The preventive effect and mechanisms of adsorbent supplementation in low concentration aflatoxin B1 contaminated diet on subclinical symptom and histological lesions of broilers

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ABSTRACT   This study aimed to investigate the subclinical symptom and histological lesions of 21-day-old and 42-day-old broilers exposure to low concentration aflatoxin B1 (**AFB$_1$**), and the preventive effect with adsorbent (Toxo-MX) supplementation. A total of 576 one-day-old Arbor Acres broilers were randomly allotted into 6 treatments 8 replicates and 12 birds per cage, fed with 0 ppb, 60 ppb and 120 ppb AFB$_1$ contamination diet with or without Toxo-MX supplementation. Results showed both 60 ppb and 120 ppb AFB$_1$ contamination significantly reduced growth performance in 21-day-old broilers ($P < 0.05$), but not in 42-day-old broilers ($P > 0.05$), however, AFB$_1$ contamination in diet caused a higher feed to gain ratio ($P < 0.05$). Broilers of 21-day-old exposure to 60 ppb and 120 ppb AFB$_1$ increased mRNA expression of hepatic inflammatory cytokines, and superoxide dismutase (**SOD**), glutathione peroxidase (**GSH-Px**) activity ($P < 0.05$), 42-day-old broilers showed a same change in 120 ppb but not in 60 ppb of AFB$_1$ contamination ($P < 0.05$). mRNA expressions of clauding-1, Zonula occludens-1 (**ZO-1**), and occludin decreased, but Bax, Bcl-2, and caspase-3 increased in 21-day-old broilers exposure to 60 ppb and 120 ppb AFB$_1$ ($P < 0.05$), broilers of 42-day-old resisted on intestinal aflatoxicosis impairment against 60 ppb AFB$_1$ contamination ($P < 0.05$), but not in 120 ppb ($P < 0.05$). Toxo-MX supplementation significantly reversed the detrimental effects on growth performance in both age broilers and reduced the accelerated feed to gain ratio caused by AFB$_1$ ($P < 0.05$). Intestinal mRNA expression of tight junction and apoptotic genes in both age broilers were recovered by Toxo-MX supplementation ($P < 0.05$). However, Toxo-MX did not restore the accelerated expression of hepatic inflammation cytokines and SOD, GSH-Px in 120ppb AFB$_1$ group ($P < 0.05$). The data demonstrated that diet supplementation with Toxo-MX reversed the detrimental effect on growth performance and intestine in broilers exposure to 60 ppb and 120 ppb AFB1. However, did not completely recovered hepatic inflammation induced by AFB$_1$.

Key words: aflatoxin B$_1$, broiler, immunity, hepatic inflammation, intestinal barrier function

INTRODUCTION

Aflatoxins are carcinogens and hepatotoxins which as the secondary metabolites produced by the fungal genus of *Aspergillus* are frequently contaminated in poultry feeds and associated with increased risk of disease outbreak and growth performance impairment (Dersjant-Li et al., 2003; Pankaj et al., 2018). The predominant aflatoxin is aflatoxin B$_1$ (**AFB$_1$**) (Rawal et al., 2010). Worldwide economic losses caused by AFB$_1$ contamination could reach billions annually (Wu, 2015; Mitchell et al., 2016). However, unlike those relative high dosage used in experiment setting (Gowda et al., 2009; Yarru et al., 2009), natural occurrence of AFB$_1$ in feed ingredients were usually between 0 and 1 mg/kg (Dersjant-Li et al., 2003). Nations in the world established aflatoxin contamination regulations which are aimed to protect human health and reduce residues in animal products: US Food and Drug Administration (**FDA**) set AFB$_1$ contamination action levels in mature

poultry feeds are less than 300 μg/kg in cottonseed meal, 100 μg/kg in corn, and 20 μg/kg in other feed ingredients. Moreover, for immature poultry are set at less than 20 μg/kg (Cai et al., 2020). The EU legal limit for AFB1 in processed cereal is 20 μg/kg (Agriopoulou et al., 2020), China set at less than 50 μg/kg (Ma et al., 2018). Furthermore, contaminated ingredients could be diluted before use, therefore, the toxic effect and prevention strategy of low concentration AFB_1 contamination should be emphasis in experiment design.

Whenever mycotoxins are produced in crops, they directly create negative impact on animal health and directly or indirectly produce serious health issues (Cao et al., 2020). Recently study reported the positive rate of AFB_1 contamination in feed ingredients could reach as high as 80.8% (Wu et al., 2016), discard the contaminated ingredient is not always practical, the treatment of AFB_1 contaminated feeds have significant economic implications for animal producer. Thus, decontamination procedures are important for both safety and economic efficiency. AFB_1 decontamination methods focused on degrading, inactivating, or removing AFB_1 by physical, chemical, and biological methods (Rawal et al., 2010; Adebo et al., 2016; Rushing and Selim, 2019). A cost-effective to the problem is to use non-nutritive and inert adsorbents in the diet to bind AFB_1 and reduced their absorption, however, the efficiency of those adsorbents is highly related to the concentration of AFB_1 because of the rapid absorption rate of AFB_1 (LI et al., 2010; Vila-Donat et al., 2018). Therefore, evaluation of the adsorbent efficiency in a natural occurrence low dosage of AFB_1 is necessary in practical production.

Liver is the principal target organ of aflatoxicosis, hepatocytes exposure to AFB_1 could activate hepatic inflammation responses, release inflammatory cytokines, and change antioxidant enzyme activity for detoxification reaction (Wang et al., 2019). The phase I of AFB_1 detoxification in poultry appears to convert the original molecules into hydrophilic compound by hydrolytic or oxidant-reduction enzymes, phase II is characterized by conjugation of original molecules or its metabolites principal with endogenous glutathione (**GSH**), a reaction catalyzed by glutathione S-transferases (**GSTs**) (Dohnal et al., 2014). Likewise, aflatoxicosis caused impairment metabolism of liver, resulted in reduction of nutrition metabolism and feed efficiency (Iqbal et al., 2005; Saleemi et al., 2020). Besides, age is another important factor that affects the resistance of poultry to AFB_1 contamination. Younger poultry is more sensitive to AFB_1 than older ones, and the resistance is associated with development of hepatic microsomal enzyme system and GSH activity, and also the age-related increasing in the expression of GST isoforms (Dohnal et al., 2014; Wang et al., 2018). After intake of AFB_1 contamination diet, AFB_1 could directly contact to intestinal epithelial cell and altered intestinal morphology and digestibility (Yunus et al., 2010, 2011; Jiang et al., 2015), inhibition cellular 20S proteasome and increase caspase-3 activity to activate apoptotic pathway (Towner et al., 2003; Amici et al., 2007). However, these changes were varied with AFB_1 dosage, length of exposure, age of poultry, species, and diet nutritional statues (Yunus et al., 2010, 2011).

Based on above information, the purpose of this study was to examine histological changes, aflatoxicosis symptoms and molecular mechanism in liver and intestine of 21-day-old and 42-day-old broilers exposure to 60 ppb or 120 ppb AFB_1, evaluate the preventive effect of dietary supplementation with a commercial smectite clay minerals adsorbent (TOXO-MX).

MATERIALS AND METHODS

Sample Preparation

AFB_1 were purchased from Pribiolab (Purity >99%, Qingdao, China), The adsorbent TOXO-MX were obtained from Trouw Nutrition, Amersfoort, The Netherlands. TOXO-MX consists of a high content of smectite clay which contained 94% dioctahedral smectite, 3% sodium feldspar, 1% each of illite and maghemite, and traces of quartz and calcite, as assessed by an X-ray diffraction study.

Diet Formulation

Diet formulation was based on NRC 1994 (Nutrient Requirements of Poultry, 1994), diet composition and nutrition level were presented in Table 1, AFB_1 were added into the premix before mixture with feed ingredients, and the final concentration of AFB_1 in the feed were determined by ELISA (Enzyme-linked Biotechnology Co., Ltd, Shanghai, China). The measured final concentration of AFB_1 is presented in Table 2. Toxo-MX (2.5 kg/T) supplementation was performed according to the products' instructions.

Animal Management

Hunan Agricultural University Animal Ethics Committee (Changsha, China) reviewed and approved all

Table 1. [1]Diet composition and nutrition level.

| Ingredients | Proportion[1] (%) | | Nutrition | | |
	0−21 d	22−42 d	Item	0−21 d	22−42 d
Corn	56.51	62.21	ME (kCal/kg)	3,039.94	3,111.17
Soybean	35.30	29.20	CP (%)	21.00	18.28
Soybean oil	4.50	4.90	Ca (%)	0.90	0.91
$CaCO_3$	1.52	1.60	P (%)	0.29	0.29
$CaHPO_4$	1.00	1.00	Lys (%)	1.27	1.09
L-Lys	0.35	0.30	Met (%)	0.47	0.41
L-Met	0.16	0.13	Thr (%)	0.83	0.75
L-Thr	0.06	0.06			
NaCl	0.30	0.30			
Premix[1]	0.30	0.30			
Total	100	100			

[1]Provided per kilogram of diet: vitamin A, 9500 IU; vitamin D3, 62.5 g; vitamin K3, 2.65 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B12, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325mg; folic acid, 1.25 mg; pantothenic, 12 mg; niacin, 50 mg; Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.35 mg.

experimental protocols. A total of 576 one-day-old Arbor Acres broilers were housed in an environmentally controlled room, and temperature was set at 34°C at first day, and gradually decreased to reach 20°C from d 28 of age and for the rest of the experiment, the average air humidity was set at 65%. Broilers with equivalent gender were completely randomized allotted to a total of 6 treatments 8 replicates and 12 birds per cage of 0 ppb, 60 ppb and 120 ppb AFB$_1$ with or without Toxo-MX supplementation. Broilers were free access to water and feed, experiment period was 42 d, include brood period of 1 d to 21 d and growth period of 22 d to 42 d. ADFI was measured by subtracting the amount of feed that was rejected from the birds that was offered, bodyweight (**BW**) were determined every 3 d, average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed efficiency (**G/F**) were calculated. Four birds from each cage were sacrificed at 21 d and 42 d, respectively. Thymus, spleens, and bursa of fabricius were weighted. A lobe of liver, duodenum, jejunum and ileum of broilers from each cage were preserved in 4% buffered paraformaldehyde (4% Formaldehyde Solution in PBS, adjusted pH to 7.4, Sinopharm, Beijing, China) for histological analysis, the remaining hepatic and enteric parts were washed by 0.90% w/v of NaCl solution, freeze in the liquid nitrogen and then stored in −80°C for further analysis.

Serum Biochemical Assay and Serum Immunoglobulin, Acute-phase Protein Quantitation

Blood was collected from brachial vein of broiler, and serum were obtained in a blood collection tube (Separable microtubes, FUCHIGAMI, 170720, Kyoto, Japan) by centrifuging at 1,500 × g for 10 min and stored at −20°C until use. The levels of serum aspartate transaminase (**AST**), alanine transaminase (**ALT**), Serum total protein (**TP**), albumin (**ALB**), alkaline phosphatase (**ALP**), and total bilirubin (**T-Bil**) were measured with an automated analyzer for clinical chemistry analysis (Mindray BS-200 Chemistry Analyzer, Shenzhen, China), Serum Gamma-Glutamyl transferase (**GGT**) were tested by a GGT instrument Specific kit (Mindray, Shenzhen, China). Serum globulin (**GLO**) is calculated using the formula: $GLO = TP - ALB$. Urea nitrogen (**BUN**) was measured by using a BUN Colorimetric Detection Kit (Thermo, Shanghai, China) according to the manufacturer's instructions.

Serum immunoglobulin (IgG, IgA, and IgM) were quantitated by using chicken immunoglobulin ELISA kit according to the manufacturer's instructions (Cusabio, Wuhan, China). Serum acute-phase protein including serum amyloid A (**SAA**) and alpha-1-acid glycoprotein (**α1-AGP**) were measured by using chicken SAA ELISA kit and chicken alpha 1 acid glycoprotein ELISA kit according to the manufacturer's instructions (Abcam, Shanghai, China).

Hepatic and Enteric Histological Lesion

Lobes of liver, duodenum, jejunum, and ileum were preserved 24 h in 4% buffered paraformaldehyde, then were dewatered gradually with 10 to 30% sucrose water, changing every 12 h. After dewatering, the liver, duodenum, jejunum, and ileum from each treatment were embedded by paraffin wax. Paraffin blocks were cross sectioned at 5 μm by a microtome system machine (Yamato, Saitama, Japan). Sliced sections of liver, duodenum, jejunum, and ileum from each treatment mounted on poly-L-lysine-coated glass slides and stained with hematoxylin-eosin, observed under a microscope (Keyence, Tokyo, Japan) and were taken at 40 × and 100 × magnification for morphological analysis. Ten villi and 10 crypts of intestines from each segment of each bird were quantified by the software in microscopic imaging system (Keyence). The villus height and crypt depth per villus were measured in each segment. TUNEL assay was performed by using a TUNEL Assay Kit (Abcam) according to manufacturer's instructions. The number of TUNEL-positive cells were counted from 10 random fields from each intestinal cross-section by the software in microscopic imaging system (Keyencen), TUNEL positive rate were calculated by average the number of TUNEL-positive cells to total number of nuclei ratio per area and normalized to TUNEL signal of negative control group.

Real-Time Quantitative PCR

Jejunal total RNA was extracted by a Total RNA II kit (Omega Bio-tek, Guangzhou, China) according to the manufacturer's manual. PrimeScript RT Reagent Kit (Takara, Tokyo, Japan) were used to synthesize the cDNA. Expressions of claudin-1, occluding, Zonula occludens-1 (**ZO-1**), B-cell lymphoma 2 (**Bcl-2**), Bcl-2-associated X protein (**Bax**), Caspase-3, and β-actin were measured by real-time quantitative PCR (**qPCR**) using One-step PrimeScript RT-PCR kit (Takara). The reaction condition consisted 12.5 μL SYBR Premix Ex Taq Ⅱ (Applied Biosystems, Foster city, CA), 2 μl cDNA, 1 μL of 10 μM 1:1 forward and reverse target primer mixture and 8.5 μL of nuclease-free water. Primers for claudin-1, occluding, ZO-1, Bcl-2, Bax, Caspase-3, and β-actin are listed in Table 3. The thermal cycling conditions were composed of 50°C for 2 min followed by an initial denaturation step at 95°C for 10 min, 45 cycles at 95°C for 30 s, 60°C for 30 s and 72°C for 30 s. Real-time PCR was carried out using an ABI PRISM 7500 Sequence Detection System (Applied Biosystems). Reactions were run in triplicate in 3 independent experiments. The geometric mean of housekeeping gene β-actin was used as an internal control to normalize the variability in expression levels. Expression data were normalized to the geometric mean of housekeeping gene GAPDH to control the variability in expression levels and were analyzed using the 2-ΔΔCT method described by previous report (Livak and Schmittgen, 2001).

Table 2. The measured concentration of aflatoxin B1 (AFB$_1$) in the diet of broilers.

Item	0 ppb AFB1	SD	60 ppb AFB1	SD	120 ppb AFB1	SD
0−21 d (ppb)	0.51	0.44	57.10	2.39	117.84	8.74
22−42 d (ppb)	0.09	0.17	62.00	4.11	126.54	10.06

The data represent mean of 5 replicates, tested sample were collected by using stratified sampling method, and AFB1 in the feed were determined by ELISA (Enzyme-linked Biotechnology Co., Ltd, Shanghai, China).

Statistical Analysis

Results were expressed as mean ± SEM. The significant differences between treatments were analyzed by the One-Way ANOVA followed Tukey's multiple comparison post-test, and by using the GLM procedure of SPSS 19.0 (SPSS Inc., Chicago, IL). ANOVA annotation uses letters (a, b, c, etc.), where treatments not sharing the same letter indicates $P < 0.05$.

RESULTS

Growth Performance

Aflatoxicosis of AFB$_1$ in poultry industry is characterized by reduction of growth performance and feed efficiency. In the present study, 60 ppb and 120 ppb AFB$_1$ contaminated diet significantly decreased BW and ADG in 21-day-old broilers (Table 4, $P < 0.05$), F:G ratio were higher compared to control group ($P < 0.05$). Diet supplementation with Toxo-MX reversed the reduction of growth performance and feed efficiency ($P < 0.05$). The 42-day-old broilers fed AFB$_1$ contaminated diet have no significant difference in BW ($P > 0.05$), however, an increased ADFI and F:G ratio has been showed ($P < 0.05$). Broilers fed both 60 ppb and 120 ppb AFB$_1$ contaminated diet increased F:G ratio during the whole experiment period ($P < 0.05$), ADG of broilers fed with 120 ppb AFB$_1$ contaminated diet significantly lower than control group ($P < 0.05$). Both 60 ppb and 120 ppb AFB$_1$ contaminated diet supplement with Toxo-MX ameliorated F:G ratio and ADG ($P < 0.05$), and had no significant differences compared with control group ($P > 0.05$).

Serum Parameters

There are a number of serum biomarkers have been validated to evaluate the toxic effect of AFB$_1$ in poultry. In the present study, a higher UA were shown in 21-day-old broilers fed AFB$_1$ contaminated diet (Table 5, $P < 0.05$), Toxo-MX supplementation in 60ppb AFB$_1$ contaminated diet recovered the UA levels ($P < 0.05$), but did not in 120 ppb AFB$_1$ contaminated diet ($P < 0.05$). Forty two-day-old broilers exposure to both 60 ppb and 120 ppb AFB$_1$ still have a higher serum UA ($P < 0.05$), but with Toxo-MX addition in AFB$_1$ contaminated diet, broilers have no significant difference in serum UA compared with control group ($P > 0.05$). BUN had no significant changes among all the treatments in both age broilers ($P > 0.05$). Serum ALP was significantly

Table 3. Primer sequences used for real-time quantitative PCR (5' to 3').

Genes	Primer sequence (5'-3')[1]	Accession number
β-Actin	F: GAGAAATTGTGCGTGACATCA	NM_205518.1
	R: CCTGAACCTCTCATTGCCA	
Bax	F: TCCTCATCGCCATGCTCAT	XM_422067
	R: CCTTGGTCTGGAAGCAGAAGA	
Bcl-2	F: GATGACCGAGTACCTGAACC	NM_205339
	R: CAGGAGAAATCGAACAAAGGC	
Caspase-3	F: TGGCCCTCTTGAACTGAAAG	NM_204725
	R: TCCACTGTCTGCTTCAATACC	
ZO-1	F: GCGCCTCCCTATGAGGAGCA	XM_413773.4
	R: CAAATCGGGGTTGTGCCGGA	
Occluding	F: CCGTAACCCCGAGTTGGAT	NM_205128.1
	R: ATTGAGGCGGTCGTTGATG	
Claudin-1	F: GCAGATCCAGTGCAAGGTGTA	NM_001012611.2
	R: CACTTCATGCCCGTCACAG	
IFN-γ	F:	

ATCATACTGAGCCAGATTGTTTCGAJ634956.1R: ACGCCATCAGGAAGGTTGTTIL-6F: AAATCCCTCCTCGCCAATCTNM_204628.1R: CTCACGGTCTTCTCCATAAACG TGF-$β1$F: CGGGACGGATGAGAAGAACTGJQ423909.1R: CAGGCACGGACCACCATATTG TNF-$α$F: GGAATGAACCCTCCGCAGTAAY765397.1R: CAGAGCATCAACGCAAAAGG TLR-4F: TTTGAAAATGTAAGGCGGCTCNM_001030693.1R: ATTTCCAGGGCTGAGTCCGAIL-$1β$F: ACTGGGCATCAAGGGCTANM_204524R: GGTAGAAGATGAAGCGGGTC

increased in broilers exposure to both concentration of AFB$_1$ in both age, Toxo-MX supplementation reversed the changes in 42-day-old broilers ($P > 0.05$), but not in 21-day-old birds ($P < 0.05$). In addition, AST, ALT, and GGT significantly increased in both 21-day-old and 42-day-old broilers fed AFB$_1$ contaminated diet ($P < 0.05$), Toxo-MX supplementation ameliorated the higher AST and ALT in the birds exposure to AFB$_1$ ($P < 0.05$), but the levels were still higher than control group ($P < 0.05$). Serum T-Bil and TP have no significant difference among the treatments in both age broilers ($P > 0.05$), however, ALB significantly decreased while GLB significantly increased in the 21-day-old broilers fed with AFB$_1$ contaminated diet than control group ($P < 0.05$), in 42-day-old broilers exposure with 120 ppb AFB$_1$ group, serum ALB and GLB was still higher ($P < 0.05$), but 60 ppb AFB$_1$ exposure group have no significant difference ($P > 0.05$). A total of 120 ppb and 60 pb AFB$_1$ contaminated diet add Toxo-MX have no significant differences of serum ALB and GLB in 21-day-old broilers ($P > 0.05$), but have lower ALB: GLB ratio ($P < 0.05$). In 42-day-old broilers, Toxo-MX supplementation reversed the changes in 60 ppb AFB$_1$ exposure ($P > 0.05$), but not in 120 ppb exposure ($P < 0.05$).

Table 4. Growth performance of broilers fed dietary contaminated aflatoxin B1 (AFB$_1$) with or without Toxo-MX.

Item	AFB$_1$ Toxo-MX	0 ppb AFB$_1$ -	+	60 ppb AFB$_1$ -	+	120 ppb AFB$_1$ -	+	SEM	P
1−21 d	Initial BW (g)	49.11	48.47	49.11	48.28	48.63	48.19	0.17	0.677
	Final BW (g)	655.36^{aW}	662.42^a	635.15^b	645.96^{ab}	611.82^c	641.78^{ab}	10.95	0.014
	ADG (g)	28.87^a	29.24^a	27.91^b	28.46^a	26.82^b	28.27^a	0.52	0.018
	ADFI (g)	44.54	44.74	44.92	43.80	44.48	43.99	0.51	0.462
	F:G	1.54^b	1.53^b	1.61^a	1.54^b	1.66^a	1.56^b	0.03	0.021
22−42 d	Final BW (g)	2,459.31	2,463.22	2,406.06	2,460.83	2,389.86	2,456.71	32.44	0.104
	ADG (g)	85.90	85.75	84.33	86.42	84.67	86.43	2.31	0.126
	ADFI (g)	147.87^c	149.22^c	156.18^{ab}	149.73^c	160.03^a	153.56^b	3.96	0.002
	F:G	1.72^b	1.74^b	1.85^a	1.73^b	1.89^a	1.78^{ab}	0.06	0.028
1−42 d	ADG (g)	57.39^a	57.49^a	56.12^{ab}	57.44^a	55.74^b	57.35^a	1.25	0.012
	ADFI (g)	96.21	96.98	100.55	96.77	102.26	98.78	2.04	0.077
	F:G	1.68^b	1.69^b	1.79^a	1.68^b	1.83^a	1.72^b	0.04	0.004

The data represent mean of 8 replicates, and different letters (abc) in the same column indicate significant differences ($P < 0.05$).

Serum Immunoglobulin and Acute-Phase Protein

AFB$_1$ has been demonstrated that involved in outbreak of infectious disease due to immunosuppression (Tessari et al., 2006), thus, we investigated serum immunoglobulin and acute-phase protein levels in both age broilers. As shown in Table 6, serum IgA and IgG concentration significantly reduced in 21-day-old broilers exposure to 120 ppb AFB$_1$ ($P < 0.05$), IgM has no significant difference ($P > 0.05$), AFB$_1$ contaminated diet with Toxo-MX addition restored the decreasing of serum IgA, IgG concentration ($P < 0.05$), but IgG concentration was still less than control group ($P < 0.05$). Forty two-day-old broilers fed with 120 ppb AFB$_1$ showed a less serum IgG concentration than control group ($P < 0.05$). Toxo-MX supplementation reversed the negative effect ($P < 0.05$), serum IgA and IgM concentration

have no significant differences among all the treatments in 42-day-old broilers ($P > 0.05$). Moreover, both 60 ppb and 120 ppb AFB$_1$ exposure showed no significant negative effect to the concentration of acute-phase proteins including SAA, α1-AGP, and α1-AT in both age birds ($P > 0.05$).

Relative Immune Organ Weight

Relative immune organ weights of the broilers in this study were presented in Table 7. Relative thymus weight significantly increased in 21-day-old broilers receiving diets with 120 ppb AFB$_1$ ($P < 0.05$), meanwhile, relative weight of bursa of Fabricius was significantly reduced ($P < 0.05$), and there was no significant difference in the relative weight of spleen ($P > 0.05$). In the 42-day-old broilers, no significant difference was

Table 5. Serum parameters of broilers fed dietary contaminated aflatoxin B1 (AFB$_1$) with or without Toxo-MX.

Item	AFB$_1$ Toxo-MX	0 ppb AFB$_1$ -	+	60 ppb AFB$_1$ -	+	120 ppb AFB$_1$ -	+	SEM	P
21 d	UA (mg/dL)	7.34^c	7.45^c	8.89^b	7.79^c	10.12^a	9.11^a	0.09	<0.001
	BUN (mg/dL)	3.42	3.30	3.21	3.54	3.45	3.24	0.15	0.724
	ALP (U/L)	126.16^b	131.54^b	179.36^a	169.93^a	174.86^a	183.78^a	9.27	0.014
	AST (U/L)	109.18^d	105.73^d	175.08^b	146.27^c	220.24^a	144.56^c	6.34	<0.001
	ALT (U/L)	3.23^d	3.44^d	44.37^a	13.27^c	42.58^a	23.02^b	2.32	<0.001
	GGT (U/L)	7.52^b	7.69^b	15.21^a	11.65^b	14.76^a	10.44^b	1.67	<0.001
	T-Bil (μmol/L)	35.92	34.85	35.60	33.11	36.11	35.26	1.18	0.237
	TP (g/L)	58.96	57.64	58.52	59.40	59.62	58.08	0.13	0.536
	ALB (g/L)	27.61^a	26.25^a	22.39^b	24.99^{ab}	21.65^b	24.92^{ab}	0.75	0.003
	GLB (g/L)	31.35^b	31.39^b	36.13^a	34.41^{ab}	37.97^a	33.16^{ab}	0.41	0.016
	ALB:GLB	0.88^a	0.84^a	0.62^c	0.73^b	0.57^c	0.75^b	0.01	<0.001
42 d	UA (mg/dL)	7.02^d	7.03^d	8.16^b	7.14^d	9.49^a	7.54^{cd}	0.10	<0.001
	BUN (mg/dL)	3.64	3.55	3.41	3.67	3.41	3.39	0.21	0.609
	ALP (U/L)	139.92^b	137.39^b	152.96^a	133.64^b	154.37^a	129.66^b	7.69	0.037
	AST (U/L)	134.86^b	140.42^b	218.80^a	154.65^b	222.56^a	164.20^b	11.66	<0.001
	ALT (U/L)	3.51^c	3.96^c	13.05^a	6.22^b	13.26^a	8.43^b	0.77	<0.001
	GGT (U/L)	10.11^c	9.69^c	26.64^a	15.42^b	27.72^a	18.56^a	2.01	<0.001
	T-Bil (μmol/L)	20.59	21.09	21.57	19.83	21.73	20.46	0.87	0.501
	TP (g/L)	58.32	59.84	60.06	60.50	61.82	60.72	0.07	0.249
	ALB (g/L)	26.18^a	26.45^a	25.61^a	26.69^a	23.04^b	24.71^b	0.36	0.018
	GLB (g/L)	32.44^c	33.39^c	34.45^c	33.81^c	38.78^a	36.01^b	0.54	0.048
	ALB:GLB	0.80^a	0.79^a	0.74^a	0.79^a	0.59^b	0.69^b	0.01	<0.001

The data represent mean of 8 replicates, and different letters (abc) in the same column indicate significant differences ($P < 0.05$).
Abbreviations: ALB, albumin; ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; BUN, Urea nitrogen; GGT, Serum Gamma-Glutamyl transferase; T-Bil, total bilirubin; TP, serum total protein.

Table 6. Serum levels of immunoglobulin and acute-phase protein of broilers fed dietary contaminated aflatoxin B1 (AFB$_1$) with or without Toxo-MX.

Item	AFB$_1$ / Toxo-MX	0 ppb AFB$_1$ -	0 ppb AFB$_1$ +	60 ppb AFB$_1$ -	60 ppb AFB$_1$ +	120 ppb AFB$_1$ -	120 ppb AFB$_1$ +	SEM	P
21d	IgA (ng/mL)	530.53[a]	552.67[a]	577.24[a]	548.64[a]	291.32[b]	464.85[a]	23.45	<0.001
	IgG (ng/mL)	1,604.65[a]	1,556.23[a]	1,448.66[a]	1,546.9[a]	945.18[c]	1,238.34[b]	62.36	<0.001
	IgM (ng/mL)	145.1	142.47	146.63	156.47	137.35	147.31	7.75	0.361
	SAA (ng/mL)	237.73	226.45	233.11	223.19	241.42	220.27	18.73	0.842
	α1-AGP (ng/mL)	146.8	146.1	133.87	133.94	132.54	140.51	19.48	0.224
42d	IgA (ng/mL)	586.07	601.66	596.57	609.1	622.82	607.85	49.92	0.701
	IgG (ng/mL)	1,418.24[ab]	1,391.94[ab]	1,350.66[b]	1,514.23[a]	1,122.44[c]	1,546.61[a]	121.13	<0.001
	IgM (ng/mL)	276.45	281.17	264.44	273.21	278.11	269.59	23.53	0.383
	SAA (ng/mL)	255.39	274.87	276.32	279.26	283.01	267.45	19.79	0.238
	α1-AGP (ug/mL)	168.55	170.73	176.08	174.96	178.71	167.68	14.26	0.524

The data represent mean of 8 replicates, and different letters (ab) in the same column indicate significant differences ($P < 0.05$).

seen in the relative immune organ weight among the treatments ($P > 0.05$).

Hepatic Histological Assay, mRNA Expression of Inflammation Genes, and Levels of Antioxidant Enzymes

Leukocyte infiltration were showed in the livers of both 21-day-old and 42-day-old broilers fed AFB$_1$ contaminated diet and the combination of 120ppb AFB$_1$ and Toxo-MX diet (Figures 1A and 1B). Relative mRNA expression of hepatic inflammation cytokines of 21-day-old broilers were shown in Figure 1C, TGFβ-1, TLR-4, and IL-1β were significantly increased in the liver of broilers fed AFB$_1$ contaminated diet ($P < 0.05$), however, a relative decreased of IFN-γ expression were showed in the birds fed AFB$_1$ contaminated diet ($P < 0.05$), and these changes were reversed when supplemented with Toxo-MX ($P < 0.05$). Moreover, hepatic antioxidant enzyme including GSH-Px and SOD were significantly increased in the broilers exposure to AFB$_1$ contamination in 21-day-age (Figure 1D, $P < 0.05$), Toxo-MX supplementation partly decreased the antioxidant enzyme increasing induced by AFB$_1$ exposure ($P < 0.05$), but still higher than control group in the 120 ppb AFB$_1$ contamination group ($P < 0.05$).

In the 42-day-old broilers, hepatic IL-6, TGFβ-1, TLR-4, TNF-α, and IL-1β mRNA expression were significantly reduced in the AFB$_1$ treatment groups (Figure 1E, $P < 0.05$), and these changes have not reversed in AFB$_1$ plus Toxo-MX diet compared to control group ($P < 0.05$). Furthermore, hepatic GSH-Px and SOD were higher in both age broilers fed AFB$_1$ contaminated diet (Figure 1F, $P < 0.05$), Toxo-MX addition reduced the accelerated GSH-Px and SOD but still higher than control group ($P < 0.05$).

Intestinal Villus Height and Crypt Depth

AFB$_1$ intake could directly contact with the intestinal villus of broilers and impaired their immune defense and absorption function. As shown in Figure 2 and Table 8, in the 21-day-old broilers with 60 ppb and 120 ppb AFB$_1$ exposure, jejunal and ileal villus height were significantly decreased ($P < 0.05$), and jejunal crypt depth increased ($P < 0.05$), which resulted in a significant lower VH:CD ratio ($P < 0.05$). Toxo-MX supplementation reversed the adverse effect in 21-day-old broilers fed AFB$_1$ contamination diet ($P < 0.05$). Aflatoxicosis at intestinal barrier remained in 42-day-old broilers, the data showed a significant shorter villus height in the jejunum and ileum ($P < 0.05$), and a significant less VH:CD ratio ($P < 0.05$), Toxo-MX also recovered the decreased villus height in 42-day-old broilers ($P < 0.05$). Interestingly, villus height and crypt depth of duodenum has no significant difference among all the treatments in both age broilers ($P > 0.05$).

Jejunal TUNEL Assay, mRNA Expression of Epithelial Cells Apoptosis, and Tight Junction Protein Genes

Jejunal TUNEL assay were presented in Figure 3A, and TUNEL positive rate were normalized by the nuclei per area quantitated TUNEL signal of control group (Figures 3C and 3F). As shown in Figure 3C, TUNEL positive rate were significant higher in 21-day-old broilers fed both 60 ppb and 120 ppb AFB$_1$ contaminated diet ($P < 0.05$), and diet Toxo-MX addition significantly reversed the increasing ($P < 0.05$). Bcl-2 mRNA expression of jejunal epithelial cells was significantly reduced, and Bax, caspase-3 significantly increased in 21-d broilers with both 60 ppb and 120 ppb AFB$_1$ exposure (Figure 3D, $P < 0.05$). Twenty one-day-old broilers with Toxo-MX supplementation significantly restored the overexpression of Bax and caspase-3 ($P < 0.05$), the decreased mRNA expression of Bcl-2 recovered in 60 ppb AFB$_1$ plus Toxo-MX ($P < 0.05$), but had not completely recovered in 120 ppb AFB$_1$ plus Toxo-MX ($P < 0.05$). Tight junction protein of claudin-1, ZO-1 and occluding were increased in 21-day-old broilers fed both 60 ppb and 120 ppb AFB$_1$ contaminated diet (Figure 3E, $P < 0.05$), tight junction protein of AFB$_1$ plus Toxo-MX groups have no significant differences when compared with control group ($P > 0.05$).
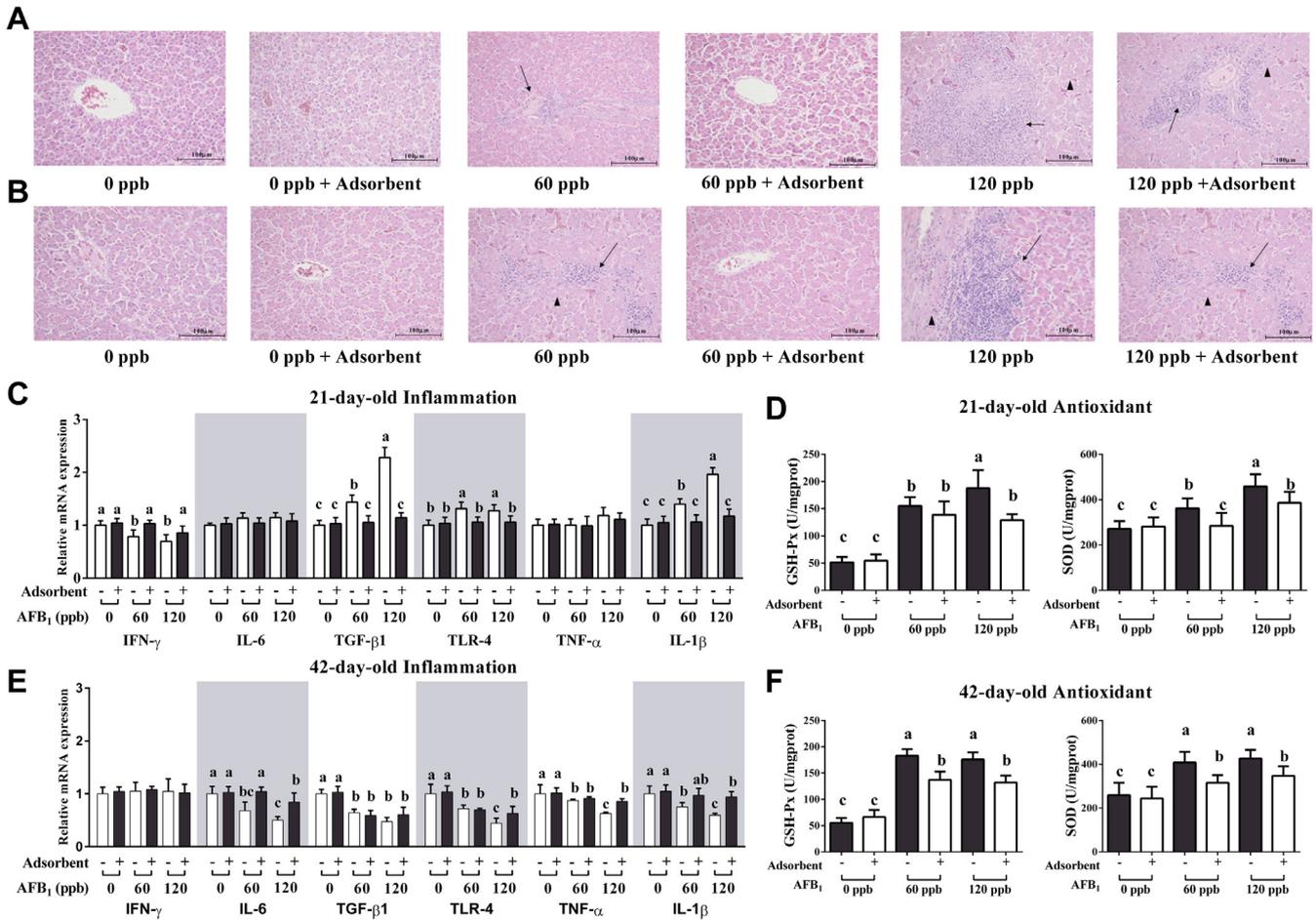
Figure 1. The mRNA expression of hepatic inflammation and antioxidant enzyme genes of broilers fed dietary contaminated AFB_1 with or without adsorbent. (A) Sliced hepatic tissue of 21-day-old broilers from each group was stained by hematoxylin−eosin (H&E) stain and was observed under a microscope (Keyence, Tokyo, Japan) with 200 × original magnification. (B) Sliced hepatic tissue of 42-day-old broilers. (C) Hepatic tissue mRNA expression of IFN-γ, IL-6, TGF-β1, TLR-4, TNF-α, IL-1β in 21-day-old broilers. (D) Hepatic GSH-Px and SOD levels in 21-day-old broilers. (E) Hepatic tissue mRNA expression of IFN-γ, IL-6, TGF-β1, TLR-4, TNF-α, IL-1β in 42-day-old broilers. (F) Hepatic GSH-Px and SOD levels in 42-day-old broilers. The data represent mean ± SD of 8 replicates, and different letters in the same column indicate significant differences ($P < 0.05$).

In 42-day-old broilers, jejunal TUNEL positive rate were only higher in 120 ppb AFB_1 treatment group compared to control group (Figure 3F, $P < 0.05$), and only in 120ppb exposure group showed a lower Bcl-2 and higher caspase-3 mRNA expression mRNA expression when compared to control group (Figure 3G, $P < 0.05$), moreover, in tight junction protein mRNA expression, the claudin-1 was still higher in 120 ppb AFB_1 exposure group (Figure 3H, $P < 0.05$), but ZO-1 and occluding have no significant difference among all the groups

Table 7. Relative immune organ weights (organ weight/body weight × 100) of broilers fed dietary contaminated aflatoxin B1 (AFB_1) with or without Toxo-MX.

Item	AFB$_1$ Toxo-MX	0 ppb AFB$_1$		60 ppb AFB$_1$		120 ppb AFB$_1$		SEM	P
		-	+	-	+	-	+		
21 d	Thymus	0.45[b]	0.43[b]	0.49[ab]	0.44[b]	0.57[a]	0.47[b]	0.019	0.017
	Spleen	0.14	0.13	0.16	0.14	0.14	0.15	0.007	0.522
	Bursa of Fabricius	0.32[a]	0.34[a]	0.26[b]	0.34[a]	0.24[b]	0.32[a]	0.011	0.021
42 d	Thymus	0.32	0.31	0.33	0.32	0.34	0.31	0.015	0.741
	Spleen	0.30	0.31	0.29	0.30	0.34	0.31	0.010	0.124
	Bursa of Fabricius	0.22	0.21	0.23	0.22	0.24	0.23	0.009	0.635

The data represent mean of 8 replicates, and different letters (ab) in the same column indicate significant differences ($P < 0.05$).
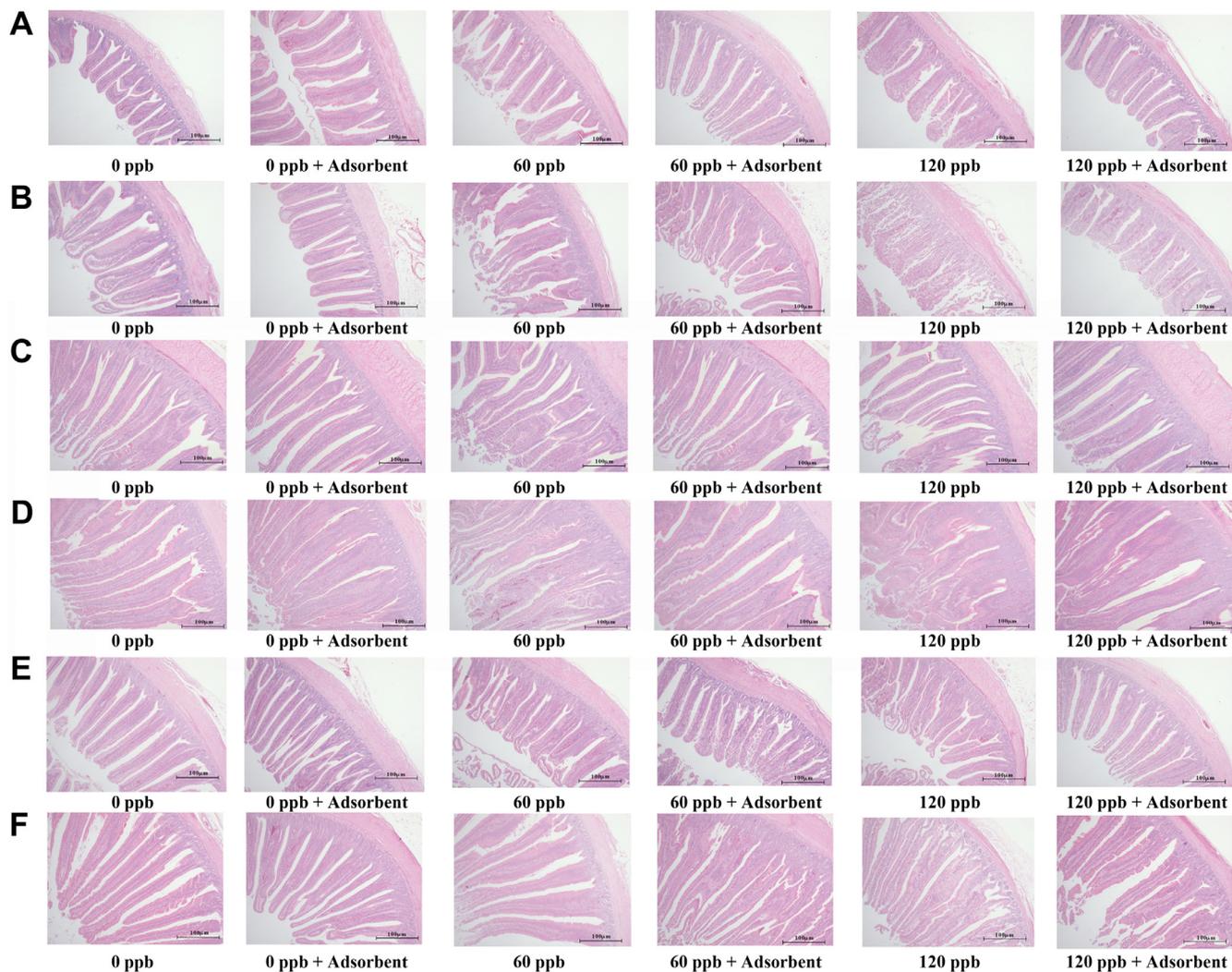
Figure 2. Representative histopathology section of intestines. (A) Sliced duodenal tissue of 21-day-old broilers from each group was stained by H&E staining and was observed under a microscope (Keyence, Tokyo, Japan) with $400 \times$ original magnification. (B) Sliced duodenal tissue of 42-day-old broilers. (C) Sliced jejunal tissue of 21-day-old broilers. (D) Sliced jejunal tissue of 42-day-old broilers. (E) Sliced ileal tissue of 21-day-old broilers. (F) Sliced ileal tissue of 42-day-old broilers.

$(P > 0.05)$. And all these changes induced by 120 ppb AFB$_1$ were recovered with Toxo-MX supplementation $(P < 0.05)$.

DISCUSSION

The toxic effect of AFB$_1$ in poultry varies with dose, length of exposure, age, species, and diet nutritional statues. Generally, young broilers are more susceptible than older ones to the toxic effect (Wang et al., 2018). In the present study, 21-day-old broilers fed both 60 ppb and 120 ppb AFB$_1$ contaminated diet showed a less BW and feed efficiency, however, 42-day-old broilers only showed less feed efficiency but had no numerical difference in BW. AFB$_1$ induced significant changes in serum ALP, ALT, AST concentration, and these changes in serum biochemical parameters became less severe with the age increasing of broilers. According to previous study (Hsieh, 1994), broilers prolonged intake of low concentration AFB$_1$ seemed to occur adaptive responses

(Dersjant-Li et al., 2003; Yunus et al., 2011), in diet with a constant concentration of AFB$_1$ contamination, the absorption of AFB$_1$ equaled to rate of elimination from the body, growth performance may numerically showed no significant differences, particularly in mature animals. Thus, the less effect on growth performance of older broilers to aflatoxicosis may due to the immunity system development and the adaptive responses to long-term chronic exposure.

Previous study has showed aflatoxin is rapidly absorbed from the small intestine into the mesenteric venous blood, and accumulated in the liver of broilers, where the AFB$_1$ and its metabolites bind with nucleic acid and proteins. Kidney has taken part in detoxification and is also among the organs where most of the aflatoxin residues are detected (Sawhney et al., 1973). Eliminated of aflatoxin appears slowly and primarily with urine and bile (Mabee et al., 1973; Sawhney et al., 1973). In this study, serum UA, hepatic SOD, and GSH-Px activity were significantly higher in both age broilers exposure to AFB$_1$ contaminated diet. Consistent with

Table 8. Intestinal villus height and crypt depth of broilers fed dietary contaminated aflatoxin B1 (AFB1) with or without Toxo-MX.

Item		AFB$_1$ Toxo-MX	0 ppb AFB$_1$ -	+	60 ppb AFB$_1$ -	+	120 ppb AFB$_1$ -	+	SEM	P
21 d	Duodenum	VH	311.38	304.40	307.75	315.37	313.83	306.68	27.46	0.559
		CD	46.79	48.48	44.38	47.99	47.45	44.46	3.40	0.302
		VH:CD	6.65	6.28	6.93	6.57	6.61	6.90	0.27	0.219
	Jejunum	VH	692.94[a]	688.87[a]	561.83[b]	685.04[a]	574.09[b]	660.97[a]	37.40	<0.001
		CD	76.12[b]	73.44[b]	91.27[a]	75.50[b]	102.85[a]	76.07[b]	15.37	0.007
		VH:CD	9.10[a]	9.38[a]	6.16[b]	9.07[a]	5.58[b]	8.69[ab]	1.43	<0.001
	Ileum	VH	268.69[a]	271.26[a]	188.17[b]	258.07[a]	183.11[b]	264.36[a]	22.98	0.023
		CD	33.33	31.84	34.85	32.74	38.52	36.66	3.39	0.124
		VH:CD	8.06[a]	8.52[a]	5.40[c]	7.88[a]	4.75[c]	7.21[ab]	1.19	0.008
42 d	Duodenum	VH	336.79	329.00	315.72	321.41	315.04	336.11	26.96	0.755
		CD	48.45	47.08	49.38	51.09	51.07	49.97	5.33	0.347
		VH:CD	6.95	6.99	6.39	6.29	6.17	6.73	0.80	0.819
	Jejunum	VH	779.32[a]	756.22[a]	556.83[b]	713.65[a]	495.47[b]	727.29[a]	41.29	<0.001
		CD	79.30[b]	78.57[b]	98.57[a]	75.33[b]	99.60[a]	79.54[b]	12.01	<0.001
		VH:CD	9.83[a]	9.62[a]	5.65[b]	9.47[a]	4.97[b]	9.14[a]	1.46	<0.001
	Ileum	VH	274.88[a]	295.44[a]	250.97[ab]	269.28[a]	213.46[b]	275.50[a]	33.24	0.008
		CD	44.70	47.38	49.04	46.64	48.42	47.65	4.38	0.217
		VH:CD	6.15[a]	6.24[a]	5.12[b]	5.77[ab]	4.41[c]	5.78[ab]	1.21	0.014

The data represent mean of 8 replicates, and different letters (abc) in the same column indicate significant differences ($P < 0.05$).

present study, hepatic SOD, GSH-Px increased in broilers exposure to AFB$_1$ had also been reported (Yang et al., 2012), and the increasing were not dose dependent, but related to hepatic protein synthesis function of broilers. Under the stimulation of low AFB$_1$ levels, broilers met the requirement of detoxification by upregulate hepatic antioxidant enzymes, however, when large amount AFB$_1$ accumulated in hepatic and inhibited protein synthesis or impaired hepatocytes, which turned into antioxidant enzymes expression impairment. Thus the detoxification responses in liver may vary with length of exposure and dose of AFB$_1$ (Yang et al., 2012; Bhatti et al., 2021). Toxo-MX supplementation in this study had not completely reversed the negative effect in liver and serum parameters, especially to the increased GSH-Px and SOD. This may because of the rapid absorption rate of AFB$_1$, while adsorbent showed less efficient binding rate with increasing AFB$_1$ concentration, or the binding of high concentration AFB$_1$ is not sufficient, the unbound AFB$_1$ were absorbed into circulation, thus broilers presented the detoxification response with higher serum UA and hepatic SOD, GSH-Px, and may resulted in growth performance depletion under high concentration AFB$_1$ exposure.

Thymus and bursa of Fabricius of broilers is the central immune organ, spleen is where immune responses toward systemically administered antigens are located. In the present study, the relative weight of thymus and bursa of Fabricius significantly decreased in broilers fed with 120 ppb of AFB$_1$ contaminated diet at the age of 21 d but have no significant differences in 42-day-old broilers, indicated that aflatoxicosis may retard the immune organ development in young broilers. Moreover, It is reported that AFB$_1$ exposure has detrimental effect on T and B lymphocytes proliferation and diversification (Chen et al., 2013; Rajput et al., 2017), and therefore, inhibit antibody production against aflatoxicosis (Qureshi et al., 1998; Ortatatli et al., 2005;

Tessari et al., 2006). Furthermore, The chronic aflatoxicosis reduced protein utilization and synthesis which also link to serum globulin production (Kubena et al., 1993; Ledoux et al., 1999), the impaired protein synthesis function could be observed in serum ALB and GLB levels before major symptom occurred (Ortatatli et al., 2005). Our study showed a significantly decreased in serum ALB concentration of broilers exposure to AFB$_1$, furthermore, serum IgG and IgA also reduced in 21-day-old broilers exposure to both concentration of AFB$_1$, and the decreasing serum IgG were remain in 42-day-old birds' exposure to 120 ppb AFB$_1$. This reduction in serum immunoglobulin was consistent with the other reports (Qureshi et al., 1998; Ortatatli et al., 2005; Chen et al., 2014). In addition, leukocyte infiltration was observed in the livers of both age broilers fed AFB$_1$ contaminated diet and 120 ppb AFB$_1$ with Toxo-MX diet, inflammation cytokines mRNA expressions, such as, TGFβ-1, TLR-4, and IL-1β were increased in 21-day-old broilers, however, decreased in 42-day-old broilers. The hepatic lesions results consistent with previous study that administration 50 μg/kg AFB$_1$ contaminated diet to broilers (Magnoli et al., 2011), another study administrated a hydrated sodium calcium aluminosilicate (**HSCAS**) reported that bioavailability of aflatoxin and its residues decreased in liver, but not prevented hepatic lesions associated with aflatoxicosis (Neeff et al., 2013). These data indicated that dietary adsorbent supplementation could not reverse the hepatic and serum inflammation induced by AFB$_1$. Hepatic immunity defense may firstly increase to against chronic aflatoxicosis and finally inhibit during long-term exposure, suggested broilers may be increasingly susceptible to disease because of suppression of immunity after long-term chronic aflatoxicosis.

Intestinal mRNA expression of tight junction protein increase is a response of recovery of impaired tight junction protein (Chen et al., 2016). Tight junction proteins
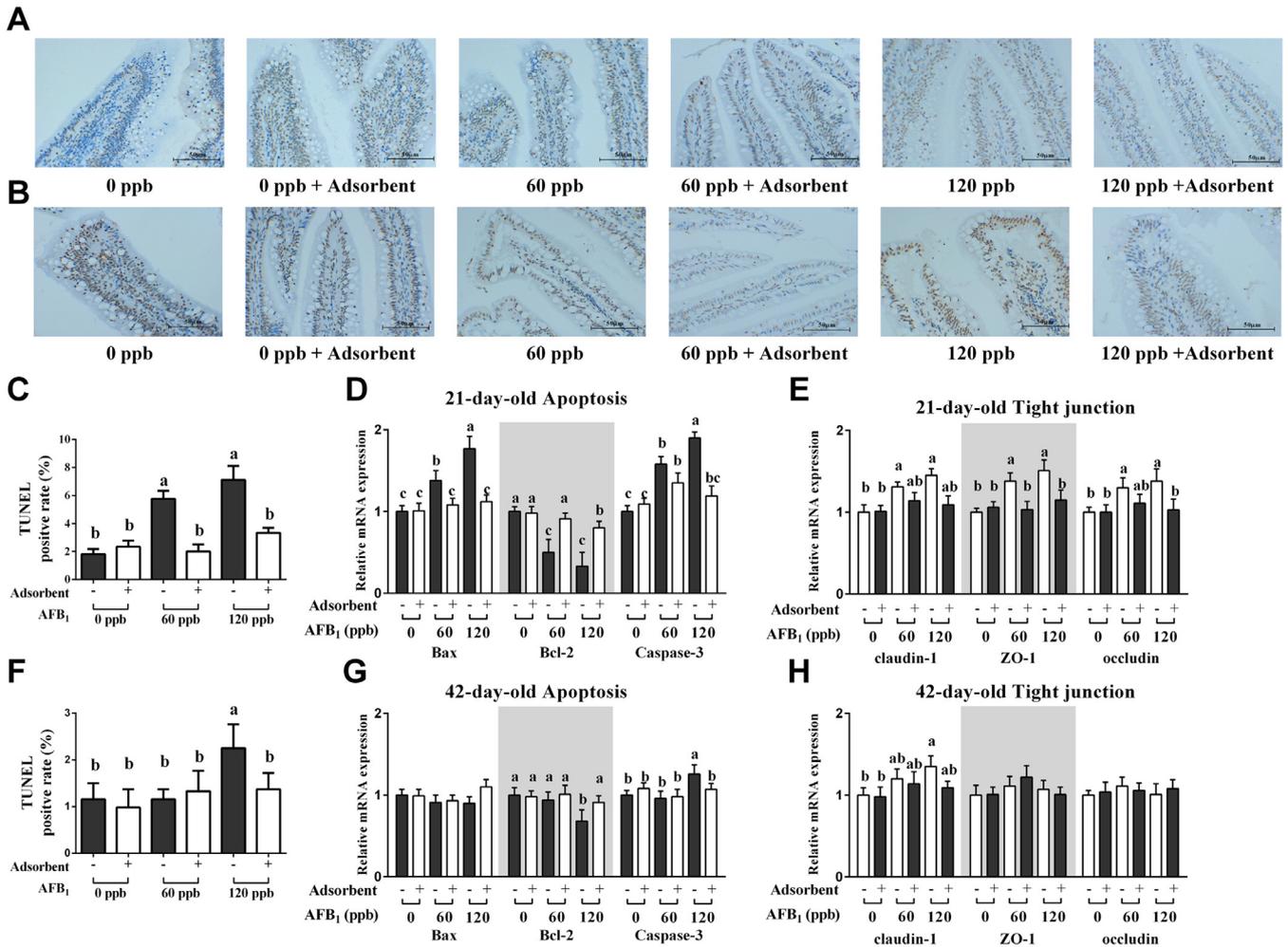
Figure 3. Jejunal TUNEL assay and mRNA expression of apoptosis and tight junction protein genes. (A) Sliced jejunal tissue of 21-day-old broilers from each group was labeled by TUNEL signal and was observed under a microscope (Keyence, Tokyo, Japan) with $400 \times$ original magnification. (B) Sliced jejunal tissue of 42-day-old broilers. (C) TUNEL positive of jejunal tissue in 21-day-old broilers. (D) Jejunal tissue mRNA expression of Bax, Bcl-2, Casepase-3 in 21-day-old broilers. (E) Jejunal tissue mRNA expression of claudin-1, ZO-1, and occludin in 21-day-old broilers. (F) TUNEL positive of jejunal tissue in 42-day-old broilers. (D) Jejunal tissue mRNA expression of Bax, Bcl-2, Casepase-3 in 42-day-old broilers. (E) Jejunal tissue mRNA expression of claudin-1, ZO-1 and occludin in 42-day-old broilers. The data represent mean $\pm$ SD of 8 replicates, and different letters in the same column indicate significant differences ($P < 0.05$).

are the crucial constituent for gut barrier function, therefore, any damage to tight junction proteins may resulted in increase of permeability and gut inflammation during AFB_1 contamination (González-Mariscal et al., 2003; Saleemi et al., 2020). This study showed an increasing of tight junction protein mRNA expression in the broilers fed AFB_1 contamination diet. In addition, aflatoxicosis induced elevation of the apoptotic gene expression in intestinal epithelial cells of 21-day-old broilers, although the elevated expression of apoptotic genes reduced with the age of broiler increasing, but still resulted in a lower villus height to crypt depth rate, suggested that aflatoxicosis impaired digestion ability of intestinal is not reversible with broiler age increasing.

The histological changes of small intestine in broilers associated with the increasing of endogenous nitrogen loss, and decreased energy and amino acid digestibility (Denli et al., 2009; Chen et al., 2016; Liu et al., 2018). Increased gut permeability may also facilitate the absorption of other antigens through the paracellular

route that are normally could not be absorbed, leading to synergistic diseases outbreak (Girgis et al., 2010; Antonissen et al., 2014). These data explained that broilers exposure to AFB_1 presented higher F:G ratio compared with control group in present study, indicated Toxo-MX supplementation may as economical solution for aflatoxicosis problem, making poultry production more cost-effective. Furthermore, Long term exposure to AFB_1 suppressed hepatic inflammatory cytokines expression in 42-day-old broiler, which may also link to intestinal impairment and increased the disease-associated antigens intake. Although Toxo-MX supplementation significantly recovered the villus height and crypt depth and decreased the overexpression of apoptotic gene in both age broilers, however, showed less effect on hepatic inflammation. Another study reported a clay adsorbent against AFB_1 contamination on intestine of young ducklings also showed ameliorated on villus height and villus/crypt ratio of the duodenum and jejunum, but did not totally protect the ducklings from the toxic effects on serum IgG and IgM of AFB_1

concentrations above 100 ppb (Wan et al., 2013). With molecular or nano-clay binders in turkey against AFB_1 aflatoxicosis showed improved on intestinal goblet cell hyperplasia, villous atrophy and diffuse lymphocytic enteritis, but with concentration above 110 ppb of AFB_1 suggested perivascular inflammatory insult and the sequela of fibroplasia (Lala et al., 2016). These accumulated data demonstrated that protection effect of adsorbents decreased with the concentration of AFB_1 increased, and absorbents are commonly more effective against the aflatoxicosis in gastrointestinal tract, but incapable for the aflatoxins that entered into circulation.

In summary, the data demonstrated that diet supplementation with Toxo-MX reversed the detrimental effect on growth performance and F:G ratio in broilers exposure to 60 ppb and 120 ppb AFB_1, protected intestinal tight junction and restored increased apoptotic gene expression. However, did not completely recovered hepatic inflammation induced by AFB_1, particularly in 120 ppb group. Suggested that Toxo-MX prevented gastrointestinal aflatoxicosis under low concentration of AFB_1 contamination, but had less effect on hepatic aflatoxicosis, particularly in young broilers.

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DISCLOSURES

The authors declare no competing interests linked to this manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101634.

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