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Detoxification of aflatoxin B_1 by lactic acid bacteria and hydrated sodium calcium aluminosilicate in broiler chickens

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ABSTRACT

Aflatoxin contamination is very common in feedstuffs across the world and finding an ideal detoxifier is urgent because of the toxic action on animals and negative effects on foods, humans, and the environment. To thoroughly eliminate the toxin, a detoxification method has changed from physical to biological. The objective of the present study was to investigate the effect of lactic acid bacteria (LAB) and hydrated sodium calcium aluminosilicate (HSCAS) on detoxification of aflatoxin B_1 (AFB_1) by assessing growth performance, digestibility, immune function, and AFB_1 residues in tissues and excreta of broiler chickens from d 0 to 21. A total of 480 female broiler chicks on d 0 were randomly allotted to 4 treatments with 6 cages of 20 chicks each for diets: positive control (PC, undetectable AFB_1), negative control (NC, PC + 40 μg AFB_1/kg), LAB (NC + 1.5×10^{10} cfu LAB/kg), and HSCAS (NC + 3.0 g HSCAS/kg). Results showed that the NC treatment reduced ($P < 0.05$) average daily gain and feed efficiency, and LAB or HSCAS supplementation improved ($P < 0.05$) the growth performance of broiler chickens, and the effect of LAB was greater than HSCAS. The LAB and HSCAS increased ($P < 0.05$) the digestibility of dry matter, crude protein, crude fat, and digestible energy by 4.0–15.0%, and improved ($P < 0.05$) immune function by modulating the relative weights of immune organs, lymphocyte percentages, and immunoglobulin contents. Additionally, residual AFB_1 in serum and organs in LAB treatment was lower ($P < 0.05$) than HSCAS. The results indicate that LAB and HSCAS can detoxify AFB_1 in the diet of broiler chickens, and LAB is more effective than HSCAS in partial biodegradation of AFB_1.

1. Introduction

Aflatoxins, produced by *Aspergillus* species of fungi, are frequently found in feedstuffs or foodstuffs. The most toxic component is aflatoxin B_1 (AFB_1), which can directly depress growth and health, induce hepatic disease and metabolic disorder in animals, and result in toxic residues in animal tissues and products (Nesbitt et al., 1962; Oguz et al., 2003). Furthermore, foods contaminated with AFB_1 have a high carcinogenic potency for humans (Abbès et al., 2016; Bedard and Massey, 2006). From the food safety's perspective, developing more productive methods to counteract the toxicity of AFB_1 have recently become a focus in animal nutrition. Clay adsorbents with a high affinity to toxins, such as hydrated sodium calcium aluminosilicate (HSCAS), are widely applied in the feed industry. Nevertheless, these adsorbents have certain limitations in terms of nutrient loss and AFB_1 adsorbed in feces, causing the secondary pollution to the environment (Andretta et al., 2011).

The toxicity of AFB_1 depends on a toxic functional group in its molecular structure, while, in this instance, the binding or decomposing AFB_1 into non-toxic metabolites will completely remove the toxicity of AFB_1 contamination. With the deeper understanding of AFB_1, microbial technology is desirable to biodegrade the toxic group into none or low toxic metabolites. Lactic acid bacteria (LAB), given its beneficial function in food fermentation and gut microflora, has been applied extensively in humans and animals. Recently, it has been found that the LAB can be a natural biological antagonist against mycotoxigenic molds and their highly toxic metabolites (Tsai et al., 2012).

El-nezami et al. (1998) observed that selected LAB from dairy removed approximately 80% of AFB_1 in liquid media. Roger et al. (2015) reported that LAB effectively inhibited the growth of *Aspergillus flavus* and degraded AFB_1 by 44–64% in fermented maize-based doughs. Also, 4 LAB strains isolated from Iranian poultry showed detoxification potentials of 19–75% for AFB_1 (Kasmani et al., 2012). *Lactobacillus plantarum* (Jebali et al., 2015) and *Lactobacillus paracasei* (Abbès et al., 2016) could effectively mitigate aflatoxins immunotoxicities in mice, and *Lactobacillus rhamnosus* (El-Nezami et al., 2000) could decrease AFB_1 concentration by 44–54% using a chicken intestinal loop technique.

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However, in farm animals, the information for the effect of LAB on detoxification of AFB_1 is very limited, especially compared with the traditional detoxifier, clay. Therefore, the objective of the present study was to investigate the effect of LAB and HSCAS on detoxification of AFB_1 by measuring growth performance, digestibility, immune function, and toxic residues in tissues and excreta.

2. Materials and methods

2.1. Lactic acid bacteria strains, aflatoxin B_1, and diets

Three LAB strains, authorized as feed additives by China Ministry of Agriculture (Version 2045-2013; Beijing, China), were obtained from Hongxiang Biological Feed Laboratory at Henan University of Science and Technology (Luoyang, China). The LAB strains consisted of *Lactobacillus acidophilus* (ACCC11073), *Lactobacillus plantarum* (CICC21863) and *Enterococcus faecium* (CICC20430), and were mixed equally and added at 1.5×10^{10} colony-forming units (cfu) per kilogram of feed.

The AFB_1 was produced using *Aspergillus flavus* from the China General Microbiological Culture Collection Center (Beijing, China). A total of 20.0 kg corn meal (mesh size, 2.00 mm) was placed in a 100 L container, with 10.0 L distilled water, and then autoclaved. The medium was inoculated with 500 mL *Aspergillus flavus* and incubated at 28 ± 1 ℃ for 7 d. The incubated corn meal was autoclaved to kill *Aspergillus flavus*, dried and ground (mesh size, 0.425 mm) for the animal feeding experiments. The AFB_1 concentrations in the moldy corn meal were estimated to be 4125 μg/kg.

The nutritive levels of positive diet (PC) were recommended by China Agricultural Standard (Version NY/T 33–2004) published by Ministry of Agriculture of China (Beijing, China), and the water contents of all ingredients and diets were controlled under 12% and stored in a cool, dry, dark, and well-ventilated place. Based on the PC diet, the negative diet (NC) was formulated using AFB_1 in the expense of corn meal, and the concentration of AFB_1 was 40 μg/kg of feed. Based on the NC diet, the LAB or HSCAS (Kemin Industries, Zhuhai, China) were added at 1.5×10^{10} cfu/kg or 3.0 g/kg, respectively. All diets were fed as mash on air-dried basis. No antibiotics were offered to broiler chickens via either feed or water throughout the experiment. Titanium dioxide was used as an external marker to determine apparent total tract digestibility of nutrients. The composition of the PC diet is presented in Table 1.

Table 1
Ingredients and nutritive values of positive control diet (as-fed basis).

Item	Content
Ingredient	
Corn (%)	54.60
Soybean meal (%)	25.70
Corn gluten meal (%)	6.00
Corn DDGS[1] (%)	6.00
Soybean oil (%)	2.50
Lys (%)	0.25
Met (%)	0.15
Salt (%)	0.40
Dicalcium phosphate (%)	2.10
Limestone (%)	1.15
Titanium dioxide (%)	0.50
Choline chloride (%)	0.15
Premix[2] (%)	0.50
Calculated composition[3]	
Metabolic energy (MJ/kg)	12.41
Crude fiber (%)	2.85
Non-phytate P (%)	0.51
Met (%)	0.51
Met + Cys (%)	0.86
Lys (%)	1.18
Analyzed content	
Crude protein (%)	21.48
Crude fat (%)	3.33
Ca (%)	0.99
Total P (%)	0.73

[1] DDGS, distillers dried grains with solubles.
[2] Provided per kilogram diet: vitamin A (retinyl acetate), 8000 IU; cholecalciferol, 1000 IU. vitamin E (DL-tocopheryl acetate), 20 IU; vitamin K, 0.5 mg; thiamin, 2.0 mg; riboflavin, 8.0 mg; d-pantothenic acid, 10 mg; niacin, 35 mg; pyridoxine, 3.5 mg; biotin, 0.18 mg; folic acid, 0.55 mg; vitamin B_{12}, 0.010 mg; Mn, 120 mg; I, 0.70 mg; Fe, 100 mg; Cu, 8 mg; Zn, 100 mg; and Se, 0.30 mg.
[3] Based on Chinese Feed Database (2010; Ministry of Agriculture of China, Beijing, China).

2.2. Broiler chickens and samples

All the experimental procedures were approved by the Animal Ethics Committee of the Henan University of Science and Technology. A total of 480 one-day-old female broiler chicks (Cobb 500; Jinyu Poultry Company, Luoyang, China) were randomly assigned to 4 treatments with 6 cages pretreatment and 20 broiler chickens per cage. All chicks were reared in 3-layer cages and given ad libitum access to diets and water throughout the experiment. The room temperature was maintained at 34 ℃ during the first 5 d and then gradually decreased to 22 ℃ on d 21–42. Broiler chickens were received continuous light for the first 24 h, and were then maintained under 16 h light and 8 h dark for the remainder of the study. Broiler chickens and feeds in each cage were weighed weekly and feed efficiency was adjusted for mortality on a cage basis. All the broiler chickens were monitored for general health at least twice a day.

During d 19 to 21, the total amount of excreta per replicate was collected and mixed, of which 10% was freeze-dried for the determination of digestibility and AFB_1 content. Care was taken during the collection of excreta samples to avoid contamination from feathers and other foreign materials. On d 21 of the experiment, 6 broiler chickens per replicate were randomly selected and killed by CO_2 suffocation, and then were dissected. Blood was immediately drawn out from the heart with a syringe and aliquoted into sterile vials. One aliquot used liquid sodium heparin (1000 IU/mL) as an anticoagulant to obtain the whole blood for lymphocyte tests, and another was allowed to clot for 4 h for serum preparation (Liu et al., 2008). After centrifugation for 10 min at $3{,}000 \times g$ at 4 ℃, the serum was aliquoted into 1-mL vials and stored at −20 ℃ for measurements. After blood collection, liver, spleen, kidney, bursa of Fabricius and thymus gland were removed, weighed, and kept in a −20 ℃ freezer until AFB_1 analysis.

2.3. Chemical and biological analysis

The nutritional components in the samples were determined according to Feed Analysis and Quality Test Technology by Zhang (2016). Gross energy was measured in an oxygen bomb calorimeter (Model 6300; Parr Instrument Co., Moline, IL, US). Dry matter was determined by drying a 2-g sample at 65 ℃ to a constant mass. The concentrations of titanium dioxide in the samples were determined using the method by Short et al. (1996).

The concentrations of AFB_1 in feed, organs, serum, and excreta were detected using an enzyme-linked immunosorbent assay kit (detection limit 2 μg/kg; Longke Fangzhou Biotech, Beijing, China). The percentages of T and B lymphocytes in peripheral blood were quantified by using the methods of erythrocyte-rosette test and erythrocyte antibody rosette test, respectively (Brain et al., 1970; Liu et al., 2008). Serum immunoglobulin (Ig) A, G, and M concentrations were measured using chicken-specific ELISA reagents according to the instructions of the