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Original Article

The effect of binder mycotoxins on the histopathology of broiler kidneys exposed to a combination of mycotoxins

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ABSTRACT

This study was made to know the histopathological changes of kidney in broiler chicken fed by mix mycotoxin (aflatoxin B1 and ochratoxin A) contaminated feed. A total of 20 chickens aged DOC to 35 days was divided into 4 groups (K-, K+, P1 and P2) each group consisted of 5 chickens. Group K- as a control without the addition of the mix mycotoxin to the fed. Group K+ feed was added with 0.1 mg/kg of aflatoxin B1 and 0.1 mg/kg of ochratoxin A. Group P1 and P2 feed were added mix mycotoxin with the same dose with addition of 1.1 g/kg mycotoxin binder in P1 group and 1.6 g/kg of mycotoxin binder in P2 group. Treatment of contaminated feed has been given for 28 days from day 8 until day 35. At the end of the treatment period (day 35), chickens were euthanized, and histopathological examination was carried out. Kruskal-Wallis test showed a significant difference ($p<0.05$) on the mean rank of the kidney cell necrosis, cell degeneration, inflammatory cell infiltration, and haemorrhage. The conclusion of this study is that the administration of mycotoxin binder has an effect in preventing and reducing the necrosis, degeneration, inflammatory cell infiltration, and haemorrhage of kidney.

Keywords: Aflatoxin B1, Ochratoxin A, Mycotoxin binder, Kidney histopathology, Broiler

Introduction

The increasing population, income, and awareness of nutrition have led to an increasing demand for broiler livestock as a source of animal protein. Based on data obtained from the Organization for Economic Cooperation and Development, meat consumption in Indonesia in 2017 only reached an average of 1.9 kg for beef, 7.7 kg for chicken, 1 kg for pork, and 0.4 kg for goat meat. Chicken meat is consumed more than beef because it has a more affordable price (Slamet *et al*., 2022). The need for broiler meat in Indonesia in 2018 reached 11.5 kg/capita/year (Wibowo *et al*., 2020). Broiler or also called meat chicken is a type of chicken resulting from livestock technology cultivation that has the characteristic of rapid growth, as a producer of meat with low feed conversion. Broiler is a type of poultry that has a fairly short harvest period of 5 weeks (Umam *et al*., 2015).

Efforts to increase broiler livestock productivity are greatly influenced by feed factors. Diverse and high-quality feed must be provided to meet complete nutritional needs. The main components of feed that are first

considered are protein and energy content. Good feed is feed that can supply all the nutrients needed by livestock in a balanced manner, such as carbohydrates, protein, fat, vitamins, and minerals (Tillman *et al*., 1998). Decrease in livestock quality and productivity can occur due to mold contamination that can damage feed. Tropical climates with high humidity levels are a factor causing mold growth, especially mold that produces mycotoxins (Miskiyah *et al*., 2010).

The presence of mycotoxins has spread to all levels of the food chain. Given the still high incidence of detected aflatoxin contamination in livestock feed, namely 98% in broiler chickens and 82.73% in laying hens, it needs attention (Martindah and Bahri, 2016). Aspergillus sp. mold can be found in various substrates, including soil, fruit leaves, and grains which are the main ingredients in the manufacture of livestock feed products from agricultural commodities (Sukmawati *et al*., 2015; Sukmawati and Miarsyah, 2017). Some of the dangers of these molds found in livestock feed products are aflatoxin and ochratoxin.

Aflatoxin is a toxic secondary metabolite produced by Aspergillus flavus. Aflatoxin B1 is in first place in the aflatoxin level. This type of aflatoxin is a very strong toxin that has a high effect on the body, namely disrupting the function of body organs (Hananto *et al*., 2015). Exposure to aflatoxin is very detrimental because it can have an impact on health such as enlargement of the kidney size (Sukmawati *et al*., 2018). Abnormalities of body organs and decreased organ function can occur due to chronic exposure to aflatoxin. Aspergillus sp. mold can also produce other types of mycotoxins, namely ochratoxins. Ochratoxin A is the most toxic type of ochratoxin compared to other types (Mally, 2012). In chickens, the effect of ochratoxin on the kidneys causes necrosis of the tubules (tubular necrotic) (Maryam *et al*., 2020).

The administration of mycotoxin binder is necessary to reduce the effects caused by mold contamination in feed. This study aims to determine the effect of mycotoxin binder on the histopathology of broiler kidneys exposed to a combination of mycotoxins (aflatoxin B1 and ochratoxin A).

Materials and methods

Research design

This study was conducted for 2 months, namely in February–April 2023. The maintenance and treatment of experimental animals in this study were carried out in the Chicken Experimental Animal Cage, Faculty of Veterinary Medicine, Airlangga University and the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University as a place to make histopathology preparations.

This study used 20 day old chicks (DOC) of the Cobb strain broiler, taken from 100 DOCs from PT. Charoen Pokphand Jaya Farm. The samples used were 20 broiler kidney organs with a ready-to-harvest age (5 weeks).

Cage preparation

The cage for broiler maintenance must be cleaned first before use and then disinfected to avoid disease contamination. Litter/husks are spread in the cage 3 days before the DOC is entered and fumigated. The thickness of the litter used is approximately 10 cm.

Preparation of experimental animals

Things to do when DOC first enters are checks that include size, whether they make noise often enough or not, full legs with clean feathers and bright eyes, agile and actively looking for food and drink, no respiratory problems or other diseases, clean anus and no white paste, weight not less than 37 g (Umiarti, 2020). The distribution of DOC to the cage is carried out after checking, behavior must be observed and its condition observed. Feed and drinking water are controlled so that they are always available. Brown sugar water can be given to restore DOC stamina lost during the trip.

Animal care for experiments

Drinking water provision in broiler maintenance is done ad libitum (available at all times). It is also necessary to pay attention to health issues, cage cleanliness and vaccination

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to form an immune system. Vaccination is carried out to prevent chickens from being infected with Newcastle Disease (ND) and Avian Influenza (AI) in accordance with the correct methods and procedures. The vaccines given are ND vaccine and AI vaccine which are given on the 7th day and the 21st day. Supplements or multivitamins can be given to reduce stress in chickens after vaccination.

Treatment of experimental animals

A total of 20 DOCs were divided into 4 treatment groups, with each treatment consisting of 5, namely:

K- = Commercial feed

K+ = Commercial feed + aflatoxin 0.1 mg/kg + ochratoxin 0.1 mg/kg feed

P1 = Commercial feed + aflatoxin 0.1 mg/kg + ochratoxin 0.1 mg/kg + mycotoxin binder 1.1 g/kg feed

P2 = Commercial feed + aflatoxin 0.1 mg/kg + ochratoxin 0.1 mg/kg + mycotoxin binder 1.6 g/kg feed

Adaptation of treatment feed began on the 8th day to the 14th day. During the adaptation of treatment feed, mycotoxin-contaminated feed was given according to the treatment but was given gradually. Feed was given ad libitum referring to the consumption standards for each growth phase.

Sampling

The sampling process was carried out when the chickens were ready for harvest (5 weeks). The chickens were slaughtered according to the treatment group and surgery was performed on the abdomen to take the kidneys which would later be made into histopathology preparations.

Preparation of histopathology preparations

The kidney organ that has been taken is put into a container containing 10% NBF solution to be used as a sample and made a histopathology preparation, then dehydration and clearing are carried out (cleaning tissue and substances carried). The next stage is embedding (making paraffin blocks), cutting (cutting paraffin blocks using a microtome), staining (painting using HE staining), and mounting (covering/gluing by object glass).

Histopathological examination of the preparation

The examination of the results was carried out using a light microscope with a magnification of 400 times. Histopathological abnormalities found in the preparation were assessed with a score seen in 5 fields of view, and in 1 field of view a score of 1–4 was given.

Data analysis

The data obtained in the form of a score of the level of change in the histopathology of the kidneys is arranged in a table for later analysis. To determine the difference in changes in the histopathology of the kidneys, the Kruskal Wallis test was performed. If there is a significant difference ($p<0.05$), it is continued with the Mann Whitney test. The analysis was carried out using the Statistical Product and Service Solution (SPSS) computer statistics program (Al-Arif, 2018).

Result

Tubular epithelial cell necrosis

The results of observations of tubular epithelial cell necrosis in the histopathology of broiler kidneys that have been identified by observing five fields of view with a magnification of 400x were analyzed using the Kruskal Wallis test showing significant differences ($p<0.05$) between treatment groups. These results indicate that the administration of mycotoxin binders has a significant effect on renal tubular epithelial cell necrosis. The differences between treatment groups were identified by continuing the analysis using the Mann Whitney test. The average value and standard deviation can be seen in table 1.

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Table 1. Mean values and standard deviations (SD) of the level of necrosis of broiler renal tubular epithelial cells in each treatment group

Treatment	Degree of Necrosis
	Mean ± SD
K- (Negative Control)	1.0^{a} ± 0.50
K+ (Positive Control)	3.5^{d} ± 0.18
P1 (Treatment with a dose of 1.1 g/kg)	2.8^{c} ± 0.39
P2 (Treatment with a dose of 1.6 g/kg)	2.0^{b} ± 0.32

Note: Different superscripts (a,b,c,d) in the same column indicate significant differences ($p<0.05$)

The results of the measurement of the level of tubular epithelial cell necrosis were analyzed using the Mann Whitney test showing a significant difference ($p<0.05$) in the group that was not exposed to a combination of mycotoxins and mycotoxin binders (K-) compared to the group that was exposed to a combination of mycotoxins (K+) and the group that was given mycotoxin binders at a dose of 1.1 g/kg (P1) and a dose of 1.6 g/kg (P2) after being exposed to mycotoxins. And there was a significant difference ($p<0.05$) in the group that was given mycotoxins (K+) compared to the group that was given mycotoxin binders at a dose of 1.1 g/kg (P1) and a dose of 1.6 g/kg (P2) after being exposed to mycotoxins. The results of the analysis showed that the administration of mycotoxin binders was most effective and the results that were close to the negative control group (K-) were group P2 with a dose of mycotoxin binders of 1.6 g/kg which could reduce the level of renal tubular epithelial cell necrosis.

Tubular epithelial cell degeneration

The results of observations of tubular epithelial cell degeneration in the histopathology of broiler kidneys that have been identified by observing five fields of view with a magnification of 400x were analyzed using the Kruskal Wallis test showing significant differences ($p<0.05$) between treatment groups. These results indicate that the administration of mycotoxin binders has a significant effect on the degeneration of broiler kidney tubular epithelial cells. The differences between treatment groups were determined by continuing the analysis using the Mann Whitney test. The average value and standard deviation can be seen in table 2.

Table 2. Mean values and standard deviations (SD) of the level of degeneration of broiler renal tubular epithelial cells in each treatment group

Treatment	Degree of Necrosis
	Mean ± SD
K- (Negative Control)	0.3^{a} ± 0.27
K+ (Positive Control)	3.0^{d} ± 0.30
P1 (Treatment with a dose of 1.1 g/kg)	2.2^{c} ± 0.27
P2 (Treatment with a dose of 1.6 g/kg)	1.4^{b} ± 0.27

Note: Different superscripts (a,b,c,d) in the same column indicate significant differences ($p<0.05$)

The results of the measurement of the level of degeneration of tubular epithelial cells were analyzed using the Mann Whitney test showing a significant difference ($p<0.05$) in the group that was not exposed to a combination of mycotoxins and mycotoxin binders (K-) compared to the group that was exposed to a combination of mycotoxins (K +) and the group that was given mycotoxin binders at a dose of 1.1 g / kg (P1) and a dose of 1.6 g / kg (P2) after being exposed to mycotoxins. And there was a significant difference ($p<0.05$) in the group that was given mycotoxins (K +) compared to the group that was given mycotoxin binders at a dose of 1.1 g / kg (P1) and a dose of 1.6 g / kg (P2) after being exposed to mycotoxins. The results of the analysis showed that the administration of mycotoxin binders was most effective and the results that were close to the negative control group (K-) were group P2 with a dose of mycotoxin binders of 1.6 g / kg which could reduce the level of degeneration of renal tubular epithelial cells.

Inflammatory cell infiltration into the interstitium

The results of observations of interstitial inflammatory cell infiltration in the histopathology of broiler kidneys that have been

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identified by observing five fields of view with a magnification of 400x were analyzed using the Kruskal Wallis test showing significant differences ($p<0.05$) between treatment groups. These results indicate that the administration of mycotoxin binders has a significant effect on inflammatory cell infiltration in the broiler kidney interstitial. The differences between treatment groups were identified by continuing the analysis using the Mann-Whitney test. The average value and standard deviation can be seen in Table 3.

Table 3. Mean values and standard deviations (SD) of inflammatory cell infiltration levels in the broiler kidney interstitium in each treatment group

Treatment	Degree of Necrosis
	Mean ± SD
K- (Negative Control)	$0.2^{a} \pm 0.17$
K+ (Positive Control)	$3.0^{d} \pm 0.43$
P1 (Treatment with a dose of 1.1 g/kg)	$2.1^{c} \pm 0.30$
P2 (Treatment with a dose of 1.6 g/kg)	$1.2^{b} \pm 0.43$

Note: Different superscripts (a,b,c,d) in the same column indicate significant differences ($p<0.05$)

The results of the measurement of the level of inflammatory cell infiltration were analyzed using the Mann Whitney test showing a significant difference ($p<0.05$) in the group that was not exposed to a combination of mycotoxins and mycotoxin binders (K-) compared to the group that was exposed to a combination of mycotoxins (K+) and the group that was given mycotoxin binders at a dose of 1.1 g/kg (P1) and a dose of 1.6 g/kg (P2) after being exposed to mycotoxins. And there was a significant difference ($p<0.05$) in the group that was given mycotoxins (K+) compared to the group that was given mycotoxin binders at a dose of 1.1 g/kg (P1) and a dose of 1.6 g/kg (P2) after being exposed to mycotoxins. The results of the analysis showed that the most effective administration of mycotoxin binders and the results that approached the negative control group (K-) were group P2 with a dose of 1.6 g/kg which could reduce the level of inflammatory cell infiltration in the renal interstitium.

Bleeding (hemorrhage)

The results of observations of bleeding (hemorrhage) in the histopathology of broiler kidneys that have been identified by observing five fields of view with a magnification of 400x were analyzed using the Kruskal Wallis test, showing significant differences ($p<0.05$) between treatment groups. These results indicate that the administration of mycotoxin binders has a significant effect on broiler kidney hemorrhage. The differences between treatment groups were determined by continuing the analysis using the Mann-Whitney test. The average value and standard deviation can be seen in Table 4.

Table 4. Mean values and standard deviations (SD) of bleeding (hemorrhage) levels in broiler kidneys in each treatment group

Treatment	Degree of Necrosis
	Mean ± SD
K- (Negative Control)	$0.3^{a} \pm 0.10$
K+ (Positive Control)	$3.0^{d} \pm 0.17$
P1 (Treatment with a dose of 1.1 g/kg)	$2.1^{c} \pm 0.44$
P2 (Treatment with a dose of 1.6 g/kg)	$1.2^{b} \pm 0.14$

Note: Different superscripts (a,b,c,d) in the same column indicate significant differences ($p<0.05$)

The results of the measurement of the level of hemorrhage were analyzed using the Mann Whitney test showing a significant difference ($p<0.05$) in the group that was not exposed to a combination of mycotoxins and mycotoxin binders (K-) compared to the group that was exposed to a combination of mycotoxins (K+) and the group that was given mycotoxin binders at a dose of 1.1 g/kg (P1) and a dose of 1.6 g/kg (P2) after being exposed to mycotoxins. And there was a significant difference ($p<0.05$) in the group that was given mycotoxins (K+) compared to the group that was given mycotoxin binders at a dose of 1.1 g/kg (P1) and a dose of 1.6 g/kg (P2) after being exposed to mycotoxins. The results of the analysis

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showed that the most effective administration of mycotoxin binders and the results that approached the negative control group (K-) were group P2 with a dose of 1.6 g/kg which could reduce the level of bleeding (hemorrhage) in the kidneys.

Discussion

Based on the results of this study, various pathological changes in the kidneys caused by mycotoxin exposure were found. Pathological lesions that appear due to the effects of mycotoxin exposure on the kidneys are tubular cell necrosis, tubular cell degeneration, inflammatory cell infiltration, and bleeding (hemorrhage).

Necrosis is cell death that occurs in living tissue or cells. The nucleus of a cell undergoing necrosis has three changes, namely pyknosis (the nucleus becomes dark, dense, and small due to shrinkage), karyorrhexis (the nucleus breaks into several segments), and karyolysis (the nucleus fades due to complete lysis) (Arimbi *et al*., 2019). Degeneration is defined as the loss of normal cell structure before death. Hydropic degeneration is a condition in which the cell cytoplasm contains water. Microscopically, in cells undergoing hydropic degeneration, there is a clear space in the cytoplasm but it is not as clear as collagen or fat (Fahmi *et al*., 2015). Inflammatory cell infiltration is a tissue response to damage caused by several pathogenic agents, dead or damaged cells, foreign objects, physical injury, irritation, radiation, or toxic substances (Arimbi *et al*., 2019). Bleeding (hemorrhage) is a condition where blood comes out of the vascular system caused by damage to the vascular wall due to ruptured tissue with the type of leakage through tears (perrexis) (Lumaksono *et al*., 2021).

The results of statistical analysis using the Kruskal Wallis test showed a significant difference between treatment groups ($p<0.05$). In further tests using the Mann Whitney test, the negative control group (K-) which was only given commercial feed without contamination of mycotoxin combinations (aflatoxin B1 and ochratoxin A) and without mycotoxin binders showed the lowest picture of tubular cell necrosis, tubular cell degeneration, inflammatory cell infiltration, and bleeding (hemorrhage) compared to other treatment groups. The positive control group (K+) which was given feed with contamination of mycotoxin combinations (aflatoxin B1 and ochratoxin A) each of 0.1 mg/kg without mycotoxin binders showed the highest picture of tubular cell necrosis, tubular cell degeneration, inflammatory cell infiltration, and bleeding (hemorrhage) compared to other treatment groups. The results of this study are in line with Moenek *et al*. (2016) which showed that exposure to mycotoxins, especially aflatoxin B1 in feed, caused changes in histopathological images in the form of degeneration, extensive necrosis, and bleeding. The results of this study are also in line with Aydin *et al*. (2003) that exposure to ochratoxin A caused histopathological lesions in the form of tubular cell degeneration, inflammatory cell infiltration, and hyperemic blood vessels in several areas.

Treatment group P1 given mycotoxin binder at a dose of 1.1 g/kg and treatment group P2 given mycotoxin binder at a dose of 1.6 g/kg, showed a lesion picture that was significantly different from the negative control group (K-) and positive control (K+). The results of the study that I have conducted show that administration of mycotoxin binder at a dose of 1.1 g/kg can reduce the occurrence of tubular cell necrosis, tubular cell degeneration, inflammatory cell infiltration, and bleeding (hemorrhage), and administration of mycotoxin binder at a dose of 1.6 g/kg is proven to be the most effective dose to reduce the occurrence of tubular cell necrosis, tubular cell degeneration, inflammatory cell infiltration, and bleeding (hemorrhage) in the kidneys exposed to a combination of mycotoxins (aflatoxin B1 and ochratoxin A). This is in accordance with the study conducted by Widiyanti and Maryam (2016) namely that administration of mycotoxin binder can reduce and overcome damage to organs exposed to mycotoxins. These results can occur because the addition of mycotoxin binders to feed is able to degrade mycotoxins

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that enter the animal's body. Mycotoxin binders that act like chemical sponges are able to bind mycotoxins and inhibit their absorption so that they can reduce the distribution of mycotoxins into the blood and target organs.

The results of the data analysis showed that there was an effect of mycotoxin binder administration in preventing histopathological damage to broiler kidneys. Mycotoxin binder with the trademark Mycofix® Plus 3.0 used in this study was able to bind molecules in the pores of the mycotoxin binder. The content of mycotoxin binders contained in the mycotoxin binder in this study included bentonite, Hydrated Sodium Calcium Aluminosilicate (HSCAS), zeolite, BBSH 797, and phytogenic substances.

Bentonite can reduce aflatoxin by up to 66% of the primary concentration, and can reduce the level of pathological damage (Ramandani *et al*., 2020). Calcium ions and protons contained in HSCAS have been studied as selective enterosorbents and can bind aflatoxins and can reduce toxicity through the bioavailability process (Widiyanti and Maryam, 2016). The selective nature of HSCAS in absorbing aflatoxins makes HSCAS not very effective in binding other types of mycotoxins. Zeolite is a hydrated alumina silicate compound that physically and chemically has the ability as an adsorbent. Research by Rajendran *et al*. (2020) and Raj *et al*. (2021) proves that the presence of zeolite compounds in mycotoxin binders is effective in reducing the effects of aflatoxicosis and reducing residues of aflatoxin B1, and can bind ochratoxin A. BBSH 797 is a commercially produced anaerobic gram-positive bacteria derived from the rumen of ruminants for detoxification. These bacteria produce epoxidase enzymes that can kill 12 and 13-epoxide rings of trichotoxin produced by deoxynivalenol (DON) and T-2. The addition of phytogenic substances has been shown to be effective in inhibiting fungal growth and aflatoxin synthesis. Phytogenic substances have antioxidant properties, are residue-free, anti-inflammatory, and are substances that reduce mycotoxin contamination in feed (Hidayat and Rahman, 2019).

The results obtained from this study are used to prevent the possibility of diseases caused by exposure to mycotoxins in feed, in addition, it is also used to control mycotoxin exposure with the aim of reducing the negative impact of mycotoxin contamination. Control is carried out by administering mycotoxin binders with an effective dose. The administration of mycotoxin binders with a dose of 1.6 g/kg of feed is so far an effective dose because it can degrade mycotoxins and reduce their negative impacts.

Conclusion

Based on the research that has been conducted, it can be concluded that the administration of mycotoxin binders in feed exposed to a combination of mycotoxins is able to prevent and reduce kidney damage in the form of tubular cell epithelial necrosis, tubular cell epithelial degeneration, interstitial inflammatory cell infiltration, and bleeding (hemorrhage) in broilers, with the use of an effective dose of mycotoxin binders.

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