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Original Article

The effect of using mycotoxin binder on daily body weight gain, protein consumption, and feed protein efficiency in broilers exposed to mycotoxins

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ABSTRACT

This research aimed to determine the use of toxin binder to average daily gain, protein consumption and protein efficiency in broiler given mycotoxin contamination. This research was an experimental study using completely randomized design (CRD) using 24 male broiler chickens randomized with 4 treatments with each treatment consisting of 6 broiler chickens. C was the control treatment consisting only commercial feed. P1 treatment was the addition of commercial feed, aflatox in and ochratoxin with the amout of 1 mg/kg feed for each. P2 treatment was the addition of commercial feed, aflatoxin with the amount of 1 mg/kg feed, ochratoxin 1 mg/kg feed, and and toxin binder as much as 1.1 g/kg feed. P3 treatment was the addition of commercial feed, aflatoxin with the amount of 1 mg/kg feed, ochratoxin 1 mg/kg feed, and and toxin binder as much as 1.6 g/kg feed. The result showed that P2, control and P3 treatment have the highest average daily gain. The highest to lowest protein cons umption showed in following order P1, P3, C, P2 treatment. P2 treatment have the highest protein efficiency showed by having the highest average daily gain with less production cost. Mycotoxin binder given in feed contaminated with aflatoxin and ochratoxin can help to reduce the negative effect of mycotoxin as to in creasing protein consumption and average daily gain resulting to more efficient in protein use and minimalizing the loss of production caused by mycotoxin contamination.

Keywords: Mycotoxin binder, aflatoxin, ochratoxin, protein consumption, average daily gain

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Introduction

Broilers are one of the sources of animal protein that is chosen by the community because they have high productivity, fast growth and relatively short maintenance period so that the costs used for feed and maintenance are less (Maharjan *et al*., 2021). The quality of feed as a component contributing 60-70% of the total production cost is important to maintain (Chia *et al*., 2019). Animal feed comes from several types of grains and other materials that are susceptible to mold contamination (Pereira *et al*., 2019). Mycotoxin-producing molds can grow on food or feed, both before and during harvest or during improper storage (Bhat *et al*., 2010).

The geographical location and climate conditions of Indonesia as a tropical country with high humidity are ideal habitats for mold. Mold as a pathogenic microbe produces toxic secondary metabolite compounds in the form of mycotoxins (Venkatesh and Keller, 2019). Aflatoxin and ochratoxin are types of dangerous mycotoxins produced by the genus Aspergillus

which commonly contaminate agricultural commodities (Navale *et al*., 2021). The effects of mycotoxin contamination on broiler growth are often associated with decreased protein use and energy conversion due to decreased nutrient efficiency and metabolic disorders (Wang and Hogan, 2019). One of the important nutrients for broiler growth is protein because protein is the main component of the formation of tendons and muscles in broilers.

Feed protein efficiency measures how efficient broilers are in converting protein contained in feed into a certain amount of body weight (Rocha *et al*., 2022). Feed protein efficiency is influenced by protein consumption and body weight gain, while protein consumption in broilers is influenced by the amount of feed consumption (Muharlien *et al*., 2020). The amount of feed intake that enters the body depends on the health condition of the broiler (Remus *et al*., 2014). Consumption of feed exposed to mycotoxins has the potential to cause disorders in the absorption and metabolism of protein organs such as the intestines and liver (Xu *et al*., 2022). Disorders in the intestines as the first digestive tract passed by mycotoxins will disrupt homeostasis, affect the structure of tissue and microflora bacteria of the digestive tract so that the function of nutrient absorption is not optimal (Liew and Mohd-Redzwan, 2018). Exposure to mycotoxins in feed, if consumed, will cause disorders in liver physiology, thereby reducing liver function in protein synthesis (Awuchi *et al*., 2022).

In sick conditions, broilers will lose their appetite because the energy to digest food is diverted by the body to repair tissue which causes a decrease in feed consumption (Kpomasse *et al*., 2021). A decrease in the amount of feed consumption will reduce the value of protein consumption resulting in low body weight gain and causing a decrease in the efficiency of broiler feed protein (Kriseldi *et al*., 2018). Efforts that can be made to increase the productivity of broilers exposed to mycotoxins are through the use of mycotoxin binders which play a role in binding mycotoxins in feed (Xu *et al*., 2022). Mycotoxin binders with a working principle like chemical sponges will bind mycotoxins in the digestive tract to then be excreted with feces, thereby minimizing the absorption and metabolism of mycotoxins which cause disruption to the work of the absorption organs and protein metabolism which causes a decrease in broiler productivity (Kihal *et al*., 2022). The purpose of this study was to determine the effectiveness of mycotoxin binder as a mycotoxin binder in increasing broiler productivity by observing daily body weight gain, protein consumption, and feed protein efficiency in broilers exposed to mycotoxins.

Materials and methods

Research design

This study used a Completely Randomized Design (CRD) with four treatment groups. The number of broiler chickens used in this study was 24. Each treatment group consisted of six broilers. The study was conducted for a total of 35 days from February to April 2023. The research and data collection location was in the Experimental Animal Cage, Faculty of Veterinary Medicine, Airlangga University, Surabaya. Proximate analysis including protein content and dry matter test of feed was conducted at the Animal Feed Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya.

Treatment

In this study, experimental animals used were DOC broiler strain Cobb 500 obtained from PT. Charoen Pokphand which were then divided into 4 treatment groups, namely:

C: Commercial feed

P1: Commercial feed + aflatoxin 0.1 mg/kg feed + ochratoxin 0.1 mg/kg feed

P2: Commercial feed + aflatoxin 0.1 mg/kg feed + ochratoxin 0.1 mg/kg feed + mycotoxin binder 1.1 g/kg feed feed

P3: Commercial feed + aflatoxin 0.1 mg/kg feed + ochratoxin 0.1 mg/kg feed + mycotoxin binder 1.6 g/kg feed feed

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Weight gain data collection

The research data included daily body weight gain obtained by weighing the broilers every week on day 14, day 21, day 28 and day 35. Daily body weight gain was calculated starting from the third week by subtracting the broiler weight on day 21 from the broiler weight on day 14 then divided by 7. Daily body weight gain in the fourth week was calculated by subtracting the broiler weight on day 28 from the broiler weight on day 21 then divided by 7, and daily body weight gain in the fifth week was calculated by subtracting the broiler weight on day 35 from the broiler weight on day 28 then divided by 7. The body weight gain in the third, fourth, and fifth weeks for each treatment sample was then added up and averaged for further analysis using ANOVA.

Protein consumption data collection

Protein consumption data is obtained from the amount of feed consumption. The amount of feed consumed by broilers is calculated every week on the 21st day, the 28th day, the 35th day and is known by subtracting the amount of feed given from the remaining feed. Broiler protein consumption is calculated by multiplying the amount of feed consumption and the percentage of crude protein then multiplied by the percentage of dry matter known through feed analysis. Protein consumption in the third, fourth, and fifth weeks for each treatment sample is then added up and averaged for further analysis using ANOVA.

Crude protein analysis

Crude protein analysis is carried out to determine the percentage of crude protein content of a feed consisting of 2 stages. The first stage is destruction which is carried out by inserting 0.5 grams of sample into a Kjeldahl flask and adding 10 cc of saturated sulfuric acid. The liquid is then heated until the color of the solution becomes clear and then left to cool and does not smoke. After cooling, the liquid in the Kjeldahl flask is put into a measuring flask and added with distilled water to the point of 250 cc then transferred into an Erlenmeyer flask to be shaken. After being homogeneous, a sample of 10 cc is taken for the distillation and titration process.

The second stage is the distillation process using a marcam steel apparatus with an Erlenmeyer flask containing a mixture of 10 cc of boric acid, 2 drops of methyl red, and 3 drops of bromocresol green placed on the outlet. A total of 10 cc of the destruction sample was inserted into the marcam steel inlet, then 5 cc of 40% NaOH was added. The 2000 cc distillation flask was filled with 1000 cc of distilled water, then heated to boiling and the Erlenmeyer flask was filled with 50 cc. In the Erlenmeyer flask that had been filled with 50 cc, 0.01 N sulfuric acid was added by dripping it slowly while shaking the Erlenmeyer flask. This step was carried out until the liquid in the Erlenmeyer flask turned pink and lasted for some time, indicating that the titration process was complete. The percentage of crude protein was calculated using the following formula:

$$Crude\ protein\ content = \frac{Titration\ volume\ x\ 0.01\ x\ 0.014\ x\ 6.25\ x\ 25}{Sample\ weight}\ x\ 100\%$$

Dry matter analysis of feed

Dry matter analysis of feed was conducted to determine the percentage of crude protein content of a feed. Feed groups K, P1, P2, and P3 were each taken as much as 5 grams. The first step, the aluminum cup was heated in an oven at a temperature of 105° C for 1 hour to remove the water content in the cup. The cup was then cooled in a desiccator for 5 minutes to avoid the formation of water vapor by the air. The cooled cup was then weighed, the weighing results were recorded. After obtaining the weight of the empty cup, 5 grams of feed sample was added to the cup. The cup containing feed was then put into an oven at a temperature of 105 ° C for 2 hours. After the oven process was complete, the cup containing feed was removed and cooled in a desiccator for 5 minutes. After cooling, the cup containing feed was then weighed, the weighing results were then recorded. The percentage of dry matter can be calculated by dividing the weight of dry feed by the weight of feed before being ovened multiplied by 100%.

Data analysis

The data obtained include daily body weight gain, protein consumption and feed protein efficiency analyzed using ANOVA analysis of variance. If there is a significant difference, it is continued with Duncan's Test.

Result

The results of one-way ANOVA data processing presented in table 1, show significant differences in several treatment groups which were then continued with the Duncan Test. Based on the results of the Duncan test, it is known that group P1 which was given feed exposed to mycotoxins had the lowest daily body weight gain compared to the control group, P1, and P2. The addition of mycotoxin binder to groups P2 and P3 as much as 1.1 and 1.6 g/kg of feed was able to significantly increase body weight gain in broilers exposed to mycotoxins compared to group P1 ($p<0.05$), but did not experience a significant increase compared to the control group ($p>0.05$).

Table 1. Average and standard deviation (SDD) of daily body weight gain, protein consumption, and protein feed efficiency in broilers given mycotoxin binder and exposed to mycotoxins

Treatment	Daily Body Weight Gain ± SD (g/head/day)	Protein Consumption ± SD (g/head/day)	Feed Protein Efficiency ± SD (%)
C	64.65^{b} ± 4.25	19.99^{c} ± 0.51	364.43^{a} ± 19.72
P1	57.50^{a} ± 2.41	15.45^{a} ± 0.34	426.18^{b} ± 17.68
P2	68.78^{b} ± 4.78	20.51^{d} ± 0.19	377.28^{a} ± 22.49
P3	67.17^{b} ± 3.63	17.50^{b} ± 0.26	435.22^{b} ± 27.08

Note: Different superscripts in the same column indicate significant differences ($p<0.05$)

The results of Duncan's test on protein consumption showed a significant difference in each treatment group with the lowest amount for group P1. The addition of mycotoxin binder of 1.1 g/kg feed in group P2 significantly increased protein consumption compared to the control group, P1, and P3 ($p<0.05$) 0.05). The highest to lowest protein consumption was shown by groups P1, P3, C, and P2, respectively.

The difference in feed protein efficiency was shown by groups P3 and P1 which experienced a significant increase compared to groups P2 and C ($p<0.05$). The average feed protein efficiency had the highest value in groups P3 and P1 and the lowest feed protein efficiency value in groups P2 and C.

Discussion

Daily weight gain

Weight gain is a series of body metabolism that indicates growth in broilers (Gorenz *et al.*, 2024). Table 1 shows that the group exposed to mycotoxins without the addition of mycotoxin binder had the lowest daily weight gain which was significantly different from the control group and the group with the addition of mycotoxin binder. Aflatoxin contamination in feed causes negative effects that affect the daily weight gain (Average Daily Gain) of broilers due to decreased protein consumption and impaired protein metabolism (Yunus *et al.*, 2011).

Before being metabolized by the body, the protein contained in the feed will first be absorbed by the microvilli in the digestive tract and then synthesized into amino acids used in the formation of meat (Kiela and Ghishan, 2016). Direct contact with feed contaminated with mycotoxins causes damage to the digestive tract epithelium which has the potential to disrupt the process of absorption of feed nutrients (Yunus *et al.*, 2011). The epithelium plays a role in maintaining the balance of digestive tract tissue by proliferating lost or damaged cells through the apoptosis mechanism (Ramachandran and Madesh, 2000). Mycotoxins can affect the apoptosis mechanism of the digestive tract, thereby disrupting the cell proliferation process (Liew and Mohd-Redzwan, 2018).

The results of statistical analysis showed a significant increase in daily body weight gain for the control group and the group given additional mycotoxin binder in the feed. Phycophytic elements or algae in mycotmycotoxin binder contain nutrients such as beta carotene which will

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be converted into vitamin A in the body and play a role in the proliferation of digestive tract cells such as the intestines (Wang *et al*., 2022). Phytogenic compounds in the form of complex terpenoids contained in mycotmycotoxin binder play a role in reducing inflammation (Riaz *et al*., 2023). Active phytogenic compounds together with Vitamin A play a role in maintaining the health of the digestive tract mucosal membrane and increasing nutrient absorption in the digestive tract so that good growth is produced (Shehata *et al*., 2022). Mycotoxin binder as a mycotoxin binder in the digestive tract acts as a preventive agent to prevent the absorption of mycotoxins by the digestive tract through mycotoxin excretion with feces (Papatsiros *et al*., 2023).

Protein consumption

Broiler protein consumption is influenced by the amount of feed consumption and the nutritional content of feed such as crude protein and dry matter (Beski *et al*., 2015). The results of the study on protein consumption showed the lowest amount in the group exposed to mycotoxins without the addition of mycotoxin binders which was significantly different from the control group and the group with the addition of mycotoxin binders. The results of the proximate analysis showed that the crude protein content p would be the lowest in group P1. The decrease in feed nutritional content can be caused by exposure to mycotoxin-producing molds that have the potential to damage the structure of feed during storage, both physically, chemically, and biologically so that it has the potential to reduce palatability and reduce nutritional value (Kępińska-Pacelik and Biel, 2022). The low protein consumption in the group exposed to mycotoxins without the addition of mycotoxin binders was also caused by low feed consumption. The amount of feed consumed depends on the health condition of the broiler. When sick, chickens will lose their appetite because the energy to digest food is diverted by the body to fight infection and tissue repair so that feed consumption will decrease (Yasar and Forbes, 1999). In healthy conditions, chickens will consume sufficient feed to support their energy and metabolic needs.

The results of statistical analysis showed the highest protein consumption in the group with the addition of mycotoxin binder as much as 1.1 g/kg feed, in line with the increase in feed consumption and the increase in crude protein content in the feed compared to the group exposed to mycotoxins without the addition of mycotoxin binder. Mycotoxin binder consists of phycophytic elements such as microalgae containing protein. Nutritional and toxicological studies show the suitability of algae biomass as a feed supplement to replace conventional protein (Wu *et al*., 2023). Protein-producing microalgae from the species *Chrorella* sp., *Arthrospira* sp., *Porphyrium* sp., and *Haematococcus* sp. have been studied to be able to increase poultry productivity and health (Patel *et al*., 2021). The addition of protein from the species *Spirulina* sp. can increase poultry immunity which is indicated by an increase in the number of white blood cells (Alaqil *et al*., 2023). It is estimated that around 30% of current algae production is used in animal feed, especially poultry because of the promising prospects and benefits for commercial ventures (Wu *et al*., 2023).

Feed protein efficiency

Feed protein efficiency indicates how efficient livestock are in using protein for growth, which is expressed in the ratio between body weight gain and protein consumption. Lower body weight gain than protein consumption will result in low feed protein efficiency. Based on the research results shown in table 1, the use of mycotoxin binder with a dose of 1.1 g/kg feed can increase feed protein efficiency and produce higher body weight gain with lower protein consumption and feed consumption so that it can reduce production costs in broiler conditions contaminated with feed containing mycotoxins.

Broiler growth is obtained from the results of feed protein metabolism that produces amino acids. One of the organs that plays a role in protein metabolism is the liver. The liver is responsible for most of the proteins circulating in the body so that liver tissue damage will disrupt

the protein utilization mechanism and affect the growth performance and overall health of the broiler (Ahmad *et al*., 2022). The liver has a detoxification function that converts toxic compounds or poisons into substances that are physiologically inactive through the action of bile enzymes (Tan *et al*., 2024). The occurrence of toxin binding with liver cells for a long period of time has the potential to cause physiological function disorders due to abnormalities in the liver cell parenchyma. Mycotoxin binder plays a role in deactivating toxins through the work of the BBSH 797 microbial strain which produces specific enzymes to degrade mycotoxin functional groups and convert them into non-toxic metabolite compounds that are safe for the liver (Schatzmayr *et al*., 2006). The hepatoprotective properties of mycotoxin binder through flavonolignan binding with liver receptors will prevent toxins from entering the liver cell membrane (Quesada-Vázquez *et al*., 2024). The effect of flavonolignans on broilers has been shown to be effective against hepatotoxicity and has a positive effect on feed consumption and body weight gain (Tan *et al*., 2022).

Conclusion

Administration of the mycotoxin binder Mycofix® Plus 3.0 at a dose of 1.1 grams/kg of feed can increase daily body weight gain, protein consumption, and feed protein efficiency in broilers exposed to aflatoxin B1 and ochratoxin A types of mycotoxins in feed.

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