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Effect of Low Levels of Aflatoxin B1 on Performance, Serum Biochemistry, Hepatocyte Apoptosis and Liver Histopathological Changes of Cherry Valley Ducks

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Abstract: This study was conducted to investigate the effects of feeding corns naturally contaminated with AflatoxinB1 (AFB1) on performance, serum biochemistry, liver histopathological changes and percentage of hepatocyte apoptosis of Cherry Valley Ducks. Sixty, 1 day old ducks randomly divide into groups and were fed corn-soybean meal uncontaminated or contaminated AFB1. AFB1-contaminated diet significantly decreased the Body Weight (BW) gain, feed intake and feed conversion rate ($p<0.05$). The concentration of serum protein and the activity antioxidant enzyme were significantly decreased ($p<0.05$), the activity of serum biochemical enzyme and hepatocyte apoptosis were significantly increased ($p<0.05$) in AFB1-contaminated group. From histological study, liver tissue of ducks receiving AFB1-contaminated diets had markedly bile duct hyperplasia and vacuolar degeneration.

Key words: AFB1, performance, serum biochemistry, hepatocyte apoptosis, ducks

INTRODUCTION

Aflatoxins are secondary metabolites of molds that have adverse effects on humans, animals and crops that result in illnesses and economic losses. The worldwide contamination of foods and feeds with aflatoxins is a significant problem (Zain, 2011). Poultry, especially turkeys are extremely sensitive to the toxic effects of AFB1 (Carnaghan *et al*., 1966). A study examining the effects of AFB1 on the development of liver lesions in poultry, it has been reported ducks was more susceptible than turkeys and chickens (Coker, 1979). In study, ducks developed hepatic lesions by dietary exposure to 30 μg kg^{-1}, turkeys at 300 μg kg^{-1} while chickens responded to 500 μg kg^{-1}. AFB1 of feed could decrease growth performance and apparent digestibility of nutrients, change digestive enzyme activities of duodenum contents in duck. 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; Meissonnier *et al*., 2008).

Liver injury is quite common in human disease and a certain degree of hepatocyte apoptosis is characteristic of a healthy liver. Various liver diseases are associated with inordinate increase or decrease in hepatocyte apoptosis which may induce cell death (Malhi *et al*., 2006). Apoptosis is a specialized process of cell death that is part of the normal development of organs and tissue maintenance but may also occur as a response to various environmental stimuli indicating toxicity. Since, apoptosis can play a critical role in the development of cancer, the ability of toxins to induce apoptosis appears to be related to their toxicological effects (Dragan *et al*., 2001).

Recently, it is accepted that oxidative stress is an apoptosis inducer. One of manifestations of AFB1-induced toxicity is oxidative stress (Souza *et al*., 1999; Chandra *et al*., 2000). There may be some relationship between apoptosis and oxidative stress. However, hepatocyte apoptosis caused by AFB1 are limited to ducks.

The aim of this study was to investigate the adverse efficacy of AFB1 in growing of Cherry Valley Ducks by observing their effects on growth performance, serum biochemistry, liver histopathological changes and percentage of hepatocyte apoptosis of Cherry Valley Ducks.

MATERIALS AND METHODS

Experimental birds and diets

AFB1 production: *Aspergillus flavus* (CICC2219), purchased from China Center of Industrial Culture

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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 corns for 1 week to allow AFB1 production. The AFB1 levels in the corn powder were measured by HPLC as described previously (Hwang and Lee, 2006). The dried AFB1-contaminated corns were incorporated into the basal diet to provide the desired level of 50 μg AFB1/kg of diet.

Animal and experimental design: Sixty, 1 day old Cherry Valley Ducks (50±1 g) were randomly divided into two treatment groups (thirty in each group) fed at the animal research center of the Sichuan Agriculture University. Ducks were initially maintained at 32°C and the temperature was gradually reduced by 3°C per week to reach a temperature of 21°C by the end of week 3. This temperature was maintained for the duration of the experiment. Ducks were fed corn and soybean meal-based dietary (Table 1) treatments for 14 days (days 8-21). The control diet was formulated to meet the minimum nutrient requirements of turkeys according to the NRC. The basal diet contained 6 μg of AFB1/kg of diet as determined by the techniques described. The experimental diets for each treatment were as follows: treatment 1: basal diet; treatment 2: AFB1-contaminated diet (50 μg kg^{-1}). Broilers were monitored daily for signs of morbidity and mortality.

Table 1: Composition and proximate analyses of the basal diet

Items	Diet (0-3 weeks)
Ingredient	
Yellow corn[1]	57.20
Soybean oil meal	28.00
Wheat middling and red dog	4.00
Meat and bone meal	5.00
Vegetable oil	2.00
Monocalcium phosphate	1.70
Oyster shell	0.70
dl-Methionine	0.15
Lysine	0.08
NaCl	0.20
Vitamin and mineral mix[2]	1.00
Total	100.00
Proximate composition of the test diets	
CP	214.00
Crude fat	50.00
Cruder fiber	25.00
Calcium	9.50
Methionine	5.00
Tryptophan	2.30
ME (kcal kg^{-1})	3100.00

[1]AFB-unonminated corn was replaced by AFB-contaminated rice according to the proportion in experimental diets. [2]Vitamin and mineral mix provided the following (mg/kg of feed): vitamin A: 10×10^6 IU; vitamin D3: 3×10^6 IU; vitamin K: 33 g, vitamin B1: 1 mg; vitamin B2: 2.5 mg; vitamin B6: 2.5 mg; vitamin B12: 0.0125 mg; folic acid: 0.25 mg; nicotinic acid: 25 mg; calcium pantothenate: 10 mg; biotin: 0.01 mg; choline chloride: 240 mg; manganese: 87.5 mg; iron: 60 mg; copper: 7.5 mg; zinc: 68.75 mg; I: 1.0 mg; Se: 0.2 mg and butylated hydroxytoluene, 0.312 mg

Pathological examination: When ducks reached 21 days old, the feeding trial was terminated and 10 ducks from each treatment was selected at random and killed by cervical dislocation. Selected animals were weighed before euthanasia. Then, blood samples were collected and centrifuged, the plasma was stored at -20°C until use. Biochemical determinations including Total Protein (TP), Albumin (ALB), serum glutamic-pyruvic transaminase (ALT), glutamic oxaloacetic transaminase enzymes (AST), Glutathione S-Transferase (GST), Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-PX) and Maleic Dialdehyde (MDA) from the serum for each group were measured with commercially available kit (Nanjing Jiancheng Biological engineering research) according to the manufacturer's recommended procedure.

Hepatocyte apoptosis: Hepatocyte apoptosis analysis was performed using a commercially available kit (Becton, Dickinson and Company) by following the manufacturer's recommendations. Briefly, a few drops of RPMI were added to tissue and then minced until complete tissue disaggregation was achieved. Suspended cells were filtered using a 50 μm pore size mesh and then centrifuged at 1000 rpm for 10 min. Cells were resuspended in PBS, counted and washed by calcium buffer then centrifuged at 1500 rpm for 5 min. Some cells were used to assess cell viability by trypan blue method. The pellet was resuspended and counted. Annexin-PI apoptotic assay was carried out using Annexin V-FITC kit (Becton, Dickinson and Company). FAC scan Becton-Dickinson (BD) flow-cytometer was used and data were analyzed using cell Quest Software.

Histopathology: After pathological examination, the same livers were fixed in 10% neutral buffered formalin. Fixed tissues were trimmed, embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin. Tissues sections from all birds were then examined microscopically.

Statistical analysis: Data for all response variables were reported as means±SE and subjected to one-way ANOVA. When significant differences were obtained, differences between means were determined by the SPSS 17.0.

RESULTS AND DISCUSSION

Growth performance: The effects of dietary AFB1 on growth performance are shown in Table 2. A significant effect was observed on BW gain and feed conversion rate about T2.

Table 2: Effects of AFB1 on growth performance

Treatments	Growth performance		
AFB1 (μg kg^{-1})	BW gain (g)	Feed intake (g)	Feed conversion rate
0	565.3^{a}	1011.9^{a}	1.79^{a}
50	410.2^{b}	865.5^{b}	2.11^{b}

Table 3: Effects of AFB1 on serum antioxidant enzyme

Treatments				
AFB1 (μg kg^{-1})	SOD (U ML^{-1})	GSH-Px (U ML^{-1})	MDA (nmol mL^{-1})	Percentage of hepatocyte apoptosis
0	35.67±0.77^{a}	121.71±18.13^{a}	6.46±0.49^{a}	6.63±0.39^{a}
50	29.46±1.33^{b}	103.10±12.02^{b}	12.38±0.45^{b}	9.97±0.65^{b}

^{a,b}Means within a column without a common superscripts differ statistically ($p<0.05$)

Compared with the control group, body weight gain was significantly reduced for the ducks fed AFB1-contaminated diet, the percentage reduction was 27.4% ($p<0.05$), respectively. The feed intake was decreased by 14.4% ($p<0.05$). There were higher feed conversion ratio for AFB1-treated ducks and it was increased by 15.0% ($p<0.05$), respectively.

Antioxidant enzyme activity and hepatocyte apoptosis assay: The effects of dietary AFB1 on serum antioxidant enzyme activity and hepatocyte apoptosis variables are shown in Table 3. The activity of serum SOD and GSH-Px decreased by 17.4 and 15.3%, compared with control group ($p<0.05$). The content of MDA was increased by 47.8% compared with control group ($p<0.05$). The percentage of hepatocyte apoptosis was increased by 50.4%, compared with control group ($p<0.05$). The hepatocyte apoptosis array spot diagram shown in Fig. 1.

Serum biochemical assay: The effects of AFB1-contaminated diet on serum biochemical variables are shown in Table 4. The content of TP and ALB were decreased by 30.9 and 23.1%, compared with control group ($p<0.05$). The activity of serum AST, ALT and GST were increased by 105.15, 198.3 and 54.0%, compared with control group ($p<0.05$).

Histopathological examination: Results of histological analysis showed that there was significant damage in the liver tissues of broilers receiving AFB1 alone (Fig. 2). Liver tissue from this treatment had vacuolar degeneration of hepatocytes, bile duct hyperplasia and hypertrophy compared with the tissue of birds fed on the uncontaminated diet.

The result indicate that AFB1 can significantly affect duck production. The effect of AFB1-contaminated diet on body weight gain, feed intake and feed conversion ratio of ducks after feeding for up to 3 weeks are showed in Table 2.

Table 4: Effects of AFB1 on serum biochemical enzyme

Treatments					
AF (μg kg^{-1})	TP (g L^{-1})	ALB (g L^{-1})	AST (U L^{-1})	ALT (U L^{-1})	GST (U ML^{-1})
0	28.67±2.15^{a}	13.02±1.01^{a}	7.47±2.15^{a}	15.91±2.38^{a}	13.97±1.67^{a}
50	19.81±3.94^{b}	10.03±1.60^{b}	15.32±1.98^{b}	32.05±2.75^{b}	21.51±1.55^{b}

^{a,b}Means within a column without a common superscripts differ statistically ($p<0.05$)

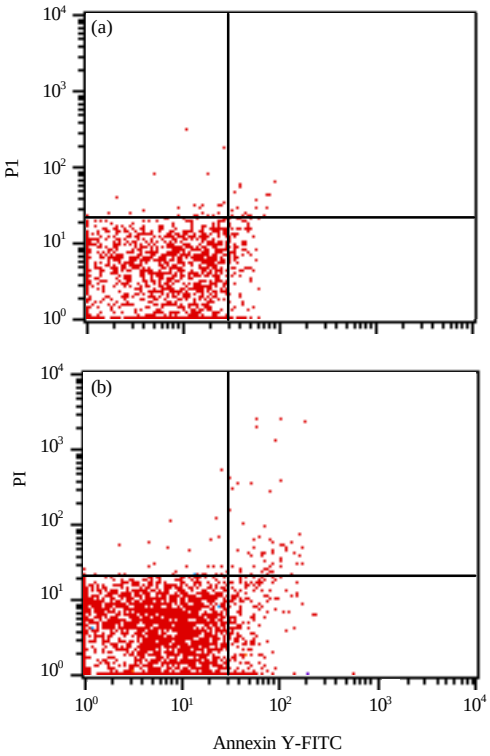

Fig. 1: Flow cytometry shows percentage of hepatocyte apoptosis; a) Control group and b) AFB1 group

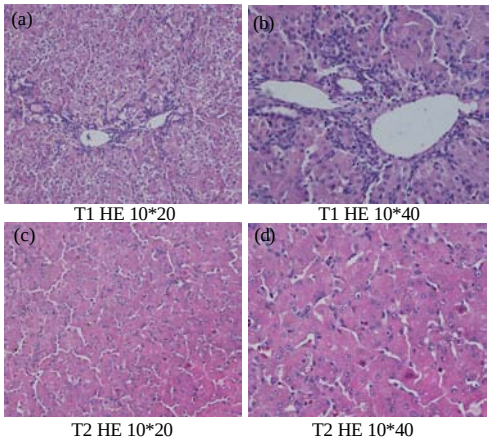

Fig. 2: Photomicrographs (optical microscopy) of hematoxylin and eosin-stained ducks liver sections

Consumption of the AFB1-contaminated diet (AFB1, 50 μg kg^{-1}) reduced feed intake, BW gain and caused poor feed conversion rate compared with the control diet (AFB1, 0 μg kg^{-1}). The toxic effects produced by AFB1 were in general agreement with previous studys found in chicks or ducks (Huff *et al*., 1992; Scheideler, 1993; Abo-Norag *et al*., 1995; Magnoli *et al* ., 2011). The adverse effects of AFB1 on growth performance have been related with a decrease in the protein (Denli *et al*., 2009), probably as a consequence of a deterioration of the digestive and metabolic efficiency of the birds (Han *et al*., 2008). In the present study, researchers observed significant reduction in the concentration of serum TP and ALB which could be attributed to differences on the levels of AFB1 in the diets.

The activity of serum antioxidant enzyme significantly the AFB1-treated groups as shown in Table 4. AFB1 mediated cell injury may be due to the release of free radicals which initiate lipid peroxidation (Jodynis-Liebert *et al*., 2006). Peroxidative damages induced oxidative stress and decrease the antioxidant capacity (Shi *et al*., 2012). In present study, researchers found the hepatocyte apoptosis rate significantly increased in the AFB1-treated groups. So, the results indicate that AFB1 can cause oxidative stress which may induce hepatocyte apoptosis.

Serum enzymes significantly increased were observed in the AFB1-treated groups. As shown in Table 3, a marked increase was found in serum ALT, AST and GST activities of AFB1-treated groups. Serum enzymes were used as the biochemical indicators for hepatic damage. This is because these enzymes are localized in hepatocytes, the serum activities presumably increase as a result of cellular membrane damage and leakage (Kaplan, 1993).

In the present study, serum ALT, AST and GST activity significantly increased, indicating a release of these enzymes from the liver injured by AFB1 treatment, the pathologic change of liver may relation to the change of serum biochemistry enzyme. These results were also found in chickens as reported by other studies (Matur *et al*., 2010; Denli *et al*., 2009). Above all, the present study indicated that AFB1-contaminated diet decreased the growth performance of ducks. The oxidative stress may induce hepatocyte apoptosis. The pathologic change of liver can explain the change of serum biochemistry enzyme.

CONCLUSION

The results showed that Cherry Valley ducks performance, the activity of antioxidant enzyme, serum biochemical enzyme and percentage of hepatocyte apoptosis were adversely affected by feeding borne AFB1.

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