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Effect of Phyllosilicates as Toxin Binder on Productivity, Intestinal Morphology, and Liver Toxicity in Broiler Fed AFB_1 Contaminated Feed

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ABSTRACT

Aflatoxin B_1 is a toxin produced by the fungus *Aspergillus flavus* which reduces the development and function of organs in broilers. The aim of this study was to determine the effect of adding binder toxin from different bentonite to feed contaminated with AFB_1 on productivity, intestinal morphology, and liver toxicity in broilers. A total of 60-day old chick male broilers were placed in 12 pens. Each treatment consisted of three replicates, each replicate containing five broilers. Treatment in the study consisted of P0 (control, basal diet, without the addition of AFB_1), P1 (P0 + 100 μg/kg AFB_1 + 4 g/kg calcium bentonite Type A), P2 (P0 + 100 μg/kg AFB_1 + 4 g/kg calcium bentonite Type B), and P3 (P0 + 100 μg/kg AFB_1 + 4 g/kg calcium bentonite Type B + kerolite + saponite). Treatment diets were given to broilers from day 22[rd] to 35[th] (finisher phase). The results showed that the toxin binder on AFB_1 contaminated feed had no effect on feed consumption, body weight gain and feed conversion ($p>0.05$). Addition of toxin binder on AFB_1 contaminated feed increased the relative weight of the duodenum ($p=0.024$), although P3 was not significantly different. Treatments had no effect on villus length, crypt depth, and ratio of villus length to crypt depth ($p>0.05$), but decreased villus width ($p=0.013$). The addition of toxin binder tended to decrease the villus area ($p=0.055$). SGOT and SGPT did not show differences between treatments. AFB_1 contamination with the addition of toxin binder showed signs of toxicity on liver histopathological observations. Based on the research, it can be concluded that the addition of binder toxin in feed contaminated with AFB_1 can reduce the negative effect on the development of intestinal villus and chemical effect to the liver. Toxin binder Type B has the best efficacy for reduce the negative effect.

Keywords: Aflatoxin, Broiler, Feed, productivity, Toxin binder

Introduction

Corn is a main energy source in broiler feed. However, corn is very susceptible for fungus infection and mycotoxin contamination that can be started from the field until to the storage. Mycotoxin contaminations are a big issue in animal feed industry due to its impact on animal health and performance. Mycotoxins are toxic compounds generally caused by the fungus *Aspergillus flavus*. Indonesia, which has a tropical climate, makes it easy for fungi to grow, resulting in very high mycotoxin contamination in feed raw materials (Mahato *et al*., 2019; Nuryono *et al*., 2012). Previous study indicated high occurrence and levels of aflatoxin B_1 (AFB_1) contamination in feed raw materials and commercial complete feed from Indonesia (Sumantri *et al*., 2017).

Mycotoxins are secondary metabolites of fungi which are synthesized during growth. Mycotoxins in feed include AFB_1, ochratoxin (OTA), deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin, and fumonisin (FUM). This type of mycotoxin is produced by the toxigenic fungi of the genera *Aspergillus*, *Penicillium*, and *Fusarium* (Widiyanti and Maryam, 2017). The type of mycotoxin that has been widely studied is AFB_1 which is included in the category of class I carcinogenic compounds (Hamid *et al*., 2013; Marchese *et al*., 2018) and cause a decrease in productivity in poultry (Fouad *et al*., 2019; Monson *et al*., 2015).

Poultry livestock has a high level of toxicity to aflatoxin compared to other livestock (Diaz *et al*., 2008). AFB_1 contamination in feed has an impact on increasing intestinal mucin secretion, decreasing absorption of feed nutrients, increasing feed conversion ratio (FCR), metabolic disorders, causing liver damage, impaired immunity, and decreased productivity (Fouad *et al*., 2019; Magnoli *et al*., 2011; Xu *et al*., 2022). The degression in performance in productivity causes huge economic losses for breeders.

One of the methods to reduce AFB_1 toxicity in poultry is the addition of toxin binders in feed (Zabiulla *et al*., 2021). Toxin binders are compounds that can bind to mycotoxins in the digestive tract so that these toxic compounds can be excreted through the excreta (Ahlberg *et al*., 2019; Oguz *et al*., 2022). The most widely used binder toxins in the poultry world are phyllosilicate, including bentonite, zeolite, montmorillonite, and hydrated sodium calcium aluminosilicate (Rejeb *et al*., 2020). Bentonite is a clay containing aluminum silicate which is capable of binding aflatoxins. Bentonite has a hollow surface with aluminium (Al) minerals that can bind aflatoxin (Hojati *et al*., 2021; Oguz *et al*., 2022; Pappas *et al*., 2016). As much as 25 mg of bentonite-based adsorbent or toxin binder can absorb AFB_1 (200 ng/mL) within 15 minutes (Nuryono *et al*., 2012). Bentonite in vitro can bind AFB_1 0.54 ppb with effectiveness of 88-95% (Oguz *et al*., 2022). The use of a smectite clay-based toxin binder is able to bind AFB_1 better because it has a large surface area, allows ion exchange capacity, and has the ability to expand in water (Zabiulla *et al*., 2021). AFB_1, which can be excreted, from the bird's body will reduce the effect of less than optimal villus growth (Galarza-Seeber *et al*., 2016; Wang *et al*., 2018) and reduce broiler liver damage (Yunus *et al*., 2011; Zabiulla *et al*., 2021). This study aims to determine the effect of adding toxin binders derived from various types of bentonites on productivity, intestinal morphology, and liver toxicity in broiler fed AFB_1 contamination.

Materials and Methods

Research management and procedures have been approved by the research ethics committee of the Faculty of Veterinary Medicine Universitas Gadjah Mada (UGM) with ethical clearance number 00013/EC-FKH/Eks./2021.

Production and analysis. AFB_1. *Aspergillus flavus* was grown on PDA (Merck, Germany) as an initial culture to produce AFB_1 on the substrate. A. flavus was incubated for 7 days at 30°C. Fungi are stored at 5°C to make them dormant. The AFB_1 production method on corn media uses the method used by Anes *et al*. (2020). The moisture content in the corn was adjusted to 25% by adding sterile distilled water. The corn was then sterilized by autoclaving (YX-24HDD, GEA, Indonesia) for 15 minutes at 121°C and 105 atm pressure. Corn substrate was weighed 250 g and placed in a plastic tube. The substrate was inoculated with 1 mL of a mixture of A. flavus and 10% Tween 80 solution (Merck, Germany). The substrate that had been inoculated with A. flavus was then incubated for 7 days at 30°C.

AFB_1 levels were analyzed using the Agraquant ELISA Aflatoxin B_1 ELISA kit protocol (Romers Labs, Singapore). The sample preparation stage for AFB_1-contaminated maize samples involved weighing 2 grams of the sample, which was then placed into an extraction tube. 10 ml of 70% methanol (Merck, Germany) was added to the tube and vortexed. The sample was filtered using Whatman number 1 paper (Cytiva, China), and the supernatant was transferred to a microtube for the AFB_1 determination stage. For the determination, 200 μL of conjugate solution was added to the dilution wells. Standard solutions (0, 4, 10, 20, and 40 ppb) and the supernatant from the sample were added to the dilution wells. The mixture from the dilution wells was transferred to antibody-coated wells and incubated for 15 minutes. The solution was washed with distilled water five times. Substrate solution (100 μL) was added to the antibody-coated wells and incubated for 5 minutes. Stop solution (100 μL) was added to the antibody-coated wells. The standard and sample solutions in the antibody-coated wells were read at a wavelength of 450 nm and a differential filter of 630 nm to obtain their absorbance values. The absorbance values obtained were used to calculate the AFB_1 concentration using the Microsoft Excel software provided by Romers Labs, Singapore.

Feed treatment and broiler maintenance. A total of 60 DOC male broilers were used in the study for 35 days of rearing. Broilers have been vaccinated with ND1, Gumboro and ND2 in the hatchery. Feed treatment consists of: P0 = Control (basal diet, without the addition of toxin binder and AFB_1); P1 = P0 + 4 g/kg calcium bentonite Type A + 100 μg/kg AFB_1; P2 = P0 + 4 g/kg calcium bentonite Type B + 100 μg/kg AFB_1, and P3 = P0 + 4 g/kg calcium bentonite Type B + kerolite and saponite + 100 μg/kg AFB_1. Each treatment consisted of three replicates with 5 birds per repetition. Cacium bentonite Type A (Terana 313) is produced by PT Clariant Indonesia and cacium bentonite Type B (TOXISORB®Classic) produced by Clariant Germany. Feed treatment is given from 22 to 35 days of age (finisher phase). Chickens aged 1 to 21 days were fed commercial feed with chemical composition consisting of metabolite energy (ME) 2800 kcal/kg, crude protein (CP) 21%, ether extract (EE) 5%, crude fiber (CF) 5%, ash 7%).

Broilers are maintained in a pen with a size of 1 x 1 m. Closed House is sanitized and fumigated 2 weeks before maintenance begins using formalin (Formades, Medion, Bandung, Indonesia). Temperature and wind speed are set according to broiler needs based on age. At the beginning of maintenance (d 1-3) the temperature of the cage was kept at 32°C and reduced by 3°C every week until it reached 23°C at the end of the period. Food and water were given ad libitum. Feed consumption was recorded from the start of feed treatment from day 21^{rd} to d 35^{th}. Body weight was calculated on day 21^{rd} and 35^{th}.

Sampling. On the day 35^{th} of rearing, one broiler from each replicate in each treatment was taken based on body weight that was close to the average body weight in the colony. Boilers fasted for five hours before blood collection. Blood samples were taken through the branchial vein using a 3 mL syringe (Onemed, Surabaya, Indonesia) and then collected in a tube (Vaculab EDTA K3, Onemed, Surabaya, Indonesia) with *ethylenediaminetetraacetic-acid* (EDTA) anticoagulant. Blood samples were stored at -20°C before analysis. Broilers were then necropsied after being slaughtered, then samples of the jejunum and liver were fixed with 10% formaldehyde solution for histomorphological tests.

Analysis of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT). Measurement of SGOT and SGPT levels each using 10 μL of blood serum sample added 1000 μL of SGOT mix reagent (GOT-110100) and SGPT (GPT-10100). Samples were incubated at room temperature for one minute and then read with a Microlab 300 spectrophotometer (Thermo Fisher Genesys 10s UV-Vis, USA) with a wavelength of λ340 nm.

Hematoxylin-Eosin staining. The jejunum and liver organs that had been fixed with formaldehyde solution 10% were then transferred to a 70% alcohol container as a stop point. The protocol for making histopathological preparations with hematoxylin-eosin (HE) staining refers to Kiernan (2008). The incisions of the jejunum and liver were placed in hematoxylin for 30 seconds then washed with distilled water. After that, the incision was put back in alcohol (30, 50, and 70%). The incisions were placed in eosin for 15 minutes and washed again with distilled water. The final step is deparaffinization of the incision with serial alcohol from high to low concentration. All stages of HE staining were carried out at room temperature.

Intestinal morphology and liver histopathology analysis. The morphology of the jejunum and liver was observed with an XSZ-107 BN binocular microscope (Zhejiang, China) and photographed with Optilab Advance MTN 004 with 3 fields of view. The magnification of the lens used on the jejunum is 100X and 400X on the liver. Intestine images were analyzed using Image Raster 3 software on the variable length of villus, width of villus, depth of crypts, and area of villus. The length of villi was measured from the crypt border to the tip of the villus. The width of villi was measured on both sides of the central part of the small intestine villi. The depth of the crypt was measured from the crypt border to the border of the small intestine wall. The area of the villi was measured by measuring the perimeter of the entire villus. Each measurement was repeated 3 times and averaged. Analysis of liver damage and identification of aflatoxicosis was carried out on liver images by the Pathology and Anatomy Laboratory, Faculty of Veterinary Medicine UGM.

Statistical analysis. The data obtained were analyzed using analysis of variance from a one-way randomized design with the SPSS version 25 application with a probability value of less than 5%. Data with significant differences will be tested further with Duncan's Multiple Range Test. A completely randomized design mathematical model with a unidirectional pattern (Gomez and Gómez, 1976).

Table 1. Formulation and composition of treated feed

Ingredients	(%)
Corn	52.75
Soy bean meal (SBM)	21.88
Corn gluten meal (CGM)	3.71
Meat bone meal (MBM)	4.00
Palm oil	3.50
L – Lysine	0.24
DL – Methionine	0.17
L – Threonine	0.08
Limestone/CaCO3	0.60
Salt	0.18
Sodium bicarbonate	0.18
Choline chloride	0.10
Trace mineral mix	0.11
Vitamin mix	0.25
Toxins binders	0.40
Total	100%
Nutrient composition	
Water content	11.43
Fat	6.50
Coarse fiber	2.63
Ash	7.30
Proteins	23.52
EM (Kcal/kg)	3100

bran 0%+21% of coffee pulp meal.

Results and Discussion

Effect of the addition of toxin binder on the productivity of the finisher phase

The effect of the addition of binder toxin to AFB_1 in AFB_1 contaminated feed on feed consumption of broiler productivity during the finisher period is shown in Table 2. The addition of bentonite toxin binder to feed contaminated with AFB_1 at a level of 100 μg/kg has no significant effect on reducing feed consumption to consumption feed, body weight gain, and broiler FCR ($P>0.05$).

The result of Yunus *et al.* (2011) research showed that AFB_1 contamination below 100 μg/kg had no effect on broiler productivity. However, an increase in the AFB_1 level in the feed causes a decrease in body weight and an increase in feed conversion (Indresh *et al.*, 2013; Mohaghegh *et al.*, 2017). Aflatoxin contamination can be categorized as realistic (<0.3 mg/kg), occasional (<0.3-2 mg/kg), and unrealistic (<2 mg/kg) (Grenier and

Table 2. Feed consumption, body weight gain, and FCR of finisher phase broilers fed calcium bentonite on feed contaminated with AFB_1

Treatment[1]	Feed consumption (g)	Body weight gain (g)	Feed conversion ration
P0	2.339,57±76.25	1.315±27.83	1.78±0.06
P1	2.659,10±475.90	1.524±366.95	1.77±0.28
P2	2.512,87±312.48	1.327±166.22	1.90±0.03
P3	2.334,96±109.02	1.331±118.15	1.76±0.07
p-value	0.09	0.30	0.82

[1]P0 (basal feed without the addition of toxin binder and AFB_1), P1 (P0 + 100 μg/kg AFB_1 + 4 g/kg calcium bentonite Type A), P2 (P0 + 100 μg/kg AFB_1 + 4 g/kg calcium bentonite Type B), and P3 (P0 + 100 μg/kg AFB_1 + 4 g/kg calcium bentotine Type B + kerolite + saponite).

Applegate, 2013). Broiler has a lower sensitivity level than turkey, quail, and duck. This is related to the kinetics of the cytochrome P450 enzyme in converting AFB_1 to AFBO. Therefore, aflatoxin contamination at a realistic level tends not to affect broiler productivity (Diaz and Murcia, 2019; Lozano and Diaz, 2006; Murcia *et al.*, 2011; Rawal and Coulombe, 2011).

The addition bentonite in toxin binder capable of binding to AFB_1 so that it is not absorbed by the small intestine. This is in accordance with the statement from McClure *et al.* (2014) that the binder toxin made from bentonite has the ability to bind AFB_1. Indresh *et al.* (2013) stated that bentonite binds to AFB_1 in the small intestine so that it can prevent the absorption of AFB_1 by the small intestine. Bentonite has a layer that is positively charged and binds to AFB_1 which is negatively charged. Therefore, the addition of toxin binder can prevent a decrease in the productivity of broilers fed high amounts of AFB_1 contaminated feed (Nazarizadeh and Pourreza, 2019; Zabiulla *et al.*, 2021).

Effect of toxin binder addition on liver weight and SGPT SGOT broiler levels

The addition of toxin binder to AFB_1 contaminated feed had no significant effect (P>0.05) on liver weight and SGPT SGOT broiler levels (Table 3). hContamination of 100 µg/kg AFB_1 in feed has not affected relative liver weight because it is included in the low dose toxicity level (Fouad *et al.*, 2019) but can increase broiler liver weight (Śliżewska *et al.*, 2019). The result of Riahi *et al.* (2021) research showed that addition of toxin binder made from zeoliteable to reduce liver weight in broilers fed mycotoxin-contaminated feed. Administration of binder toxins with various active ingredients such as calcium bentonite, zeolite, kerolite, and saponite is able to bind AFB_1 to cations in the interlayer (Fowler *et al.*, 2015; Akbar *et al.*, 2022).

The addition of toxin binder to feed contaminated with AFB_1 did not significantly affect broiler SGPT and SGOT levels. AFB_1 contamination in high amounts (0.5 mg/kg) will increase SGOT and SGPT levels (Amiridumari *et al.*, 2013; Liu *et al.*, 2022; Valchev *et al.*, 2014). AFB_1 contamination level of 100 ppb has no effect on SGOT and SGPT levels because broilers are still able to carry out the detoxification process in the liver. The research results have similarities with Saminathan *et al.* (2018) that AFB_1 contamination at a level of 100 µg/kg had no effect on SGOT and SGPT levels, however AFB_1 contamination at a level of 1 mg/kg significantly increased SGOT levels by 7.93% and SGPT by 12.48% (Farooqui *et al.*, 2019; Attia *et al.*, 2019). Nazarizadeh and Pourreza (2019) reported that the addition of 1 g/kg mycotoxin binder in feed contaminated with AFB_1 at a level of 2 mg/kg reduced the activity of SGPT and SGOT enzymes caused by impaired hepatocyte cell necrosis. The secretion of these two enzymes is an indicator of damage to hepatocyte cells and functions to assist protein synthesis (Senanayake *et al.*, 2015).

Effect of of toxin binder addition on broiler liver histopathology

The effect of adding toxin binder to feed contaminated with AFB_1 on broiler liver histopathology is shown in Figure 1. Histology of the control treatment (P0) liver was normal or no signs of aflatoxicosis were found. The addition of calcium bentonite Type A (P1) to feed contaminated with AFB_1 found hydropic and focal degeneration of lymphocyte follicles. Effect of adding toxin binder with the active ingredient European calcium bentonite on feed treatment (P2) of hydropic degeneration, liver cell necrosis, and focal lymphocyte follicles. Treatment of P3 feed with the active ingredient calcium bentonite + kerolite and saponite found signs of liver damage in the form of hydropic degeneration, proliferation of bile ducts, and hepatocyte necrosis. The addition of a type B toxin binder is thought to reduce the effects of liver damage with the fewest signs AFB_1 (Figure 1 P2). Contamination causes liver damage characterized by increased fat synthesis, vascular degeneration, lobe inflammation (Kraieski *et al.*, 2017; Sumantri *et al.*, 2018). The higher the AFB_1 contamination in the feed can increase the level of aflatoxicosis effect (Śliżewska *et al.*, 2019; Hua *et al.*, 2021). The ability to bind toxin binders will have an impact on the level of liver damage. The higher activation of AFB_1 by toxin binder will prevent the accumulation of AFB_1 in the liver, thus minimizing the detoxification process and liver damage. The binding ability of AFB_1 by toxin binder is influenced by the particle size of the material, surface area, the optimum pH of the material for work. The result of Oguz *et al.* (2022) research showed that different types of toxin binder materials have different mycotoxin binding abilities. The binding ability is affected by the content of materials that will bind to the active site of mycotoxin. Raw materials that are able to bind mycotoxin optimally will prevent the maximum level of toxicity (Zabiulla *et al.*, 2021; Rashidi *et al.*, 2020; Tarasova *et al.*, 2020). Study on duck showed low levels of AFB_1 in feed (30 to

Table 3. Broiler liver weight and SGPT SGOT enzyme activity in broilers treated with calcium bentonite on feed contaminated with AFB_1

Parameter	Treatment[1]				p-value
	P0	P1	P2	P3	
Liver weight (g)	42.33±1.53	37.00±1.00	42.33±5.13	45.33±1.02	0.460
Liver weight (%)	2.86±0.15	2.34±0.40	2.81±0.44	2.96±0.80	0.570
SGPT[2] (U/l)	7.43±2.00	8.63±1.89	8.03±2.11	11.63±1.60	0.104
SGOT (U/l)	171.85±5.95	209.83±56.84	217.60±25.00	221.70±48.41	0.441

[1]P0 (basal feed without the addition of toxin binder and AFB_1), P1 (P0 + 100 µg/kg AFB_1+ 4 g/kg calcium bentonite Type A), P2 (P0 + 100 µg/kg AFB_1+ 4 g/kg calcium bentonite Type B), and P3 (P0 + 100 µg/kg AFB_1+ 4 g/kg calcium bentotine Type B+kerolite+saponite).
[2]SGPT (Serum glutamic oxaloacetic transaminase), SGOT (serum glutamic pyruvic transaminase).

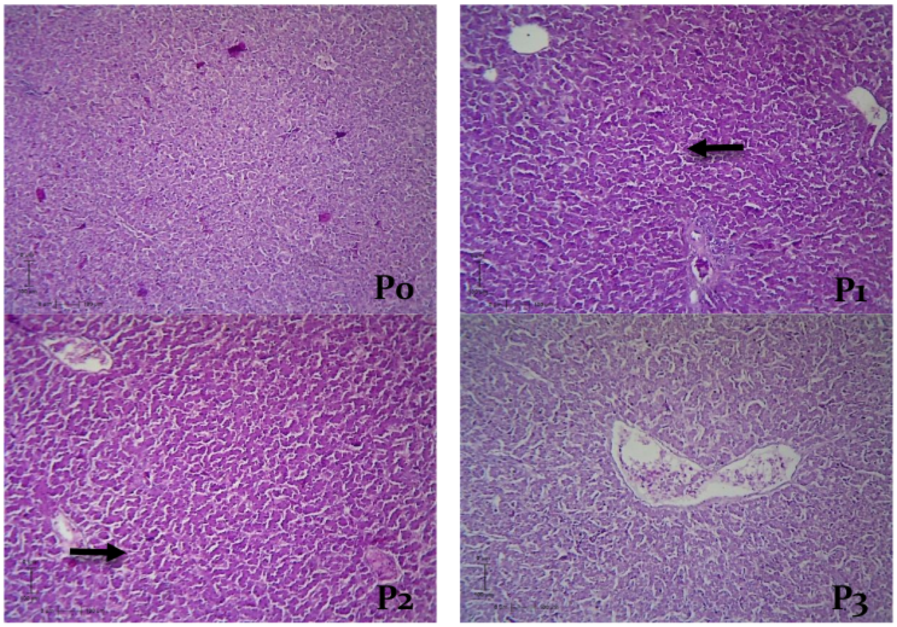

Figure 1. Histopathology of liver treated with toxin binder on feed contaminated with AFB_1 (400X magnification). P0 (basal feed without the addition of toxin binders and AFB_1), P1 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type A), P2 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type B), and P3 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentotine Type B +kerolite+saponite).

70 ppb) resulted in mild acute liver vacuoles degeneration and 2% zeolite inclusion in AFB_1 contaminated feed could reduce the hepatic lesions (Sumantri *et al*., 2018).

The effect of the addition of toxin binder on the morphology of the small intestine of broilers

The length and weight of the small intestine are shown in Table 4. The addition of toxin binder to feed contaminated with AFB_1 had no effect on the length of the duodenum, jejunum and ileum. Kövesi *et al*. (2020) reported that exposure to aflatoxin 50 to 100 ppb is included in a low exposure dose which does not make a difference to the length of the gastrointestinal tract, but AFB_1 contamination of 700 ppb can increase the length of the jejunum and duodenum (Yunus *et al*., 2011).

The addition of toxin binder in AFB_1-contaminated feed significantly increased the weight of the duodenum (P=0.024) and ileum (P=0.049) compared to the control but had no effect on the weight of the jejunum (P=0.367). AFB_1 contamination of less than 200 μg/kg in the feed does not cause a decrease in small intestine weight (Manafi *et al*., 2012). Holanda and Kim (2022) showed that AFB_1 contamination with a level of 950 μg/kg could inhibit the growth and performance of the intestinal tract of broilers, and reduce the weight of the small intestine of broilers by 11%. The presence of AFB_1 in the small intestine causes inflammation of the epithelial cells as an immune response so that the swelling can increase the weight of the small intestine (Domingues *et al*., 2021; Rashidi *et al*., 2020).

Effect of toxin binder addition on the histomorphology of small intestinal villus in broilers

The effect of adding toxin binder to feed contaminated with AFB_1 on the villus morphology of the small intestine of broilers is shown in Table 5. Addition of toxin binder to feed contaminated

Table 4. Effect of adding calcium bentonite to feed contaminated with AFB_1 on the length and weight of the small intestine of broilers

Parameter	Treatment[1]				p-value
	P0	P1	P2	P3	
Gastrointestinal length					
Duodenum (cm)	28.33±0.58	28.67± 0.58	27.67± 2.52	30.00±4.58	0.749
Jejunum (cm)	70.00±7.81	74.67±11.72	77.33±17.21	75.33±3.79	0.875
Ileum (cm)	68.00±6.93	69.67± 5.13	59.67±23.59	70.00±1.00	0.725
Gastrointestinal weight					
Duodenum (%)	0.54±00.7^{a}	0.89±0.14^{b}	0.79±0.14^{b}	0.73±0.06^{ab}	0.024
Jejunum (%)	1.06±0.23	1.22±0.08	1.59±0.66	1.36±0.15	0.367
Ileum (%)	0.79±0.13^{a}	0.73±0.06^{a}	0.84±0.20^{ab}	1.10±0.13^{b}	0.049

[1]P0 (basal feed without the addition of toxin binder and AFB_1), P1 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type A), P2 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type B), and P3 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentotine Type B+kerolite+saponite).

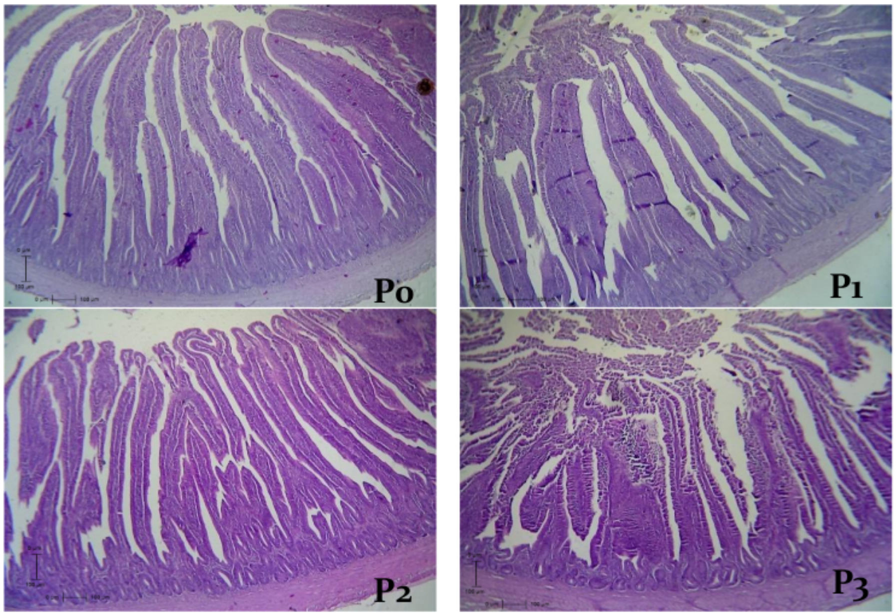

Figure 2. Histology of small intestinal villus (jejunum) of broilers fed AFB_1-contaminated feed with the addition of calcium bentonite (100X magnification). P0 (basal feed without the addition of toxin binders and AFB_1), P1 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type A), P2 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type B), and P3 (P0 + 100 μg/kgAFB_1+ 4 g/kg calcium bentotine Type B+kerolite+saponite).

Table 5. Villus morphology of the small intestine of broilers fed AFB_1-contaminated feed with the addition of calcium bentonite

Parameter	Treatment[1]				p-value
	P0	P1	P2	P3	
Villus length (mm)	1.61±0.14	1.85±0.10	1.63±0.12	1.61±0.23	0.249
Villus width (mm)	0.26±0.03^{b}	0.16±0.01^{a}	0.15±0.04^{a}	0.19±0.04^{a}	0.013
Crypte depth (mm)	0.28±0.02	0.29±0.05	0.30±0.02	0.34±007	0.443
Area (mm^2)	0.44±0.10^{b}	0.29±0.02^{a}	0.30±0.05^{a}	0.32±0.05^{a}	0.055
Villus/crypta length ratio	5.67±0.18	6.55±1.41	5.52±0.78	4.97±1.74	0.479

[1]P0 (basal feed without the addition of toxin binder and AFB_1), P1 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type A), P2 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentonite Type B), and P3 (P0 + 100 μg/kg AFB_1+ 4 g/kg calcium bentotine Type B+kerolite+saponite).

with AFB_1 decreased the width of the villus (P=0.013) and tended to decrease the area of villus (P=0.055) in the small intestine of broilers. However, it did not significantly affect villus length, crypt depth, and ratio of villus length to crypt depth (P>0.05).

The AFB_1 contamination at a dose of 60 μg/kg will decrease the villus width by 20%, exposure to AFB_1 with a higher level of 250 ppb decreases the villus width by up to 52%, the higher the AFB_1 exposure level will reduce the wider villus width (Xie *et al.*, 2022; Ashry *et al.*, 2022). At a contamination level of 82.4 ppb, it can reduce the villus area by 13% (Yang *et al.*, 2012). Aflatoxin compounds in the small intestine reduce the ability of tight junctions in the phenomenon of leaky gut syndrome, thereby inhibiting the development of villus and triggering a decrease in the area of absorption of broiler nutrients (Galarza-Seeber *et al.*, 2016). In addition, aflatoxin causes pathological damage to the intestinal mucosa which reduces cell proliferation (Wang *et al.*, 2018). The use of toxin binders in research Dogi *et al.* (2017) able to reduce the effect of AFB_1 on villus length and crypt depth in broilers fed AFB_1 contaminated feed with a level of 100 μg/kg. Research result (Alharthi *et al.*, 2022) with the addition of 0.4% bentonite to 250 ppb AFB_1 contamination was able to increase the height, width, and area of the villus compared to positive controls contaminated with AFB_1.

Conclusions

The conclusion of the research that has been done isthe addition of a toxin binder made from phyllosilicate in feed contaminated with AFB_1 100 μg/kg had no effect on the productivity of the finisher phase broilers, although it reduced the villus area. Calcium bentonite able to reduce the effects of liver damage due to AFB_1 contamination in feed.

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