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RESEARCH ARTICLE

# Effects of aflatoxin-detoxifizyme on growth performance and liver biochemical indices of broilers fed with aflatoxin $B_1$

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**Abstract** **Aflatoxin-detoxifizyme (ADTZ) was evaluated for its ability to reduce the deleterious effects of aflatoxin $B_1$ ($AFB_1$) in broiler diets. A total of 624 one-day-old AA broilers were randomly divided into 3 treatment groups with 8 replicates each. The control, $AFB_1$ and ADTZ treatments were fed with basal diet, basal diet + 0.1 mg/kg $AFB_1$ and basal diet + 0.1 mg/kg $AFB_1$ + 0.3% ADTZ, respectively. The trials lasted 42 days. The results showed that: (1) $AFB_1$ diminished the growth performance of broilers significantly ($P < 0.01$). (2) The $AFB_1$ supplement caused significant alteration of related liver enzyme activities and significantly decreased the body's antioxidant capacity. (3) Related parameters were returned to normal levels by a 0.3% ADTZ addition to the $AFB_1$-contaminated diet. All the results in the trials indicate that ADTZ has the ability to detoxify $AFB_1$ and recover the negative effects of $AFB_1$.**

**Keywords** aflatoxin-detoxifizyme (ADTZ), aflatoxin $B_1$($AFB_1$), broilers, growth performance, liver biochemical indices

## Introduction

It is reported that as much as 25% of crop products in the world are mildewed annually, completely losing about 2%–3% of food (fed) values (Richard, 1989). The aflatoxin $B_1$ ($AFB_1$) is very toxic and exists in a wide range of mycotoxins. Its high toxicity to both animals and humans makes $AFB_1$ the most dangerous mycotoxin (Wilson et al., 1994). Liver is the target organ of $AFB_1$, long-term intake of diets containing $AFB_1$ can cause a reduction of broiler growth performance and antiviral ability, liver dysfunction, and even cancer (Quezada et al., 2000). In the feed industry, the most common method to control $AFB_1$ is the application of nonnutritive adsorptive materials so as to reduce the concentration of mycotoxin in the gastrointestinal tract of animals. But these adsorbents may also adsorb other feed nutrients, resulting in the decrease in their nutritional value. Reports show that hydrated aluminosilicate and yeast cell wall polysaccharide have non-selective adsorption to vitamin C and vitamin E, in which hydrated aluminosilicate has stronger adsorption to both of them (Chen et al., 2008). Therefore, more advanced and efficient methods of detoxifying $AFB_1$-containing feedstuffs are in great demand. Aflatoxin-detoxifizyme (ADTZ) is a protease from edible fungus *A. tabescens*, it can specifically act on the 8, 9 double bond, the toxic part of $AFB_1$, to open the ring structure and degrade $AFB_1$ into a nontoxic substance. This study was conducted to examine the influence of $AFB_1$ on the growth performance and related liver biochemical indices of broilers and the antitoxic effect of ADTZ.

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## Materials and methods

### Chemicals

Aflatoxin-detoxifizyme (7000 U/g) was provided by Guangzhou Co-win Bioengineering Co., Ltd.

$AFB_1$ (purity > 99%) was purchased from Beijing Rapidbio Co., Ltd.

### Animals and experimental design

624 one-day-old AA broilers were randomly divided into 3 treatment groups with 8 replicates each. The birds were given 24 h illumination and access to the assigned diet and water ad

libitum. Trials were conducted in two phases: 1–21 d and 21–42 d. All birds were assigned to one of the three treatments: the control, $AFB_1$ and ADTZ treatment, which were fed with basal diet (Table 1), basal diet + 0.1 mg/kg $AFB_1$ and basal diet + 0.1 mg/kg $AFB_1$ + 0.3% ADTZ, respectively.

**Table 1** Composition and nutrient level of basal diet

| Item | 1–21 d | 21–42 d |
|---|---|---|
| Ingredient | | |
| Corn | 54.05 | 59.29 |
| Soybean meal | 37.11 | 32.99 |
| Soybean oil | 2.65 | 2.72 |
| Fish meal | 2.00 | 1.00 |
| Methionine | 0.13 | 0.04 |
| Lysine | 0.00 | 0.01 |
| Dicalcium phosphate | 1.37 | 1.42 |
| Limestone | 1.32 | 1.16 |
| Salt | 0.37 | 0.37 |
| Premix[a)] | 1.00 | 1.00 |
| Total | 100 | 100 |
| Nutrient levels | | |
| ME (MJ /kg) | 12.12 | 12.33 |
| CP | 22 | 20 |
| Ca | 1.00 | 0.90 |
| AP | 0.46 | 0.43 |
| Lys | 1.13 | 1.00 |
| Met + Cys | 0.86 | 0.72 |

[a)] means premix per kg diet for the period from 1 to 21d: consists of 10000 IU vitamin A, 1000 IU vitamin D3, 20 IU vitamin E, 0.5 mg vitamin $K_3$, 2.0 mg vitamin $B_1$, 8.0 mg vitamin $B_2$, 10.0 mg pantothenic acid, 35.0 mg nicotinic acid, 3.5 mg vitamin $B_6$, 0.05 mg biotin, 0.55 mg folic acid, 0.01 mg vitamin $B_{12}$, 1300 mg choline, 4 mg flavomycin, 40 mg salinomycin, 100 mg Fe, 8.0 mg Cu, 100 mg Zn, 120 mg Mn, 0.7 mg I, 0.3 mg Se, and that from 21 to 42 d consists of 8000 IU vitamin A, 750 IU vitamin D3, 15 IU vitamin E, 0.5 mg vitamin $K_3$, 2.0 mg vitamin $b_1$, 5.0 mg vitamin $B_2$, 10.0 mg pantothenic acid, 35.0 mg nicotinic acid, 3.5 mg vitamin $B_6$, 0.05 mg biotin, 0.55 mg folic acid, 0.01 mg vitamin $B_{12}$, 1000 mg choline, 4 mg flavomycin, 40 mg salinomycin, 80 mg Fe, 8.0 mg Cu, 80 mg Zn, 100 mg Mn, 0.7 mg I, 0.3 mg Se.

### Collection of hepatic samples

At the end of this trial, 12 birds were randomly selected from each treatment, and then killed to obtain liver samples for subsequent determination of enzymes activities. All samples were flash frozen by liquid nitrogen and stored at −70°C.

### Measurements of growth performance and liver biochemical indices

On the 1st, 21st and 42nd day of the experiment, birds were weighed by replicates for the measurement of growth performance. Average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (F/G) were also measured.

Hepatic metabolic enzyme activities of succinic dehydrogenase (SDH), choline esterase (CHE), alkaline phosphatase (AKP) activities in the liver were measured using a 752 spectrophotometer, with kits purchased from Nanjing Jiancheng Bioengineering Institute, China.

Hepatic antioxidant indices were determined by measuring superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione peroxidase (GSH-Px) activity, and glutathione reductase (GR) activity, using the methods of xanthine oxidase, ammonium molybdate, dithio-bis-nitrobenzoic acid, and colorimetry, respectively. The concentration of malonaldehyde (MDA) in the liver was measured using the method of barbituric acid by a 752 spectrophotometer and kits purchased from Nanjing Jiancheng Bioengineering Institute, China.

### Data statistical analysis

One-way ANOVA was performed using the GLM procedure of the SPSS 13.0 software. Significant differences among treatment means were determined at $P < 0.05$ by the LSD multiple comparison test. Values were expressed as the mean ±SD.

## Results

### Growth performance

The growth performance of broilers was significantly affected by the 0.1 mg/kg $AFB_1$-containing diet (Table 2). In the first phase (from 1 to 21d) of the experiment, the ADG and ADFI of the $AFB_1$ treatment were decreased by 3.45% ($P < 0.05$) and 0.74% ($P > 0.05$), respectively, compared with the control; however, the F/G was increased by 2.72% ($P > 0.05$). The ADG and ADFI of the ADTZ treatment were raised by 3.47% ($P < 0.05$) and 2.77% ($P < 0.05$), respectively, compared with the $AFB_1$ treatment; however, the F/G was decreased by 0.71% ($P = 0.615$). In the second phase (from 21 to 42 d) of the experiment, the ADG and ADFI of the $AFB_1$ treatments were decreased by 5.62% ($P < 0.05$) and 0.88% ($P > 0.05$), respectively, compared with the control, but the F/G was increased by 4.93% ($P < 0.05$). The ADG and ADFI of the ADTZ treatment were raised by 5.58% ($P < 0.05$) and 3.40% ($P < 0.05$), respectively, compared with the $AFB_1$ treatment, but the F/G was decreased by 2.05% ($P > 0.1$). In the entire experiment (from 1 to 42 d), compared with control, the ADG and ADFI of the $AFB_1$ treatment were decreased by 5.09% ($P < 0.05$) and 0.85% ($P > 0.05$), respectively, and the F/G was increased by 4.42% ($P < 0.05$). As compared with $AFB_1$ treatment, the ADG and ADFI of the ADTZ treatment were raised by 5.06% ($P < 0.05$) and 3.27% ($P < 0.05$), respectively, and the F/G was decreased by 1.7% ($P > 0.1$).

Compared with the control, the ADFI of the ADTZ treatment in the second phase and the entire treatment course was raised significantly, and the feed efficiency of the ADTZ treatment did not reach the same level of the control.

**Table 2** Effects of ADTZ and $AFB_1$ on broiler performance

| Periods | Items | Control | $AFB_1$ | ADTZ |
|---|---|---|---|---|
| First phase (1–21 d) | ADG/g | 22.88±0.95[a] | 22.09±0.55[b] | 22.85±0.30[a] |
| | ADFI/g | 35.22±0.77[ab] | 34.95±0.61[b] | 35.92±0.65[a] |
| | F/G | 1.541±0.061 | 1.583±0.037 | 1.572±0.027 |
| Second phase (21–42 d) | ADG/g | 70.37±2.95[a] | 66.41±1.98[b] | 70.12±2.37[a] |
| | ADFI /g | 132.27±2.90[b] | 131.11±2.73[b] | 135.57±3.76[a] |
| | F/G | 1.882±0.074[a] | 1.975±0.031[b] | 1.934±0.051[ab] |
| Whole course of the experiment (1–42 d) | ADG/g | 46.62±1.49[a] | 44.25±1.03[b] | 46.49±1.17[a] |
| | ADFI/g | 83.74±1.49[b] | 83.03±1.26[b] | 85.74±1.79[a] |
| | F/G | 1.797±0.055[a] | 1.877±0.027[b] | 1.845±0.037[b] |

In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$). The same for Tables 3 and 4.

### Hepatic metabolic enzyme activities

The changes in the hepatic metabolic enzyme activities are shown in Table 3. Compared with the control, the SDH and CHE activities of the $AFB_1$ treatment were decreased by 14.52% ($P < 0.05$) and 12.56% ($P = 0.072$), respectively, whereas the AKP activity was increased by 13.57% ($P = 0.065$). In the ADTZ treatment, SDH and CHE activities were raised by 17.98% ($P < 0.05$) and 14.83% ($P = 0.06$), and AKP activity was decreased by 12.68% ($P = 0.052$), compared with that of the $AFB_1$ treatment, respectively.

The hepatic metabolic enzyme activities showed no significant difference between the ADTZ treatment and the control.

### Hepatic anti-oxidation

The antioxidant indices in liver are shown in Table 4. A significant effect of 0.1 mg/kg $AFB_1$ on the hepatic anti-oxidation of broilers was observed. In the liver, the GSH-Px, SOD, GR and CAT activities of the $AFB_1$ treatment were decreased by 10.14% ($P < 0.05$), 5.5% ($P < 0.05$), 12.08% ($P = 0.071$) and 10.07% ($P > 0.1$), respectively, compared with control, but the MDA content there was increased by 9.22% ($P = 0.061$). In the ADTZ treatment, SOD activity was raised by 5.58% ($P = 0.056$), and the GSH-Px, GR and CAT activities were raised by 8.29% ($P > 0.1$), 10.48% ($P > 0.1$) and 10.08% ($P > 0.1$), respectively, whereas MDA content in liver was decreased by 7.80% ($P = 0.082$), compared with that of the $AFB_1$ treatment.

As compared with the control, no significant difference was found in the hepatic antioxidant indices of the ADTZ treatment.

## Discussion

$AFB_1$ is a highly toxic substance and chronic poisoning caused by long-term ingestion has become most common in livestock and poultry production, resulting in the reduction of animal growth performance including feed efficiency, litter size, poultry egg production and hatching rate (Lindemann et al., 1993; Verma et al., 2002). In this trial, results showed that the performance of broilers was significantly reduced by $AFB_1$, in which ADG and ADFI were significantly decreased, while F/G was significantly increased. This result is consistent with that of Hou et al. (2008a), who reported that feeding $AFB_1$-contained diets led to a significant decrease in BW, ADG, and ADFI ($P < 0.05$) and an increase in F/G.

**Table 3** Effects of ADTZ and $AFB_1$ on hepatic metabolic enzyme activities

| Index | Control | $AFB_1$ | ADTZ |
|---|---|---|---|
| SDH (U/mg prot) | 3.49±0.61[a] | 2.99±0.58[b] | 3.52±0.45[a] |
| CHE (U/mg prot) | 58.17±10.79 | 50.75±9.67 | 58.50±8.72 |
| AKP (U/mg prot) | 879.33±140.90 | 998.67±137.14 | 872.25±178.56 |

U/mg prot means activity unit in a milligram protein. The same for Table 4.

**Table 4** Effects of ADTZ and $AFB_1$ on hepatic antioxidant indices

| Index | Control | $AFB_1$ | ADTZ |
|---|---|---|---|
| GSH-Px (U/mg prot) | 17.39±1.86[a] | 15.63±2.27[b] | 16.92±1.84[ab] |
| CAT (U/mg prot) | 9.67±1.28 | 8.70±1.04 | 9.57±1.92 |
| SOD (U/mg prot) | 30.92±1.70[a] | 29.22±2.19[b] | 30.85±2.14[ab] |
| GR (U/mg prot) | 7.85±1.29 | 6.90±1.07 | 7.63±1.35 |
| MDA (nmol/mg prot) | 0.641±0.076 | 0.701±0.077 | 0.646±0.072 |

nmol/mg prot means nmol in milligram protein.

In the ADTZ treatment, ADG and ADFI were significantly raised, while F/G decreased compared with the $AFB_1$ treatment. Broiler growth performance indices were basically returned to normal levels, but the feed efficiency did not reach the control's. It indicates that ADTZ can diminish the inhibitive effects of $AFB_1$ on broiler growth performance by decomposing $AFB_1$.

Liver is the target organ of $AFB_1$, aflatoxicosis can cause abnormal liver enzyme activities. SDH, as the first enzyme of the mitochondrial respiratory chain, is also the key enzyme of the citric acid cycle. The changes in SDH activity could indicate whether the liver function is normal or not. CHE is a sensitive parameter, reflecting the synthesis of liver cells and positive correlation between its decrease of activity and liver damage (Long et al., 2007). AKP are a group of enzymes that can hydrolyze phosphate ester under alkaline conditions, and it is widespread in tissues and body fluids. AKP activity may abnormally increase because of hepatobiliary disease. Hou et al. (2008b) reported that SDH and CHE activities in broiler liver were significantly decreased, whereas AKP activity was significantly increased after the ingestion of $AFB_1$. We found similar results, wherein compared with the control, liver SDH activity was significantly decreased and CHE activity decreased, whereas AKP activity increased in the $AFB_1$ treatment. After adding ADTZ, SDH and CHE activities were significantly raised, whereas AKP activity decreased, compared with the $AFB_1$ treatment, and there was no significant difference between them and the control. All data indicate that $AFB_1$ can damage the liver, thereby affect liver function, however, the damages can be reduced by adding ADTZ.

There is an antioxidant system in aerobiont cells, mainly including SOD, CAT, GSH-Px, GR, etc., which can be induced and synthesized by the increasing number of free radical compounds. Their content and activities affect the levels of active oxygen radicals *in vivo* and also the concentration of MDA as the terminal metabolites of lipid peroxidation (Wills, 1966). The decline of these series of enzyme activities suggests the reduction of free radicals' scavenging ability in the liver. Shi et al. (2007) reported that with the addition of 0.1 mg/kg $AFB_1$ into growing-finishing pig diets lead to a significant reduction of liver antioxidant capacity, GSH-Px, GR, and CAT activities were significantly decreased. The results in this study also showed a significant reduction in liver antioxidant capacity in those birds fed with the $AFB_1$ diet, with significant decreases in the GSH-Px and SOD activities, with GR and CAT activities also decreased, whereas the content of MDA in the liver was increased. The above-mentioned liver antioxidant indices were basically returned to normal levels without significant differences between them and the control by the addition of 0.3% ADTZ. It indicates that $AFB_1$ can cause a significant decline in liver antioxidant indices and ADTZ has the ability to recover this negative effect of $AFB_1$.

Overall, the results demonstrate that exposing broilers to 0.1 mg/kg $AFB_1$ in their diet is associated with a number of negative effects manifested by reductions in ADG and ADFI and a change in several liver parameters. Moreover, we found that ADTZ is safe for broilers and its use to detoxify $AFB_1$-contaminated feed is possible because it can degrade $AFB_1$ into a nontoxic substance and helps birds recover from the negative effects of $AFB_1$.

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