover that helps renewal of the villus as required in response to normal sloughing or inflammation, pathogens or their toxins, and the high demand for tissue (Rashidi *et al.*, 2020). Decreased villus height and deeper crypts led to decreased nutrient absorption and increased secretion in the gastrointestinal tract (Xu *et al.*, 2004). We have shown that adding toxin binders could improve morphology. We believed that toxin binders could remove AFB1. Langrová *et al.* (2019) reported that plant toxin binders reduce the nega-

tive effects of aflatoxin on the intestinal bacterial population. In relation to the antimicrobial activity of medicinal plant compounds, it has been found that these compounds cause disruption in the cytoplasmic membrane, stopping the penetration of stimulating protons, smoothing the flow of electrons and active transport, as well as coagulation of bacterial contents and by eliminating pathogenic bacteria, they can improve intestinal morphology.

## CONCLUSION

It could be stated that AFB1 showed adverse effects on growth performance, immune responses, carcass traits and intestinal morphology. However, herbal-based toxin binders could alleviate some negative effects of AFB1 on immune responses and intestinal morphology. It could be recommended to use herbal-based supplements for alleviation of the adverse effects of AFB1.

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