

# Effects of feeding corn naturally contaminated with aflatoxin on growth performance, apparent ileal digestibility, serum hormones levels and gene expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase in ducklings

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**Objective:** A 14-d trial was conducted to determine the effects of feeding corn naturally contaminated with aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) on growth performance, apparent ileal digestibility, serum hormones levels and gene expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase in ducklings.

**Methods:** A total of 704 ducklings were blocked on the basis of sex and body weight (BW), and then allocated randomly to one of the following two treatments: i) CON, basal diet and ii) AFB<sub>1</sub>, diets with 100% of normal corn replaced with AFB<sub>1</sub> contaminated corn. There were 22 pens per treatment and 16 birds per pen. The concentration of AFB<sub>1</sub> was 195.4 and 124.35 µg/kg in the contaminated corn and AFB<sub>1</sub> diet, respectively.

**Results:** The AFB<sub>1</sub> decreased average daily gain, average daily feed intake, d 7 BW, final BW in the whole trial, and feed conversion ratio (FCR) during d 8 to 14 and d 1 to 14 by 10% to 47% (p<0.05), while FCR during d 1 to 7 was increased (p<0.05). AFB<sub>1</sub> did not affect mortality to 7 d of age, and then increased to 5.8% from 8 to 14 d of age (p<0.01). Apparent ileal gross energy digestibility was reduced by AFB<sub>1</sub>, whereas apparent ileal digestibility of dry matter, nitrogen, and amino acid was improved (p<0.01). Feeding AFB<sub>1</sub> diets increased serum concentration of leptin and insulin-like growth factors-1 (IGF-1) (p<0.05), but had no effect on neuropeptide Y, ghrelin, cholecystokinin-8 or insulin (p>0.05). Dietary treatments did not influence relative expression of jejunal Na<sup>+</sup>, K<sup>+</sup>-ATPase gene (p>0.05).

**Conclusion:** Taken together, feeding corn naturally contaminated with AFB<sub>1</sub> reduced growth performance, improved apparent ileal digestibility, and affected serum leptin and IGF-1 in ducklings from d 1 to 14.

**Keywords:** Aflatoxin B<sub>1</sub>; Ducklings; Leptin; Nutrient Digestibility; Performance

## INTRODUCTION

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) produced by *Aspergillus* species is the most toxic of aflatoxins (AF) subgroup [1]. China has the largest number of ducks on the earth [2]. Ducks are the most susceptible species to AFB<sub>1</sub> among all the poultry species because they cannot efficiently metabolize AF [3-4]. Corn, as the major energy source, accounts for more than 50% of duck feed in China. Furthermore, it was reported that the corn was extremely susceptible to the AFB<sub>1</sub> with the incidence rate being above 82% in China [5].

The effects of AFB<sub>1</sub> on growth performance, hepatic functions, immunity, intestinal morphology and blood profiles have been already documented in ducks [6-9]. Feeding naturally AFB<sub>1</sub> contaminated diets (120.02 µg/kg in the starter diet and 153.12 µg/kg in the grower diet) compromised growth performance and intestinal morphology, changed digestive physiology and development in ducks [10]. A recent study explored how dietary crude protein (CP) concentration and semi-pure AFB<sub>1</sub> affects the nutrient digestion and absorption in 14-d Pekin ducks, in

which 200 µg/kg AFB<sub>1</sub> caused adverse effects on performance primarily through decreased feed intake and the influence on nutrient digestion processes (jejunum morphology, digestive enzyme activity, and apparent energy digestibility) [11]. Higher dietary CP can increase growth performance regardless of AFB<sub>1</sub> without interactive effects [11]. Recent studies revealed the impact of AFB<sub>1</sub> on the gastro-intestinal tract [10,11], which was supported by a study that observed the biotransformation of AFB<sub>1</sub> to the toxic AFB<sub>1</sub>-exo-8,9-epoxide (AFBO) also occurred in the intestinal tract [12]. AFB<sub>1</sub> is known to be a potent inhibitor of protein synthesis [13,14] because AFBO, as its metabolite, may be able to interact with DNA and RNA in poultry [15].

Based on the above results, we hypothesized that the detrimental effects of AFB<sub>1</sub> on ducks were correlated to nutrient absorption transporter and several hormones involved in ingestion and digestion. However, very limited information is available on serum hormones and gene expression of nutrient absorption carrier in ducks fed diets naturally contaminated with AFB<sub>1</sub>. Therefore, the objectives of the current study were to explore the effects of feeding corn naturally contaminated with AFB<sub>1</sub> on growth performance, apparent ileal digestibility, serum hormones levels and gene expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase in ducklings.

## MATERIALS AND METHODS

### Analysis of dietary mycotoxins

Dietary mycotoxins concentrations were measured by enzyme-linked immunosorbent assay method (kits, Neogen Company, Lansing, MN, USA; Microplate Reader, Model 680, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The detection limits of the assay kits were 2 to 200 µg/kg for AFB<sub>1</sub>, 2 to 25 µg/kg for ochratoxin, 0.025 to 0.25 µg/kg for T2 toxins, 1 to 6 µg/kg for fumonisins, 0.025 to 0.25 µg/kg for deoxynivalenol, and 0.02 to 0.5 µg/kg for zearalenone, respectively.

### Experimental design and duck husbandry

The Animal Welfare Committee of Southwest University of Science and Technology approved the animal care protocol used for this experiment. A total of 704 one-d-old Cherry Valley ducklings with an average initial BW of 55.9±0.2 g were weighed, tagged, and randomly allotted to 44 pens on the basis of sex and body weight. All ducklings were housed in an environmentally controlled room.

This 2-wk trial consisted of 2 treatments with 22 pens per treatment and 16 birds per pen (8 males and 8 females) in a randomized complete block design. Birds were fed from 1 to 14 d of age. All diets (Table 1) were formulated to meet or exceed the NRC [16] requirements for ducks, and the dietary treatments were control (CON) and 100% contaminated corn. Diets were fed in pellet form and feed and water were provided *ad libitum*.

### Sampling and measurements

**Table 1.** Diet composition (as-fed basis)

Item	Starter <sup>1)</sup>
Ingredients (%)	
Corn	59.05
Soybean meal (CP 46%)	30.60
Wheat flour	5.00
Soybean oil	0.70
Rice bran	0.50
Calcium phosphate	1.65
Limestone	1.10
sodium chloride	0.30
Choline chloride (50%)	0.10
DL-Met (99%)	0.18
L-Lys≡HCl (78%)	0.07
Cr <sub>2</sub> O <sub>3</sub>	0.50
Vitamin premix <sup>2)</sup>	0.10
Trace mineral premix <sup>3)</sup>	0.15
Analytical composition	
ME (MJ/kg) <sup>4)</sup>	12.14
Crude protein (%)	19.72
Lys (%)	1.10
Ca (%)	0.97
P (%)	0.63

CP, crude protein; ME, metabolizable energy.

<sup>1)</sup> Provided starter diets during wk 1 to 2.

<sup>2)</sup> Provided per kilogram of diet: vitamin A, 2,500 IU; vitamin D<sub>3</sub>, 400 IU; vitamin E, 10 IU; vitamin K<sub>3</sub>, 0.5 mg; vitamin B<sub>1</sub>, 2.0 mg; vitamin B<sub>6</sub>, 2.5 mg; vitamin B<sub>12</sub>, 0.02 mg; nicotinic acid, 55 mg; pantothenic acid, 10 mg; folic acid, 1.0 mg; and biotin, 0.1 mg.

<sup>3)</sup> Provided per kilogram of diet: 60 mg Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O); 8 mg Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O); 60 mg Zn (ZnSO<sub>4</sub>·7H<sub>2</sub>O); 50 mg Mn (MnSO<sub>4</sub>·H<sub>2</sub>O); 0.1 mg Se (Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O); and 0.2 mg I (KI).

<sup>4)</sup> Calculated values.

The ducklings were weighed and feed intake was recorded on d 1, 7, and 14, and average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated. Dead birds were weighed daily, and the mortality was recorded as it occurred.

At the end of the experiment, 8 ducklings (4 males and 4 females) were randomly selected from each pen and blood samples were collected from the jugular vein into a sterile syringe and stored at 4°C. Samples were then centrifuged at 3,000×g for 15 min and serum was separated. After blood collection, the same ducks were sacrificed by cervical dislocation. The lower 2/3 ileal digesta was collected by flushing with reverse osmosis water for nitrogen (N), gross energy (GE), dry matter (DM), ether extract (EE), chromium (Cr), and amino acid (AA) determination. A section of mid jejunum was gently scraped with a glass slide, and mucosa was frozen in liquid nitrogen, and stored at -80°C for relative gene expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase analysis.

All diets and ileal samples were analyzed for DM, N, EE, crude ash, calcium, and phosphorus according to AOAC [17,18]. Cr was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan) [18]. Energy was determined by measuring the heat of combustion in the samples using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline,

**Table 2.** Primer sequences of gene for Na<sup>+</sup>, K<sup>+</sup>-ATPase

Gene	Sequences of forward primer	Sequences of reverse primer
Na <sup>+</sup> , K <sup>+</sup> -ATPase	CGTGGCATTGTTATTAGGAC	GTATTCAAGGATCAGCGAGA

IL, USA). Amino acid contents were determined, following acid hydrolysis with 6 N HCl at 110°C for 24 h, using an AA analyzer (Biochrom 20, Pharmacia Biotech, Cambridge, England). Before acid hydrolysis, Met and Cys were oxidized with formic acid. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C.

The serum neuropeptide Y (NPY), ghrelin, leptin, cholecystokinin-8 (CCK-8), insulin, insulin-like growth factors-1 (IGF-1) levels were analyzed by radioimmunoassay method using kits (Shanghai Yuanye Biotechnology Co. Ltd., Shanghai, China) according to the manufacturer's instructions. Automatic radioimmunoassay  $\gamma$  counter (H-7500, Hitachi, Tokyo, Japan) was used for determination.

Total RNA extraction, reverse transcription reaction and quantitative real-time polymerase chain reaction (PCR) of Na<sup>+</sup>, K<sup>+</sup>-ATPase gene was determined as previously described [19]. Briefly, total RNA of tissues was extracted with RNAiso Reagent (TaKaRa, Kyoto, Japan) and reverse-transcribed with RT Reagents (TaKaRa, Japan) according to manufacturer's instructions. Quantitative real-time PCR was performed using 96-well iCycler iQTM Real-Time PCR Detection System (Bio-Rad, USA). The gene-specific primers used are listed in Table 2 and purchased from TaKaRa (Japan). The PCR system consisted of 12.5 mL SYBR Green PCR Master Mix (TaKaRa, Japan), 2.0 mL of cDNA, 8.5 mL of PCR-grade water and 2.0 mL of primer pairs (100 mM forward and 100 mM reverse) for a total volume of 25 mL. All samples were assayed in triplicate. Cycling conditions were as follows: 94°C for 10 s, and 40 cycles involving a combination of 94°C for 5 s, 55.5°C for 20 s and 72°C for 15 s. Relative gene expression to the house-keeping gene ( $\beta$ -actin) was performed in order to correct for the variance in amounts of RNA input in the reactions. However, the relative gene expressions compared to the house-keeping gene were calculated [20]. The primer sequences of gene for Na<sup>+</sup>,

K<sup>+</sup>-ATPase are presented in Table 2.

### Statistical analysis

Data were analyzed by analysis of variance using the T-test procedure of SAS (SAS Inst. Inc., Cary, NC, USA) with the pen being the experimental unit. Variability in the data was expressed as the standard error of means. Probability values less than 0.05 were considered significant.

## RESULTS

### Mycotoxins levels of corn and diets

Concentrations of various mycotoxins, as well as their regulatory guidance concentration, in corn and diets are presented in Table 3. The dietary AFB<sub>1</sub> was 2.87 and 124.35  $\mu$ g/kg for CON and AFB<sub>1</sub>, respectively. Only AFB<sub>1</sub> exceeded the regulatory guidance concentration of Chinese National Standard (GB 13078-2001), while others did not exceed the regulatory limits of Chinese National Standard (GB 13078.2-2006, GB 13078.3-2007, and GB 21693-2008) and European Commission [21].

The GE and nutrient composition between normal corn and contaminated corn are presented in Table 4, which showed few differences.

### Growth performance

From d 1 to 7, feeding AFB<sub>1</sub> diets decreased d 7 BW, ADG, and ADFI by 22%, 31%, and 23%, respectively ( $p < 0.05$ ), while FCR increased by 11% ( $p < 0.05$ ) (Table 5). From d 8 to 14, birds fed AFB<sub>1</sub> diets had lower ADG, ADFI, and FCR ( $p < 0.05$ ) with the reduction of 41%, 47%, and 10%, respectively. In the whole trial, the AFB<sub>1</sub> reduced final BW, ADG, ADFI, and FCR by 34%, 39%, 43%, and 8% ( $p < 0.05$ ). Mortality was increased by AFB<sub>1</sub> diets ( $p < 0.01$ ) from d 8 to 14 and d 1 to 14.

**Table 3.** The concentration of mycotoxins in corn and diets

Mycotoxins	Normal corn	Contaminated corn	CON <sup>1)</sup>	AFB <sub>1</sub> <sup>1)</sup>	Limits	
					Corn	Complete diet <sup>2)</sup>
AFB <sub>1</sub> ( $\mu$ g/kg)	3.80	195.4	2.87	124.35	50.0 <sup>3)</sup>	10.0
OA ( $\mu$ g/kg)	5.25	4.35	1.18	0.75	100 <sup>3)</sup>	100
T2 (mg/kg)	0.12	0.05	0.04	0.05	-	1.00
FM (mg/kg)	1.00	3.35	1.03	4.50	60.0 <sup>4)</sup>	20.0 <sup>4)</sup>
DON (mg/kg)	0.45	0.24	0.50	0.45	5.00	1.00
ZEA (mg/kg)	0.21	0.08	0.17	0.15	0.50 <sup>3)</sup>	0.50

AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; OA, ochratoxin; T2, T2 toxins; FM, fumonisins; DON, deoxynivalenol; ZEA, zearalenone.

<sup>1)</sup> CON, basal diet; AFB<sub>1</sub>, basal diet containing 100% contaminated corn.

<sup>2)</sup> Chinese National Standard (GB) 13078-2001, GB 13078.2-2006, GB 13078.3-2007 and GB 21693-2008 of China (Beijing, China).

<sup>3)</sup> GB 13078-2001 and GB 13078.2-2006 of China.

<sup>4)</sup> European Commission [21].

**Table 4.** The GE and nutrient composition between normal corn and contaminated corn

Items	Normal corn	Contaminated corn
GE (MJ/kg)	16.2	16.15
DM (%)	86.94	86.79
CP (%)	7.81	7.70
Starch (%)	64.9	65.10
Lys (%)	0.27	0.28
Met+cys (%)	0.38	0.37
Thr (%)	0.37	0.37

GE, gross energy; DM, dry matter; CP, crude protein.

**Apparent ileal digestibility**

Apparent ileal digestibility of DM, N, and AA was increased ( $p < 0.05$ ) by AFB<sub>1</sub> diets, whereas apparent ileal digestible energy digestibility was decreased ( $p < 0.05$ ) (Table 6). No difference was observed in apparent ileal EE digestibility between treatments ( $p > 0.05$ ).

**Serum hormones levels and relative expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase gene**

Serum levels of leptin and IGF-1 was increased by AFB<sub>1</sub> diets ( $p < 0.05$ ), while NPY, ghrelin, CCK-8 and insulin were not affected ( $p > 0.05$ ) (Table 7). There was no difference ( $p > 0.05$ ) in relative expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase between treatments (Table 8).

**DISCUSSION**

**Table 5.** Effects of feeding corn naturally contaminated with AFB<sub>1</sub> on growth performance in ducklings<sup>1)</sup>

Item	CON <sup>2)</sup>	AFB <sub>1</sub> <sup>2),3)</sup>	SEM	p-value
Initial BW (g)	55.98	55.82	0.25	0.30
d 7 BW (g)	217.9	169.16	4.06	<0.01
Final BW (g)	661.06	433.73	9.68	<0.01
ADG (g)				
d 1-7	23.10	15.83	2.06	0.03
d 8-14	63.31	36.99	7.58	0.02
d 1-14	43.20	26.42	4.27	0.03
ADFI (g)				
d 1-7	28.03	21.46	2.12	0.04
d 8-14	92.80	48.66	6.33	0.01
d 1-14	61.62	34.98	8.74	0.03
FCR				
d 1-7	1.21	1.35	0.02	0.02
d 8-14	1.46	1.31	0.03	0.04
d 1-14	1.43	1.32	0.02	0.03
Mortality (%)				
d 1-7	0.00	0.00	0.00	0.89
d 8-14	0.00	5.80	1.35	<0.01
d 1-14	0.00	5.80	1.36	<0.01

AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; SEM, standard error of the means; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

<sup>1)</sup> Means represent 22 pens of ducks with 16 birds per pen (n = 22/group).

<sup>2)</sup> CON, basal diet; AFB<sub>1</sub>, basal diet containing 100% contaminated corn.

<sup>3)</sup> The AFB<sub>1</sub> fed group contained 124.35 µg/kg from d 1 to 14.

**Growth performance**

The detrimental effects of AFB<sub>1</sub> on growth performance may be due to anorexia, listlessness, impaired liver function, and inhibition of protein synthesis and lipogenesis [22,23]. As expected, the ADG and ADFI was reduced by AFB<sub>1</sub> (124.35 µg/kg) in the current study, which was in agreement with a study that reported that the growth and feed intake decreased by naturally contaminated AFB<sub>1</sub> diets (120.02 µg/kg) during d 0 to 14 in Cherry valley ducklings [10]. Similar findings in ducks were observed by other

**Table 6.** Effects of feeding corn naturally contaminated with AFB<sub>1</sub> on apparent ileal digestible energy and nutrients digestibility in ducklings<sup>1)</sup>

Item (%)	CON <sup>2)</sup>	AFB <sub>1</sub> <sup>2),3)</sup>	SEM	p-value
Energy				
ADE	78.17	74.33	0.98	0.04
Nutrients				
DM	71.13	73.82	0.10	<0.01
EE	93.23	92.15	0.20	0.09
N	83.37	85.58	0.72	0.03
AA				
Lys	87.21	90.14	0.13	<0.01
His	87.76	90.14	0.19	<0.01
Arg	90.55	92.69	0.20	<0.01
Thr	76.73	81.16	0.25	<0.01
Met	87.87	90.92	0.52	0.01
Ile	81.55	86.19	0.58	<0.01
Leu	70.96	77.63	0.53	<0.01
Phe	78.67	85.23	1.63	0.04
Val	82.79	86.58	0.19	<0.01
Asp	81.24	84.20	0.22	<0.01
Ser	80.54	84.87	0.31	<0.01
Glu	88.65	91.36	0.21	<0.01
Gly	77.68	82.43	0.28	<0.01
Ala	85.66	87.83	0.28	<0.01
Cys	83.17	85.66	0.54	0.01
Pro	84.31	86.07	0.29	<0.01
Total AA	84.80	88.11	0.14	<0.01

AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; SEM, standard error of the means; ADE, apparent digestible energy; DM, dry matter; EE, ether extract; AA, amino acid.

<sup>1)</sup> Means represent 22 pens of ducks with 8 birds per pen (n = 22/group).

<sup>2)</sup> CON, basal diet; AFB<sub>1</sub>, basal diet containing 100% contaminated corn.

<sup>3)</sup> The AFB<sub>1</sub> fed group contained 124.35 µg/kg from d 1 to 14.

**Table 7.** Effects of feeding corn naturally contaminated with AFB<sub>1</sub> on serum hormones levels in ducklings<sup>1)</sup>

Item (pg/mL)	CON <sup>2)</sup>	AFB <sub>1</sub> <sup>2),3)</sup>	SEM	p-value
NPY	175.82	172.19	9.08	0.64
Ghrelin	83.59	80.24	5.54	0.46
Leptin	6.81	8.36	0.30	0.03
CCK-8	3.92	4.44	0.57	0.28
Insulin	16.89	16.59	1.34	0.78
IGF-1	220.44	301.84	20.68	0.04

AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; SEM, standard error of the means; NPY, neuropeptide Y; CCK-8, cholecystokinin-8; IGF-1, insulin-like growth factors -1.

<sup>1)</sup> Means represent 22 pens of ducks with 8 birds per pen (n = 22/group).

<sup>2)</sup> CON, basal diet; AFB<sub>1</sub>, basal diet containing 100% contaminated corn.

<sup>3)</sup> The AFB<sub>1</sub> fed group contained 124.35 µg/kg from d 1 to 14.

**Table 8.** Effects of feeding corn naturally contaminated with AFB<sub>1</sub> on average relative expression of jejunum Na<sup>+</sup>, K<sup>+</sup>-ATPase gene in ducklings<sup>1)</sup>

Item	CON <sup>2)</sup>	AFB <sub>1</sub> <sup>2),3)</sup>	SEM	p-value
Na <sup>+</sup> , K <sup>+</sup> -ATPase	0.037	0.045	0.005	0.35

AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; SEM, standard error of the means.

<sup>1)</sup> Means represent 22 pens of ducks with 8 birds per pen (n = 22/group).

<sup>2)</sup> CON, basal diet; AFB<sub>1</sub>, basal diet containing 100% contaminated corn.

<sup>3)</sup> The AFB<sub>1</sub> fed group contained 124.35 µg/kg from d 1 to 14.

studies [6-8] with purified AFB<sub>1</sub> (from 40 to 200 µg/kg). Consistently, a recent study indicated that purified AFB<sub>1</sub> (200 µg/kg) decreased the 14 d body weight gain and ADFI of Pekin ducklings by approximately 33% [11]. Interestingly, FCR was reduced by 10% and 8% during d 8 to 14 and 1 to 14, while increased by 11% during d 1 to 7 in the study herein. In contrast, others observed that FCR was improved by purified AFB<sub>1</sub> (40 to 200 µg/kg) in ducks [6-8,24]. However, FCR was not affected by semi-purified AFB<sub>1</sub> (200 µg/kg) in Pekin ducklings [11] and naturally contaminated AFB<sub>1</sub> (from 120.02 to 128.7 µg/kg) in Cherry valley ducklings [9,10] from d 0 to 14. This inconsistency in FCR was also observed in broilers. FCR was decreased in broilers fed naturally contaminated AFB<sub>1</sub> diets (44.5 µg/kg) [25], whereas FCR was improved by purified AFB<sub>1</sub> (1,500 µg/kg) [26]. This inconsistency may be due to AFB<sub>1</sub> origins (corn naturally contaminated or inoculated with purified mycotoxins), dosage and species-specificity. The lack of AFB<sub>1</sub> effect on FCR was attributed to the ducks maturity [11]. The decrease in the rate of excreta to pass through the digestive tract in ducks may lead to the reduction in FCR [10], which was supported by some studies that reported the fusarium toxin decreased the excreta emptying rate in growing pigs and broilers, respectively [27,28]. Therefore, the reduction in ADG may be due to the decreased ADFI, whereas the reduced ADFI may be attributed to the decrease in excreta passage rate, which was supported by the decreased FCR in this study. All the deaths caused by AFB<sub>1</sub> happened in the second week because of the chronic and accumulated mycotoxicosis [9]. Nevertheless, more research is needed to evaluate the influence of naturally contaminated AFB<sub>1</sub> diets on poultry because some reaction in enzyme activities and cell wall degradation may take place.

### Apparent ileal digestibility

Several studies have revealed the AFB<sub>1</sub> effect on the gastro-intestinal tract [10,11]. It was reported the biotransformation of AFB<sub>1</sub> to the toxic AFBO was also occurred in the intestinal tract [12]. Therefore, the digestion and absorption in gastro-intestinal tract may be affected by AFB<sub>1</sub>. However, there was limited literature about the nutrient digestibility in ducks. Reduced apparent ileal digestible energy (ADE) in birds fed AFB<sub>1</sub> diets was observed in this study, which was in agreement with several studies in Pekin ducklings (200 µg/kg) from d 0 to 14 [11] and in broilers (2,000 µg/kg), respectively [29]. Purified AFB<sub>1</sub> (200 µg/kg) did not affect apparent ileal DM and N digestibility in Pekin ducklings [11]. Re-

duced N digestibility upon AFB<sub>1</sub> exposure (200 µg/kg) in Cherry Valley ducks was observed [8]. On the contrary, apparent ileal DM, N, and AA digestibility was improved by AFB<sub>1</sub> in the herein study. A study indicated that improved proenzymes were released from the injured pancreas in response to AFB<sub>1</sub> (200 µg/kg) in Cherry Valley ducks [8]. Meanwhile, improved pancreatic amylase and lipase activity was observed in Pekin ducklings given purified AFB<sub>1</sub> (200 µg/kg) [11]. The authors also proposed that the compensatory effect of the birds in response to decreased ADFI to meet their nutrient need might be a possible reason, yet the improved enzyme activities was not enough to restore the damage to growth performance from AFB<sub>1</sub>. Notwithstanding this, the reason for the increased apparent ileal DM, N, and AA digestibility requires further research to determine whether it was due to the improved enzymes or other factors.

### Serum hormones levels and relative gene expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase

Because of the dramatic reduction in ADFI by AFB<sub>1</sub>, it was hypothesized that the adverse effects of AFB<sub>1</sub> on ducks were correlated to nutrient absorption transporter and several hormones involved in ingestion and digestion. Therefore, this was an important issue in this study. Liver is the primary target organ of AFB<sub>1</sub>, which was demonstrated by other researchers [9,30] who observed hepatic physical change and impaired hepatic function. Leptin (a satiety hormone), is mainly produced in the adipocytes of white adipose tissue, and can regulate fat stores through depressed appetite and increased energy consumption [31]. Furthermore, fatty liver can be caused by AFB<sub>1</sub> [32]. Leptin was increased by AFB<sub>1</sub> in this study. Accordingly, it was supposed that leptin showed a compensatory increase to inhibit adipose synthesis and promote adipose lipolysis caused by fatty liver in response to AFB<sub>1</sub>. Although IGF-1, produced mainly in the liver, was a primary mediator of the effects of growth hormone and had growth-promoting effects, it is an important cytokine of the liver inflammation and fibrosis. Dietary AFB<sub>1</sub> had no effect on ghrelin, NPY and CCK-8 in the current study. Ghrelin (hunger hormone) and NPY were opposed by the action of leptin, which can increase appetite. CCK-8, synthesized and released by enteroendocrine cells in the mucosal lining of the small intestine (mostly in the duodenum and jejunum), suppressed hunger and feed intake through reduced rate of gastric emptying [33].

The Na<sup>+</sup>, K<sup>+</sup>-ATPase is a solute pump that pumps sodium out of cells while pumping potassium into cells, both against their concentration gradients. The Na<sup>+</sup>, K<sup>+</sup>-ATPase helps maintain resting potential, effects transport, and regulates cellular volume [34]. Particularly, it is used to transport most nutrients in the intestinal tract, which can reflect the absorption of nutrients [35]. The AFB<sub>1</sub> may affect the tight junction proteins, which were the major constituent of gut barrier for the latter function, thus any damage to these proteins' synthesis and activities may result in an increase of permeability of the selective gut barrier [11,12].

The relative expression of jejunum  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase gene was numerically increased by 22% in ducklings fed  $\text{AFB}_1$  diets in the herein study. Although the  $\text{AFB}_1$  effect on the relative expression of jejunum  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase gene was not significant, the large increase in number may partially mirror the gut permeability to some degree. Previous studies indicated that increased gut permeability may also facilitate the absorption of any presented mycotoxins [11,12].

It was hypothesized that the mechanism for the depressed feed intake by  $\text{AFB}_1$  may be as follows: decreased ADFI may be due to the improved leptin, which inhibited appetite and adipose synthesis caused by fatty liver. In addition, the increase in nutrient digestibility and feed efficiency may be due to the reduced rate of gastric emptying and improved enzyme activities.

## CONCLUSION

Based on the above findings and discussions, feeding naturally contaminated corn diets (124.35  $\mu\text{g}/\text{kg}$ ) depressed the growth performance in ducklings through the reduction of ADFI, which may partially be due to the increased leptin. The decreased FCR originated from reduced ADG which further decreased ADFI.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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