Curcumin ameliorates duodenal toxicity of AFB1 in chicken through inducing P-glycoprotein and downregulating cytochrome P450 enzymes

Ping Cheng,[*,1] Muhammad Ishfaq,[*,1] Hongxiao Yu,[*] Yuqi Yang,[†] Sihong Li,[*] Xiaotin Li,[*] Saqib Ali Fazlani,[*] Wenxin Guo,[‡] and Xiuying Zhang[*,2]

[*]*Heilongjiang Key Laboratory for Animal Disease Control and Pharmaceutical Development, Faculty of Basic Veterinary Science, College of Veterinary Medicine, Northeast Agricultural University, 600 Changjiang Road, Xiangfang District, Harbin, P R China;* [†]*Pharmacology Teaching and Research Department, School of Basic Medicine, Guizhou University of Traditional Chinese Medicine, Guiyang, P R China; and* [‡]*Technical Identification Department of Agricultural Products and Veterinary Medicine Feed, Heilongjiang Institute of Veterinary Drug and Feed Control, Harbin, P R China*

ABSTRACT It has been reported that oral intake of aflatoxin B1 (**AFB1**)-contaminated feed could cause acute, sub-chronic, or chronic toxicity in livestock and poultry. However, the harmful effect of AFB1 on the small intestine is still controversial. Therefore, blocking the entry of AFB1 into the body through the digestive tract is one of the important methods to prevent its toxicity. In the present study, 1-day-old Arbor Acres broilers were randomly divided into 6 groups including control group, curcumin control group (450 mg curcumin/kg feed), curcumin low-, medium-, and high-dose group (150, 300, and 450 mg curcumin/kg feed + 5 mg AFB1/kg feed), and AFB1 group (5 mg AFB1/kg feed). After 28 d, the samples of chickens' duodenums were collected for further analyses. AFB1 caused abnormal functional and morphological changes in the duodenum, including histological lesions, increased the length of the duodenum and depth of crypt, decreased the unit weight of the duodenum, height of villus, and the value of villus height/crypt depth. Meanwhile, AFB1 administration enhanced malonaldehyde activity, 8-HOdG level, and the mRNA expression of cytochrome P450 (**CYP450**) enzymes, and reduced superoxide dismutase, catalase, adenosine triphosphatase (**ATPase**) activity and the mRNA expression of *Abcb1*. Importantly, curcumin supplementation partially ameliorated AFB1-induced abnormal functional and morphological signs of the duodenum, alleviated AFB1-induced oxidative stress, and decreased the mRNA expression of CYP450 enzymes. Furthermore, curcumin ameliorated AFB1-induced decrease in the *Abcb1* mRNA expression, P-glycoprotein (**P-gp**) level, and ATPase activities. It has been suggested from these results that curcumin supplementation in the feed could ameliorate AFB1-induced duodenal toxicity and damage through downregulating CYP450 enzymes, promoting ATPase activities, and inducing P-gp in chickens.

Key words: chicken duodenum, aflatoxin B1, curcumin, cytochrome P450, P-glycoprotein

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INTRODUCTION

Researchers demonstrated that aflatoxin could contaminate foods or feeds such as dried fruits, oilseeds, spices, and grains (Murphy et al., 2010). Among these toxins, aflatoxin B1 (**AFB1**) is regarded as the most potent and harmful aflatoxin, and has been classified as a class I carcinogen by the International Cancer Research Institute (Muhammad et al., 2017). Its toxicity is very complex and strong, resulting in liver toxicity, growth retardation, biological malformations, digestive tract disorders, and even cancer (Vineis and Xun, 2009). It has been reported that experimental animal models fed on aflatoxin-contaminated feed showed decreased immune system function, increased susceptibility to various diseases, and reduced performance (Peng et al., 2016; Huang et al., 2019). Therefore, strict control measures are currently being followed in several

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[1]The two authors contributed equally to this work.

[2]Corresponding author: zhangxiuying@neau.edu.cn

countries to limit AFB1 contamination in food to ensure the safety of human health (Rushing and Selim, 2019). The small intestine and stomach are the main places for food absorption and conversion, and the small intestine is also recognized as the first line of defense in mucosal surfaces (Wijtten et al., 2011). However, a series of antigens such as bacteria, viruses, and toxicants could affect the functionality and morphology of the gastrointestinal tract (Yunus et al., 2011b). In fact, the toxic effects of AFB1 on the gastrointestinal tract are often inconclusive and neglected, but it primarily comes in contact with the gastrointestinal tract in the body of humans and animals. Moreover, several studies have reported that this toxin could exert more harmful effects on the small intestine (Yunus et al., 2011a; Wang et al., 2018). A study by Zhang et al. reported that AFB1 could lead to shedding of epithelial cells in chicken jejunal villus (Zhang et al., 2014). Similarly, Kumar and Balachandran have observed enteritis with mononuclear and lymphocytic cell infiltration in the intestine of broilers fed AFB1 at 4 wk of age (Kumar and Balachandran, 2009).

The involvement of cytochrome P450 (**CYP450**) enzymes in AFB1 biotransformation was well studied in the liver of animals and humans (Deng et al., 2018). It has been demonstrated that the major CYP enzymes responsible for AFB1 bioactivation into its harmful metabolite are CYP1A1, CYP1A2, CYP1A5, CYP2A6, CYP3A37, and CYP3A4 (Muhammad et al., 2018). However, the crucial role of CYP450 enzymes in AFB1-induced intestinal damage has been rarely reported. Due to the toxicity of AFB1, CYP450-mediated bioactivation of AFB1 in the gastrointestinal tract is still of major concern. In addition to CYP450, the role of P-glycoprotein (**P-gp**) in AFB1 transport is worth investigating. P-gp encoded by the MDR genes is an important adenosine triphosphate (**ATP**)-dependent membrane efflux and drug transporter protein, which is distributed in many tissues of the body (Elmeliegy et al., 2020). In recent years, the potential of P-gp in veterinary therapy has attracted public attention (Schrickx and Fink-Gremmels, 2008). Previous studies demonstrated that one major function of P-gp is to limit the uptake of toxic compounds from the outside into the body (Lugo and Sharom, 2005). Therefore, P-gp has been recognized to play an important role in barrier function of the intestine (Tsuji, 2002). However, it is still elusive whether P-gp expression affects the uptake of AFB1 in the duodenum of chicken.

As a fat-soluble phenolic pigment, curcumin is derived from the rhizome of *Curcuma longa* Linn, which is extensively used in medicinal preparations, spices, as a cosmetic, and to color butter and cheese in many Asian countries (Gupta et al., 2012; Sanmukhani et al., 2014). Numerous researchers demonstrated the anti-inflammatory, anticancer, antioxidant, and detoxification potential of curcumin in various experimental animals as well as humans (Lam et al., 2016; Dai et al., 2018). Our previous study also reported preventive effects against the AFB1-induced harmful effects in chicken liver (Li et al., 2019). Moreover, some researchers reported that curcumin could upregulate the function and expression of P-gp in Caco-2 cells (He et al., 2013). However, the role of P-gp in the transport of AFB1 and intervention by curcumin is still unknown.

In view of the intestinal tract possessing drug transporters and CYP 450 enzymes, whether P-gp and CYP 450 enzymes are involved in AFB1 transportation and bioactivation in the small intestine is still elusive. Therefore, in this study, we aimed to investigate the preventive effects of curcumin against AFB1-induced duodenum damage, and further determine the correlation of the preventive effects with the modulation of P-gp and CYP 450 enzymes expression. These findings will provide a better understanding of the mechanism of curcumin against AFB1-induced duodenum damage in chickens.

MATERIALS AND METHODS

Ethical Statement

All the experimental protocols were performed in accordance with animal ethics guidelines and approved protocols of the Northeast Agricultural University, Harbin, China.

Chemicals and Reagents

Pure AFB1 (purity $\geq$ 99.0%) was purchased from Sigma Aldrich (St. Louis, MO). Curcumin (2.5%) was purchased from Shengxing Biological Technology Co., Ltd. (Henan, China). Hematoxylin was purchased from Guangzhou Chemical Reagent Company (China) and eosin was purchased from Nanjing Chemical Reagent Factory (China). Absolute ethanol (99.0%), methanol, isopropanol, chloroform, agarose G-10, and sodium chloride (**NaCl**) were bought from Tian Li Co., Ltd. (Tianjin, China). SYBR Green PCR Master Mix was purchased from Toyobo Life Science Co., Ltd. (Shanghai, China), and M-MuLV cDNA reverse transcription kit was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The malonaldehyde (**MDA**), superoxide dismutase (**SOD**), catalase (**CAT**), and adenosine triphosphatase (**ATPase**) assay kits were bought from Nanjing Jiancheng Bioengineering Institute, China. The catalog numbers of the kit are A003-1 (MDA), A001-2 (SOD), A007-1 (CAT), and A016-2 (ATPase). The 8-hydroxy-2′-deoxyguanosine (**8-OHdG**) assay kit was bought from MEIMIAN, Jiangsu Feiya Biological Technology Co. Ltd., China (catalog number: ml037012).

Animals and Experimental Design

One hundred and twenty one-day-old Arbor Acres broilers were obtained from a commercial hatchery (Yi Nong, Harbin, Heilongjiang, China; License No. 230108799294096). As shown in Table 1, the broilers were randomly divided into 6 groups with 2 replicates of 10 birds per pen including 1) control group (**C**); 2) curcumin control group (**CC**); 3) low-dose curcumin group (**L**); 4) medium-dose curcumin group (**M**); 5) high-dose

Table 1. Specific cases of grouping and feeding.

Group	Diet
Control group	Basal corn diet
Curcumin control group	Basal corn diet + 450 mg curcumin/kg feed
Low-dose curcumin group	Basal corn diet + 5.0 mg AFB1/kg feed + 150 mg curcumin/kg feed
Medium-dose curcumin group	Basal corn diet + 5.0 mg AFB1/kg feed + 300 mg curcumin/kg feed
High-dose curcumin group	Basal corn diet + 5.0 mg AFB1/kg feed + 450 mg curcumin/kg feed
AFB1 group	Basal corn diet + 5.0 mg AFB1/kg feed

curcumin group (**H**); and 6) AFB1 group. After 3 d of acclimation, the treatment groups were served the diet for the next 28 d. Temperature and relative humidity were maintained according to the requirements of broilers. The broilers were kept on a 12 h light and dark cycle, and allowed ad libitum access to feed and water. At day 28, broilers were euthanized by CO_2 asphyxiation. The entire duodenum was collected and a part of the duodenum was processed for measurement of biomarkers of oxidative stress and histological examination, and the remaining duodenum was immediately frozen in liquid nitrogen and stored at a temperature of −80°C for further analysis.

Histopathological Observation of Duodenum Damage

The duodenum was evaluated via hematoxylin and eosin (**H&E**) staining. Histopathological procedure was carried out as mentioned in a previous study (Xun, 2015). In brief, fresh duodenum samples were immersed in neutral formalin for more than 24 h, and then the fixed duodenum samples were dehydrated in an ascending series of ethanol, cleared in xylene, and embedded in paraffin. The samples were sectioned at a 5-μm thickness, installed on glass slides, and stained with H&E; the length of the villi and the depth of the crypt were measured by the motor-3.0 photo-processing software for histopathological examination, and the ratios of villus height/crypt depth (**V/C**) were calculated. Three different fields of view were chosen, and 5 villi and crypts were selected from each field for measurement.

Measurement of Biomarkers of Oxidative Stress in Duodenum Tissues

The duodenum tissues were prepared according to the manufacturer's instruction. In brief, a small piece of duodenum was weighed (0.1 g) and immediately chilled in 0.9% NaCl at 0°C. After drying and homogenizing, centrifugation was performed at 12,000 × *g* for 15 min at 4°C, and the supernatants were analyzed for antioxidant activities. The level of 8-OHdG, and the activity of MDA, SOD, and CAT were determined by using kits according to the manufacturer's instructions.

Measurement of ATPase Activities in Duodenum Tissues

A part of the duodenum tissues collected from the beginning, middle, and end of the duodenum was weighed (0.1 g) and homogenized to make a 10% duodenum homogenate (1 mL of 0.9% NaCl mixed with 100 mg of duodenum tissue and homogenized). The tissue homogenate was centrifuged at 3,000 rpm for 10 min. The supernatant of the duodenum homogenate was collected and used to detect ATPase activities according to the manufacturer's instruction.

RNA Extraction and cDNA Synthesis

Total RNA of the duodenum was isolated using the TRIzol extraction method according to the manufacturer's instructions (Invitrogen Inc., Carlsbad,

Table 2. Primers used for quantitative real-time PCR.

Target gene	Primers	Sequence (5′-3′)	Product length (bp)
Abcb1	Forward	ACCCATCCAGACCAGATGTTG	158
	Reverse	AATGGTAATCGTGCCTTCCTTG	
CYP1A1	Forward	GCATGATGTACGCTGCCTTG	188
	Reverse	CGTTGTACAATGCGGGATGG	
CYP1A2	Forward	CTTTGGAGCAGGCTTTGACAC	162
	Reverse	GTGTAGGGCAGCATACCTCG	
CYP2A6	Forward	CTGCAGAGAATGGCATGAAG	145
	Reverse	CCTGCAAGACTGCAAGGAA	
CYP3A4	Forward	TTGCCTAACAAGGCTGCTCC	119
	Reverse	TTGCAGATCCGCTCAATCCG	
β-Actin	Forward	TGAAGCCCAGAGCAAAAGAG	135
	Reverse	TGCTCCTCAGGGCTACTCTC	

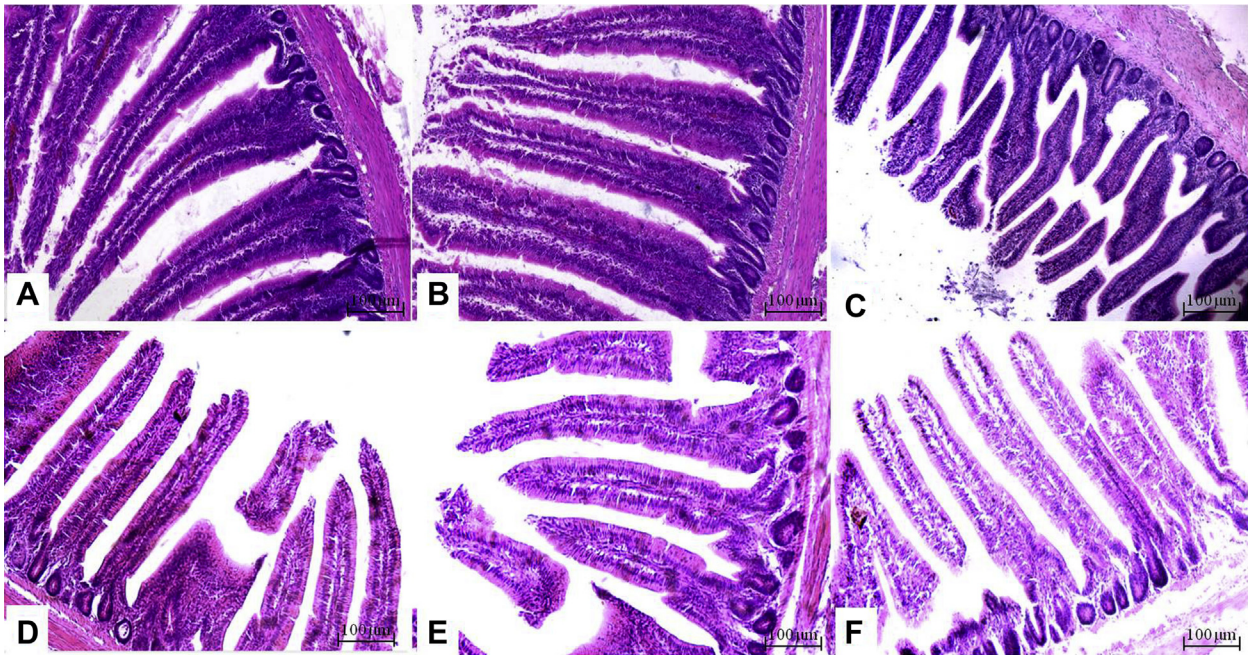

Figure 1. Histopathological observation of duodenum damage: (A) control group; (B) curcumin control group; (C) AFB1 group; (D) low-dose curcumin group; (E) medium-dose curcumin group; and (F) high-dose curcumin group.

CA) and quantified by measuring the absorbance at 260 nm. RNA quality was determined by measuring the 260/280 ratio (1.8∼2.0). M-MuLV cDNA reverse transcription kit was utilized to synthesize first strand cDNA, according to the manufacturer's protocol.

Quantitative Real-Time PCR Analysis

Real-time PCR was performed by using a LightCycler 96 (Roche, Switzerland). The mRNA expression of CYP450 (CYP1A1, CYP1A2, CYP2A6, and CYP3A4) and *Abcb1* were determined by quantitative real-time PCR. The 20-μL reaction mixture contained 0.4 μL of forward primer and 0.4 μL of reverse primer, 10 μL of SYBR Green PCR Master Mix, 1 μL of cDNA sample, and 8.2 μL of nuclease-free water. The primers used in these assays are listed in Table 2. The amplification conditions were as follows: 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 60 s, and 72°C for 60 s. The housekeeping gene β-actin was used as a reference gene for normalization. Data were analyzed according to the $2^{-\Delta\Delta Ct}$ method.

Immunohistochemical Staining

To study the localization and semiquantitative protein expression of P-gp in the duodenum, immunohistochemical staining was performed as described previously (Guo et al., 2013). Briefly, the duodenum sections were prepared and incubated overnight at 4°C with the primary antibody (C219, mouse monoclonal anti-P-gp, 1:20) (Guo et al., 2013) and at 37°C for 1 h with the secondary antibody (rabbit anti-mouse IgG horseradish peroxidase), and the P-gp immunoreactivity was visualized with DAB staining according to the manufacturer's instruction. Finally, sections were counterstained with hematoxylin, dehydrated and cleared with xylene, and coated with neutral balsam. The results were observed under a light microscope (Nikon E100, Japan). The expression levels for P-gp were semiquantitatively determined by densitometry using Image-Pro Plus 5.0 software (National Institutes of Health, Bethesda, MD).

Statistical Analysis

The unit weight of the duodenum was calculated as weight (g) per unit length (cm). All analyses were

Table 3. Curcumin attenuates AFB1-induced alteration in functionality and morphology of the duodenum in chickens*.

Items	Control	Curcumin	AFB1	Low	Medium	High
Duodenum						
Length (cm)	27.87 ± 4.79	28.23 ± 2.49	35.55 ± 3.89**	32.18 ± 2.93	30.01 ± 2.53***	27.42 ± 2.30†
Unit weight (g/cm)	0.70 ± 0.09	0.73 ± 0.14	0.47 ± 0.08**	0.53 ± 0.06	0.58 ± 0.10	0.65 ± 0.06†
Villus height (μm)	1270.67 ± 41.86	1403.92 ± 68.95	1019.64 ± 55.75**	1167.6 ± 111.07***	1291.23 ± 42.26†	1409.30 ± 76.44†
Crypt depth (μm)	200.18 ± 12.74	164.46 ± 13.22	249.43 ± 13.77**	213.46 ± 11.86†	182.59 ± 13.61†	168.58 ± 15.98†
V/C	6.34	8.53	4.09	5.47	7.07	8.36

*$P < 0.05$ and **$P < 0.01$, compared to the control group; ***$P < 0.05$ and †$P < 0.01$ compared to the AFB1 group.
Abbreviation: V/C, villus height/crypt depth.

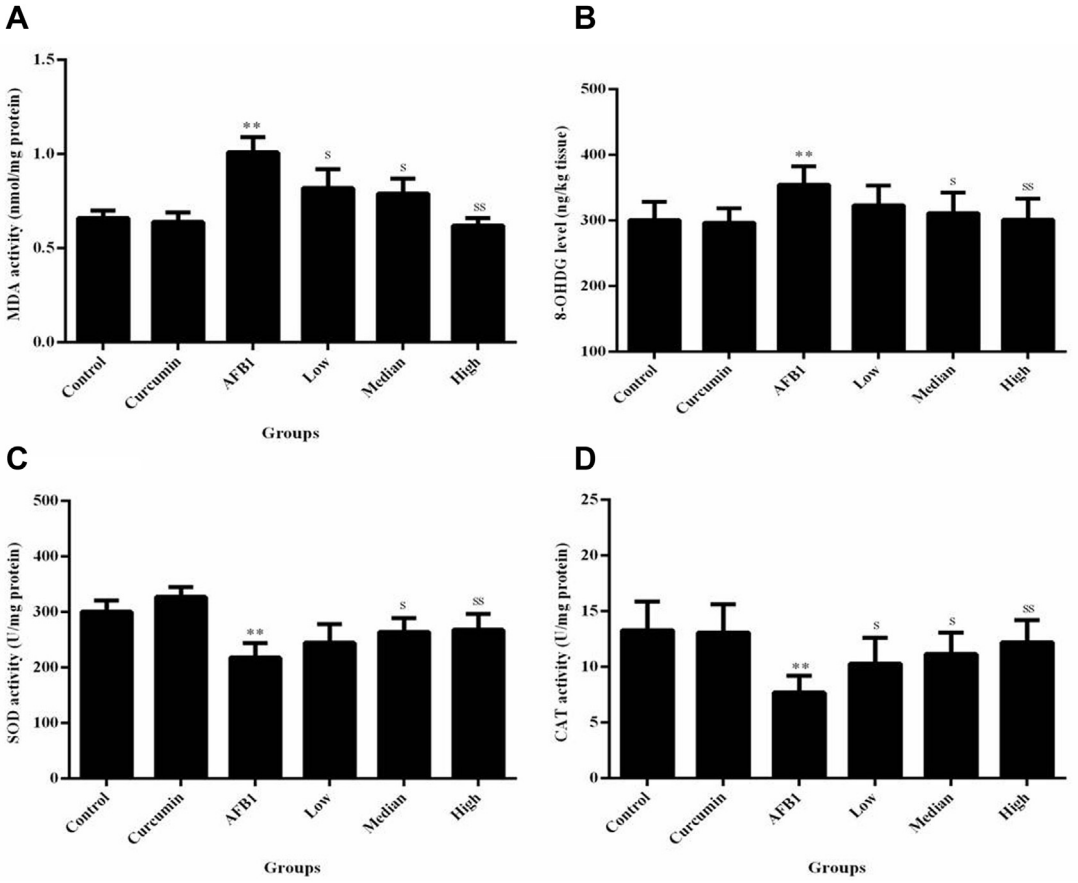

Figure 2. (A) MDA activity; (B) 8-OHdG level; (C) SOD activity; (D) CAT activity. Values are represented as mean ± SD (n = 12). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the control group; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ compared to the AFB1 group. Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CAT, catalase; MDA, malonaldehyde; SOD, superoxide dismutase.

performed with the SPSS 22.0 software (SPSS Inc., Chicago, IL). Data were expressed as mean ± SD. Statistical significance was analyzed using one-way ANOVA followed by the least significance difference test as the post hoc test. In all statistical comparisons, values of $P < 0.05$ were considered significant, and values of $P < 0.01$ were considered markedly significant.

RESULTS

Curcumin Attenuates AFB1-Induced Clinical Symptoms of Chickens

We observed no (zero) mortality in all experimental groups during the 28 d trial period. Chickens in the C and CC group showed no significant abnormal signs. Compared to the C and CC group, chickens in the AFB1 group showed symptoms of drowsiness, lethargy, and ruffled feathers. These signs were anecdotally observed to be milder or disappeared with curcumin treatment in all the 3 groups (L, M, and H) than the AFB1 group, but were not quantified and statistically analyzed.

Curcumin Attenuates AFB1-Induced Duodenum Damage in Chickens

The mucosal morphology of the duodenum was examined using paraffin section that was stained routinely with H&E. As shown in Figure 1, the duodenum from the C and CC group showed normal morphology, and the villi of duodenum were intact in structure and clear in shape. Chickens treated with AFB1 showed severe damage in the duodenum, villi fragmentation, tissue congestion, and structure ambiguity. Importantly, curcumin treatment could effectively alleviate AFB1-induced damage.

As shown in Table 3, AFB1 could induce an increase in the length of the duodenum compared to the C group. However, dietary curcumin significantly ameliorated AFB1-induced increase in the length of the duodenum in the curcumin-treated groups M ($P < 0.05$) and H ($P < 0.01$). The unit weight of the duodenum significantly decreased in the AFB1 group relative to the C group ($P < 0.01$), but curcumin could restore the decrease in unit weight of the duodenum in the curcumin-treated group H ($P < 0.01$).

The crypt depth of the duodenum significantly increased ($P < 0.01$) in the AFB1 group compared

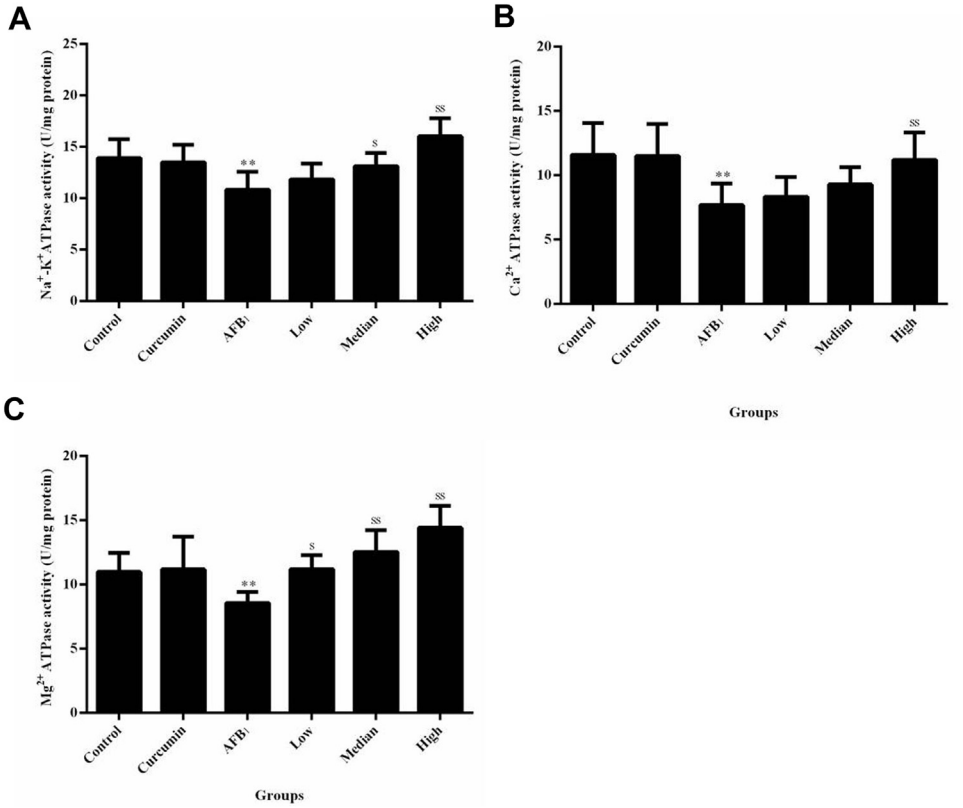

Figure 3. Activities of ATPase in the duodenum: (A) Na^+-K^+-ATPase, (B) Ca^{2+}-ATPase, and (C) Mg^{2+}-ATPase. Values are represented as mean ± SD (n = 12). $^*P < 0.05$ and $^{**}P < 0.01$, compared to the control group; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ compared to the AFB1 group. Abbreviation: ATPase, adenosine triphosphatase.

to the C group. However, dietary curcumin significantly ameliorated AFB1-induced increase in the crypt depth ($P < 0.01$). AFB1 could cause significant decrease in the villi height ($P < 0.01$) compared to the C group. Importantly, as compared to the AFB1 group, chickens in the curcumin-treated groups (L, M, and H) showed significant increase in the villi height ($P < 0.05$ or 0.01). Moreover, the ratio of V/C decreased in the AFB1 group compared to the C group, but curcumin could restore the decrease in the ratio of V/C in the curcumin-treated groups (L, M, and H) in a dose-dependent manner. This implied that curcumin could alleviate AFB1-induced duodenum damage in a dose-dependent manner (as shown in Table 3).

Curcumin Attenuates AFB1-Induced Oxidative Stress in Duodenum of Chickens

As shown in Figure 2, AFB1 and curcumin-supplemented diet alone or in combination could alter biomarkers of oxidative stress in the duodenum of chickens. Compared to the C group, AFB1 significantly downregulated the SOD and CAT activities ($P < 0.01$). However, curcumin treatment significantly ameliorated AFB1-induced decrease in SOD and CAT activities compared to the AFB1 group ($P < 0.05$ or 0.01). Compared to the C group, AFB1 significantly upregulated the activity of MDA ($P < 0.01$). However, curcumin treatment significantly ameliorated AFB1-induced increase in MDA activity relative to the AFB1 group ($P < 0.05$ or 0.01). The level of 8-OHdG was significantly upregulated in the AFB1 group compared to the C group ($P < 0.01$). In contrast, the level of 8-OHdG was significantly downregulated in the curcumin-treated group H ($P < 0.01$).

Curcumin Attenuates AFB1-Induced Decrease of ATPase Activities in Duodenum of Chickens

As shown in Figure 3, AFB1 significantly downregulated the activities of Na^+-K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase in the duodenum of chickens compared to the C group ($P < 0.01$). However, curcumin significantly ameliorated AFB1-induced decrease in ATPase activities in the curcumin-treated group H ($P < 0.01$).

Curcumin Downregulates the CYP450 mRNA Expression in Duodenum of Chickens Treated With AFB1

As shown in Figure 4, AFB1 could significantly increase the mRNA expression of CYP3A4, CYP2A6, CYP1A1, and CYP1A2 in the duodenum of chickens relative to the C group at day 28 ($P < 0.01$). In

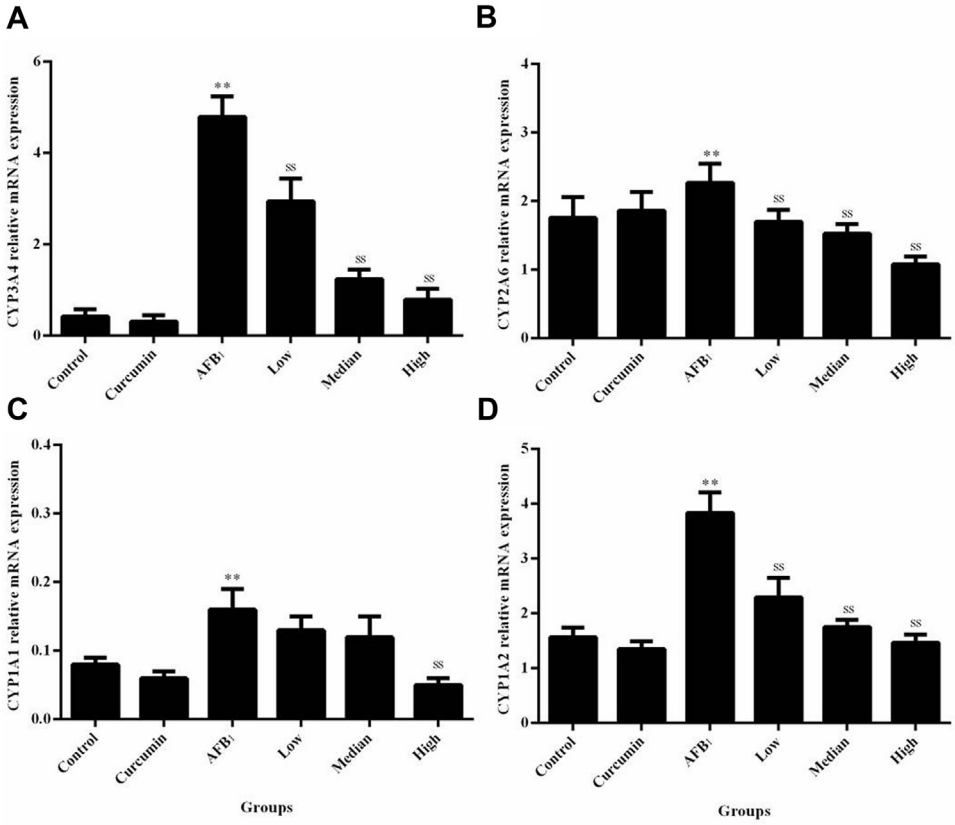

Figure 4. The mRNA expression levels of CYP3A4 (A), CYP2A6 (B), CYP1A1 (C), and CYP1A2 (D). Values are represented as mean ± SD (n = 12). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the control group; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ compared to the AFB1 group.

contrast, compared to the AFB1 group, curcumin markedly reduced AFB1-induced increase in the mRNA expression of CYP3A4, CYP2A6, and CYP1A2 ($P < 0.01$) in the curcumin-treated groups (L, M, and H). Furthermore, chickens in the curcumin-treated group (H) displayed significant decrease in the mRNA expression of CYP1A1 ($P < 0.01$).

Curcumin Upregulates the Abcb1 *and P-gp Expression in Duodenum of Chickens Treated With AFB1*

As shown in Figure 5A, AFB1 could significantly reduce the mRNA expression of *Abcb1* in the duodenum of chickens compared to the C group at day 28 ($P < 0.01$). Interestingly, chickens in the

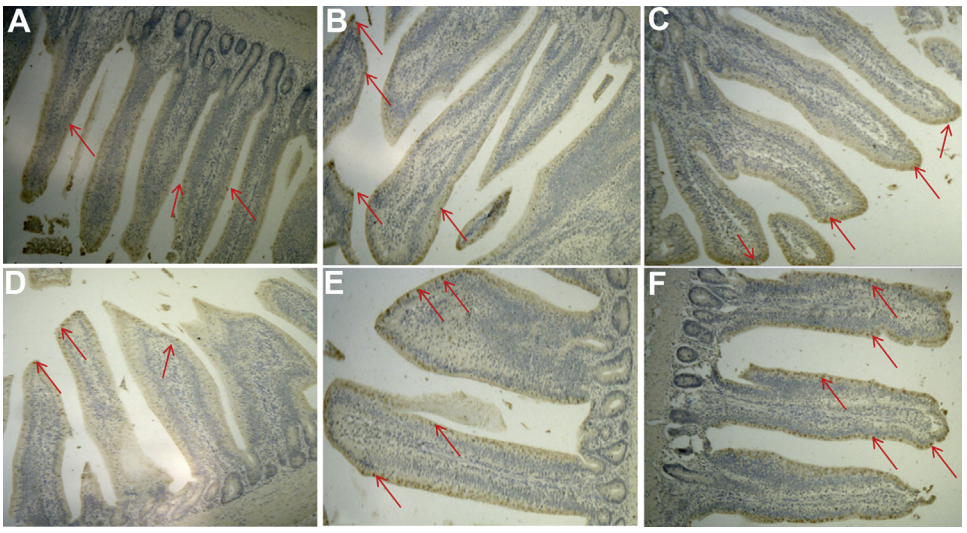

Figure 5. Immunohistochemical staining of P-glycoprotein in the duodenum. (A) Control group; (B) curcumin control group; (C) AFB1 group; (D) low-dose curcumin group; (E) medium-dose curcumin group; and (F) high-dose curcumin group.

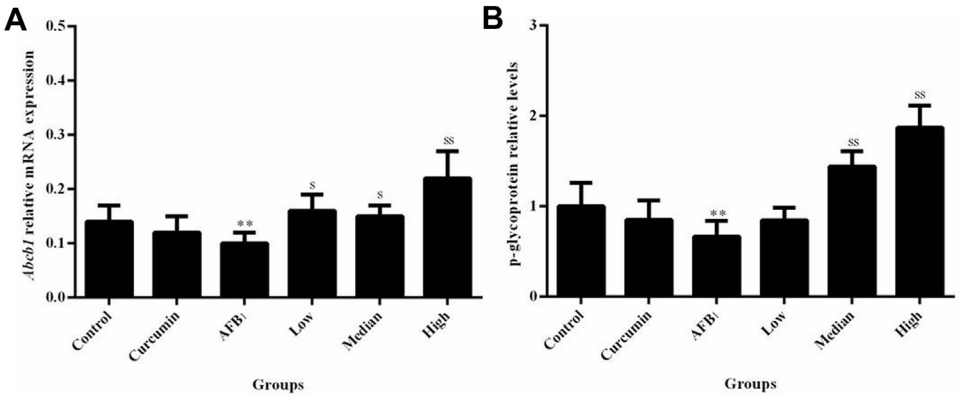

Figure 6. The mRNA expression level of *Abcb1* (A) and the expression of P-glycoprotein (B) in the duodenum. Values are represented as mean ± SD (n = 12). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the control group; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ compared to the AFB1 group.

curcumin-treated groups (L, M, and H) showed significant increase in the mRNA expression of *Abcb1* compared to the AFB1 group ($P < 0.01$). Thus, the decreased expression of *Abcb1* mRNA induced by AFB1 was upregulated by curcumin in a dose-dependent manner. As shown in Figures 5 and 6B, the expression of P-gp significantly decreased in the AFB1 group compared to the C group at day 28. However, curcumin could significantly increase the expression of P-gp in the curcumin-treated groups (M and H) in a dose-dependent manner ($P < 0.01$).

DISCUSSION

The small intestine is an important organ due to the following 3 functions: nutrition, immune system, and gut microbiota, which is regarded as the first line of defense against pathogenic bacteria and toxins (Wijtten et al., 2011). Previous studies have demonstrated that the intestinal villus height and crypt depth could be used as important indicators for growth, digestion, and absorption of the small intestine (Jiang et al., 2015). When intestinal villus shows atrophy, suggesting the villus absorptive cells reduces and the secretory cells increase, which could result in the deterioration of intestinal absorptive capacity. The crypt depth could be used to reflect the colonization rate and the maturity of the crypt cell (Liu et al., 2012). AFB1 is extremely toxic, and has carcinogenic, teratogenic, and mutagenic effects on mammals and poultry (Kalpana et al., 2012). It has been reported that duck fed on maize naturally contaminated with AFB1 had a negative effect on intestinal function and morphology, and altered digestive physiology and growth performance (Prasad et al., 2016). In the present study, histopathological examination revealed that AFB1 could induce significant alteration in the functionality and morphology of the duodenum, including histological lesions, increase the length of the duodenum and the depth of the crypt, decrease the unit weight of the duodenum, the height of villus, and the value of V/C. However, curcumin treatment could promote the recovery of normal functionality and morphology of the duodenum. It indicated that consumption of feed containing AFB1 would result in decreased nutrient digestion and absorption capacity of chickens, but curcumin could alleviate the AFB1-induced intestinal toxicity; these results were in according with previous studies (Prasad et al., 2017).

It is well known that direct or indirect action of AFB1 could induce oxidative stress (Huang et al., 2019). In the present study, AFB1-contaminated feed increased MDA activity, which is an indicator of lipid peroxidation, and decreased antioxidant activities such as SOD and CAT. Apparently, curcumin treatment could ameliorate AFB1-induced oxidative stress. These results are in line with a previous report that demonstrated that curcumin ameliorated AFB1-induced alteration in glutathione, SOD, CAT, and MDA activities (Li et al., 2019). This may be due to the ability of curcumin to scavenge free radicals by restoring antioxidant enzymes activities and alleviated oxidative stress (El-Bahr, 2015; Benzer et al., 2018). Furthermore, previous studies have demonstrated the antioxidant ability of curcumin against AFB1-induced liver damage in chicken and rat (Sujatha and Sashidhar, 2010; Zhang et al., 2016). It has been reported that AFB1-induced reactive oxygen species could promote the generation of 8-OHdG leading to oxidative DNA damage (Topal et al., 2017). 8-hydroxy-2′-deoxyguanosine is regarded as a biomarker of DNA oxidative damage, which can cause mutation and accelerate the process of AFB1-induced carcinogenesis (Liu et al., 2018). Our previous study demonstrated that AFB1 could significantly increase the levels of reactive oxygen species and 8-OHdG in the liver of chickens (Li et al., 2019). Therefore, in the present study, the toxicity of AFB1 in the duodenum was further confirmed by analyzing the 8-OHdG level. Our results showed that the 8-OHdG level was enhanced in the AFB1-fed group compared to C, and was effectively decreased by curcumin treatment. The results are consistent with our previous study which revealed that curcumin treatment could ameliorate AFB1-induced oxidative DNA damage by reduction of 8-OHdG (Li et al., 2019).

Moreover, P-gp is an important member of the ATP-binding transport family, which can pump exogenous substances out of the cell with the energy generated by

the hydrolysis of ATP, thereby reducing the concentration of the drug in the cell (Peachey et al., 2017). Various components in fruits and vegetables and traditional Chinese medicines in daily life can regulate the activity of P-gp, and could thereby affect the intestinal absorption of P-gp substrates (Bhutto et al., 2018; Zhang et al., 2019). It has been reported that quercetin and rutin significantly reduce the oral bioavailability of cyclosporine through activating P-gp (Yu et al., 2011). Furthermore, curcumin was found to reduce the expression of P-gp in human cervical cancer drug-resistant cells in a dose-dependent manner (Anuchapreeda et al., 2002). Curcumin plays a pivotal role in conferring protection from endogenous and exogenous toxins, and simultaneous administration of curcumin with anti-carcinogenic or anti-toxicogenic properties might alter the pharmacokinetics of co-administrated drugs by P-gp induction (He et al., 2013; Dai et al., 2020). Therefore, curcumin is recognized as a promising natural modulating drug (Das et al., 2010). In the present study, the mRNA expression of *Abcb1* and P-gp level were significantly reduced in the duodenum of chickens in the AFB1 group. However, curcumin increased the mRNA expression of *Abcb1* and activated P-gp, suggesting that it could effectively ameliorate AFB1-induced duodenum damage in chickens by activating P-gp.

Na^+-K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase are the Na^+, K^+, Mg^{2+}, Ca^{2+} ion pumps in cells, which are also regarded as markers of cell impairment during toxic exposure (Sun et al., 2018). It has been reported that the activities of Na^+-K^+-ATPase in renal tissues were decreased in broilers fed a diet contaminated with AFB1. The combination of AFB1 with Na^+ and K^+ activated sites may be responsible for the decrease of Na^+-K^+-ATPase activities (Liang et al., 2015). In this study, AFB1 caused a decrease in the activities of Na^+-K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase in the duodenum of chickens. It has been speculated that the cytotoxic effect and duodenum impairment induced by AFB1 may be associated with ion exchange imbalances caused by intracellular accumulation of Na^+ and Ca^{2+}. Importantly, curcumin treatment could ameliorate the AFB1-induced decrease in activities of ATPase, and promote restoring normal cellular function.

It is well understood that CYP450 enzymes are often responsible for the metabolism of various exogenous and endogenous compounds (Peter Guengerich et al., 2003). Several herbs including *Citrus paradise*, *Hypericum perforatum*, and *Ginkgo biloba* have been reported to influence CYP450-mediated metabolism (von Moltke et al., 2004; Girennavar et al., 2007; Zhang et al., 2018). The inhibitory effect of curcumin toward the in vitro CYP3A-mediated metabolism of testosterone has been demonstrated in rat liver microsomes (Kim et al., 2015). Meanwhile, it was found that dietary curcumin could inhibit CYP2A6-mediated bioactivation of AFB1 in broilers' liver and negatively modulated AFB1 biotransformation in our previous study (Muhammad et al., 2017). In this study, the expression of CYP450 enzymes was significantly increased in the AFB1 group, showing the increased conversion of AFB1 into its toxic metabolites. However, curcumin treatment inhibited the expression of CYP450 enzymes. High exposure of the intestines to curcumin upon oral administration could lead to an inhibitory effect toward CYP450 enzymes, suggesting that curcumin may have implications for alleviation of AFB1-induced toxicity in the intestines.

Curcumin demonstrates versatile pharmacological effects such as antioxidant, anticancer, and anti-inflammatory activities, and radioprotective and hepatoprotective effects in experimental animals as well as humans (Lam et al., 2016). Numerous studies have shown that curcumin can reduce the toxicity of AFB1 by inhibiting harmful metabolites of AFB1 and can reduce the damage caused by AFB1 (Limaye et al., 2018). Our previous study has shown that curcumin could be applied as a natural drug to alleviate AFB1-induced hepatotoxicity and carcinogenicity through inhibiting the generation of AFB1-DNA adduct (Li et al., 2019). Furthermore, preventive effects of curcumin against AFB1 were demonstrated to be associated with modulating CYP450 function (Chen et al., 2017). Taken together, we can speculate from the findings of this study that curcumin could alleviate AFB1-induced toxicity and damage through downregulating the expression of CYP450 enzymes, promoting ATPase activities, and inducing P-gp in the duodenum of chickens. However, indepth studies are needed to investigate the inhibitory mechanism of curcumin against CYP450 enzymes and the mechanism of curcumin inducing P-gp expression. In summary, our findings revealed a better understanding of the preventive mechanism of curcumin against AFB1-induced damage in the duodenum of chickens, and demonstrated the potential of curcumin in veterinary therapy.

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DISCLOSURES

The authors declare no competing financial interest.

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