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Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B_1 levels

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Abstract

The objective of this study was to investigate toxic effects of aflatoxin B1(AFB_1) on growth performance, organs, hepatic enzyme activities, apparent digestibility of nutrients and digestive enzyme activities in ducks. Ninety 1-day-old Cherry Valley commercial ducks were designed to three treatment groups with three replicates of ten birds each. Group I (control) was fed conventional feed free of AFB_1, group II or III was fed the diets containing 20 μg/kg or 40 μg/kg AFB_1-contaminated rice respectively. The feeding trial lasted 6 weeks. The results were that decreased body weight gain and feed intake, increased feed to gain ratio and selected organ weights (liver, kidney and pancreas) were observed in AFB_1-treated groups. The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly increased in AFB_1-contamined groups. The apparent digestibility of crude protein (CP) was significantly lower while activities of digestive enzyme from duodenum contents including protease, chymotrypsin, trypsin and amylase were increased in AFB_1-treated group. These results indicated that AFB_1 of feed could decrease growth performance and apparent digestibility of nutrients, change digestive enzyme activities of duodenum contents in duck.

Keywords: Aflatoxin B1; Duck; Digestive enzyme; Nutrient digestibility; Performance

1. Introduction

Aflatoxins, a closely related group of polysubstituted bisfuranocoumarins, are toxic compounds produced in contaminated grains produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Among the aflatoxins, aflatoxin B1(AFB_1) has been much concerned due to its carcinogenicity, mutagenicity, and teratogenicity (Ismail and Rustom, 1997; Smela et al., 2001; Mishra and Das, 2003). Animals may develop various health problems when they exposure high levels of the toxin. Occurrence of aflatoxin in poultry and animal feedstuffs is quite common in many countries, and causes great economical loss in terms of growth retardation or meat production and the residues of aflatoxins in liver and eggs and other edible tissues (Charmley et al., 1995; Bintvihok et al., 2002).

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Characteristics of aflatoxicosis from different type of animals are various. Aflatoxicosis in poultry is characterized by weakness and anorexia with lower growth rate, poor utilization, decreased weight gain and egg production, increased bruising and hemorrhaging, increased susceptibility to environmental and microbial stresses, and increased mortality (Kryukov et al., 1992; Bailey et al., 1998; Kubena et al., 1998; Verma et al., 2002; Mendoza et al., 2006). Some consider the duckling to be the most sensitive poultry species. Liver is the target organ of aflatoxins and hepatobiliary damages are associated with alterations in liver function enzymes. Aflatoxicosis is also associated with biochemical, haematological, pathological changes and immune functions changes (Sur and Celik, 2005).

AFB_1 has been suggested as a factor in human liver cancer and classified as a class I human carcinogen (Robens and Richard, 1992; IARC, 1993; Fink-Gremmels, 1999). Chronic exposure to aflatoxin may significantly change productivity and animal farming trends, and undergo a great risk to the consumer (Robens and Richard, 1992). The changes of digestive enzyme activities and nutrient digestibility are closely related to animal growth and weight gain. The present study was conducted to examine the changes of growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks exposure to aflatoxin B_1 contents.

2. Materials and methods

2.1. Preparation of aflatoxin B_1 and analysis

Aspergillus flavus (CICC2219), purchased from China center of industrial culture collection, was cultured on potato dextrose agar (PDA) and incubated for 5–6 days. The cultured mixtures were suspended in distilled water, mixed in 20% moisture rice for 1 week to allow AFB production. The AFB_1 content was measured by ELISA test kits (Beijing Laboratory Biotech Co., LTD, China). The dried AFB_1-contaminated rice was incorporated into the basal diet to provide the desired level of about 20 or 40 μg AFB_1/kg of diet.

2.2. Animals and treatment

Ninety 1-day-old Cherry Valley commercial ducks, provided by Huzhou Poultry Industry Co., LTD, China, were randomly divided into three treatment groups with three replicates of ten birds each. Group I was fed conventional feed free of AFB_1 and served as the control, group II or III was fed the diets containing about 20 μg/kg or 40 μg/kg AFB_1-contaminated rice respectively. Ducks were fed for 6 weeks under standard management conditions with feed and water available *ad libitum*. All diets contained adequate levels of nutrients as recommended by the National Research Council (1994) and the composition of diets and the content of AFB_1 is presented in Table 1. Body weight and feed consumption were recorded weekly.

2.3. Digestibility experiment

For the determination of nutrient digestibility, the total fecal collection method was used. The 36 ducks were kept in individual cages, and given their respective experimental diets, 12 birds per diet. Feed and water were available *ad libitum*. Collection of fecal material was undertaken through three consecutive days, after an adaptation period of 1 week. The amount of feed consumed and feces were recorded daily. Feces were stored in closed plastic containers at 4 °C during the collection period. At the end of this period, collected excreta were mixed thoroughly and a 500 g sample was taken from

Table 1
Composition and nutrient levels of the diets

Item	0–3 week	4–6 week
Ingredients (%)		
Corn	19.20	26.20
Rice [a]	38.00	38.00
Soybean meal	28.00	19.00
Wheat middling	4.00	8.00
Fish meal	5.00	2.00
Vegetable oil	2.00	3.00
$CaHPO_4$	1.70	2.10
Stone meal	0.70	0.35
Methionine	0.15	0.06
Lysine	0.08	0.06
Salt	0.20	0.30
Premix [b]	1.00	1.00
Composition [c]		
ME, MJ/kg [d]	12.52	13.10
DM, %	93.23/94.15/95.20 [e]	93.66/94.20/95.23 [e]
CP, %	18.82/19.46/20.25 [e]	15.12/15.65/16.43 [e]
EE, %	3.62/3.81/4.14 [e]	5.05/5.17/5.38 [e]
CA, %	7.23/7.11/6.85 [e]	5.76/5.72/5.66 [e]
Ca, %	1.46/1.38/1.20 [e]	1.07/1.02/0.94 [e]
P, %	0.73/0.74/0.76 [e]	0.55/0.59/0.66 [e]
AFB_1(μg/kg)	0/21.21/41.69 [e]	0/20.48/40.71 [e]

[a] AFB-free rice was replaced by AFB-contaminated rice according to the same proportion in experimental diets.

[b] Premix supplied per kilogram: 0–3 weeks: vitamin A, 3000 IU; vitamin D_3, 600 IU; vitamin E, 8 IU; vitamin K, 2 mg; vitamin B1, 3 mg; riboflavin, 5 mg; D-pantothenic acid, 11 mg; nicotinic acid, 60 mg; vitamin B6, 3 mg; biotin, 0.1 mg; choline, 1650 mg; folic acid, 1 mg; vitamin B_{12}, 0.02 mg; Fe, 96 mg; Mn, 30 mg; Zn, 60 mg; Cu, 8 mg; Se, 0.15 mg; I,0.45 mg. 4–6 weeks: vitamin A, 2500 IU; vitamin D_3, 500 IU; vitamin E, 8 IU; vitamin K, 2 mg; vitamin B1, 3 mg; riboflavin, 5 mg; D-pantothenic acid, 11 mg; nicotinic acid, 55 mg; vitamin B6, 3 mg; biotin, 0.1 mg; choline, 1400 mg; folic acid, 1 mg; vitamin B_{12}, 0.02 mg; Fe, 96 mg; Mn, 80 mg; Zn, 60 mg; Cu, 8 mg; Se, 0.15 mg; I,0.45 mg.

[c] Measured value. ([4]Calculated value).

[d] Calculated value.

[e] Three values came from Group I (control), Group II and Group III, respectively.

Table 2
Effects of aflatoxin B_1(AFB_1)on growth performance of ducks after feeding for 6 weeks

Item	Control	Group II	Group III
Initial weight (g)	56.0±0.9	56.0±0.8	55.5±0.8
Final weight (g)	2087±39	1821±58*	1582±73*
ADG (g)	48.21±2.5	42.52±2.5*	37.44±2.7*
ADFI (g)	142.20±4.6	140.73±3.7	130.28±3.5*
FCR	2.95±0.02	3.31±0.04*	3.48±0.04*

Data are expressed as mean±SE of 30 ducks each treatment.
*Significantly different ($P<0.05$) when compared to control values.
Control (Group I) was fed conventional feed free of AFB_1, group II or III was fed the diets containing about 20 μg/kg or 40 μg/kg AFB_1-contaminated rice respectively.

respective homogenized fecal sample and were dried in a drying oven at 70 °C and ground in a Wiley Mill to pass through a 1 mm screen prior to chemical analysis.

2.4. Sample collection

At the end of feeding trial, 18 ducks were randomly selected from each treatment and fasted overnight before sampling. The ducks were euthanatized by severing the jugular vein. Blood samples were centrifuged at 2200 g for 10 min, and serum was separated and packed in Eppendorf tubes respectively. Liver, kidney, pancreas and heart were removed, cleaned and weighed respectively. The samples of duodenum contents were collected and snap-frozen in liquid nitrogen. The samples of serum and intestinal contents were stored at −70 °C until required for analysis.

2.5. Chemical analysis

The proximate analyses of excreta were carried out according to the methods of AOAC (1990). Calcium was determined by method of titration with 0.1 N EDTA. Total phosphorus was determined colorimetrically using a molybdovandate reagent with a UV–visible spectrophotometer (Ultrospec, 2000, Sweden) (AOAC, 1990). The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the procedure of Reitman and Frankel (1957) by estimating their hydrazone at 510 nm. The samples of duodenum contents (0.2 g) were homogenized with 4 ml ice-cold saline (0.9% NaCl). They were stored at 4 °C for 24 and then centrifuged at 6000 g for 15 min. The supernatant was stored at −70 °C for assay of enzyme activities. Protease, trypsin, chymotrypsin and amylase were measured according to the methods described by Lhoste et al. (1993).

2.6. Data analysis

All data measured in the study were analyzed by comparing means according to least significant difference test, using the general linear model procedure of SAS (version 6.12). Data are expressed as mean±SE. A significant level of 0.05 was used.

3. Results and discussion

3.1. Growth performance and relative organ weight

AFB_1 can significantly affect duck health and production. The effect of AFB_1-contaminated-feed on body weight (BW) and feed conversion ratio (FCR) of ducks after feeding for up to 6 weeks are showed in Table 2. Compared with the control group, BW was significantly reduced for the ducks fed diet containing AFB_1, the percentage reduction was 12.7% and 24.2%, respectively. There were higher FCR for AFB_1-treated birds and it was increased by 12.2% and 18.0%, respectively.

Data presented in Table 3 show the effects of dietary AFB_1 treatments on relative organ weight. The diet containing AFB_1 significantly increased relative liver, kidney, and pancreas weights compared to the control. But AFB_1 did not affect relative weight of heart of ducks.

In the current study, toxicity of AF was expressed as reduced body weight gain, higher feed/gain ratio, increased relative organ weight. The toxic effects produced by AF were in general agreement with previous reports found in chicks or ducks (Huff et al., 1992; Scheideler,1993; Abo-Norag et al., 1995; Goswami et al., 1998; Aravind et al., 2003). Dersjant-Li et al. (2003) have reviewed the impact of dietary aflatoxins on the performance and growth rate of pigs and broilers suggesting a relationship between aflatoxin in diet with the growth rate.

3.2. Serum ALT and AST activities

Serum enzymes were used as the biochemical indicators for hepatic damage. Significantly increased enzyme activities were observed in the AFB_1-treated groups. As shown in Table 4, a marked increase by 9.6% and 13.8%, 32.1% and 43.8%, respectively, was found in serum ALT and AST activities of AFB_1-treated groups.

Table 3
Effects of aflatoxin B_1(AFB_1)on relative weight of selected organs of ducks

Item	Control	Group II	Group III
Liver (%)	2.44±0.05	2.95±0.06*	3.10±0.07*
Kidney (%)	0.71±0.03	0.83±0.04*	0.90±0.04*
Heart (%)	0.61±0.01	0.64±0.02	0.66±0.02
Pancreas (%)	0.36±0.03	0.49±0.03*	0.57±0.04*

Data are expressed as mean±SE of 18 ducks each treatment.
*Significantly different ($P<0.05$) when compared to control values.
Control (Group I) was fed conventional feed free of AFB_1, group II or III was fed the diets containing about 20 μg/kg or 40 μg/kg AFB_1-contaminated rice respectively.

Table 4
Effects of aflatoxin B_1(AFB_1)on the activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of ducks

Item	Control	Group II	Group III
AST (IU/L)	44.12±1.13	48.35±1.20*	50.23±1.40*
ALT (IU/L)	16.25±0.66	21.46±0.67*	23.37±0.75*

Data are expressed as mean±SE of 18 ducks each treatment.
*Significantly different ($P<0.05$) when compared to control values.
Control (Group I) was fed conventional feed free of AFB_1, group II or III was fed the diets containing about 20 μg/kg or 40 μg/kg AFB_1-contaminated rice respectively.

Alterations in serum levels of ALT and AST are liver specific and have been considered as a tool for studying varying cell viability and changes in cell membrane permeability (Novelli et al., 1995). In the clinical, serum ALT and AST activities represent biomarkers for liver function. They exist in mitochondria of hepatocyte and play a vital role in metabolism of protein. In the present study, serum ALT and AST activities significantly increased, indicating a release of these enzymes from the liver injured by AFB_1 treatment. These results were also found in chickens as reported by other papers (Kececi et al., 1998; Raju and Devegowda, 2000; Aravind et al., 2003).

3.3. Apparent digestibility of nutrients

The digestibility values are presented in Table 5. The apparent digestibility of crude protein (CP) was significantly lower by 8.5% and 12.8% respectively in AFB_1-treated group than that of control. The data showed a decrease in nutrient utilization, particularly nitrogen, caused by dietary AFB_1. However, there were no effects of AFB_1 on apparent digestibility ether extract (EE), Ash, Calcium (Ca), total phosphorus (TP).

Table 5
Effects of aflatoxin B_1(AFB_1)on apparent digestibility of crude protein (CP), ether extract (EE), Ash, Calcium (Ca), total phosphorus (TP) of ducks

Item	Control	Group II	Group III
CP (%)	63.75±1.46	58.31±1.35*	55.62±1.28*
EE (%)	85.12±1.78	82.75±1.75	80.84±1.85
Ash (%)	44.72±1.10	42.74±0.98	39.85±0.93
Ca (%)	46.54±1.13	46.74±1.01	45.75±1.10
TP (%)	70.38±1.83	69.96±1.79	67.46±1.73

Data are expressed as mean±SE of 12 ducks each treatment.
*Significantly different ($P<0.05$) when compared to control values.
Control (Group I) was fed conventional feed free of AFB_1, group II or III was fed the diets containing about 20 μg/kg or 40 μg/kg AFB_1-contaminated rice respectively.

Studies indicated that AF could stimulate the forepart of gastrointestinal tract directly and cause pathologic changes (Huff et al., 1986). These would affect nutrients absorption and decrease apparent digestibility of nutrients. The present study showed the decreased digestibility of CP. The same results were found in chickens as reported by Li (1998). This result suggests that the decrease of apparent digestibility of CP may be one of the reasons that resulted in decrease of growth performance in AFB_1-treated group.

3.4. Digestive enzyme activities of duodenum contents

As shown in Table 6, AFB_1 had an effect on digestive enzyme activities of duodenum content. Markedly increased activities of digestive enzyme including protease, chymotrypsin, trypsin and amylase were observed in the AFB_1-treated groups.

The literature about the effect of AFB_1 on digestive enzyme activities is scarce. The present results demonstrated a tendency for an increase in the specific activity of digestive enzyme in the duodenum content of ducks receiving AFB_1-contamined feed. The possible reason may be ascribed to increased proenzyme released from injured pancreas caused by AFB_1. However, the increase of these digestive enzymes is abnormal and pathologic and the digestion of nutrients in the intestine did not enhanced, as shown the present results. When acute or chronic pancreatitis happens, proenzyme will be greatly released from animal pancreas cells. This will cause the increased activities of digestive enzyme of intestinal tract (Largmani, 1990).

Above all, the present study indicated that AFB_1 of feed could decrease duck growth performance and apparent digestibility of nutrients, change digestive enzyme activities of duodenum contents and cause pathologic change of some organs. The reduction of body weight gain may be related to decreased digestibility

Table 6
Effects of aflatoxin B_1(AFB_1)on digestive enzyme activities of duodenum contents of ducks

Item	Control	Group II	Group III
Protease (U/g)	6.74±0.13	8.06±0.19*	8.72±0.17*
Chymotrypsin (U/g)	5.35±0.14	6.22±0.15*	6.63±0.15*
Trypsin (U/g)	4.22±0.18	5.29±0.16*	5.84±0.17*
Amylase (U/g)	496.1±14.8	521.9±15.7	544.5±15.3*

Data are expressed as mean±SE of 18 ducks each treatment.
*Significantly different ($P<0.05$) when compared to control values.
Control (Group I) was fed conventional feed free of AFB_1, group II or III was fed the diets containing about 20 μg/kg or 40 μg/kg AFB_1-contaminated rice respectively.

of nutrients, while the increase of digestive enzyme activities is abnormal.

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