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Research Article

# EFFECT OF ANTI-MOLD AND MYCOTOXIN BINDER ON CORN QUALITY AND BROILER PERFORMANCE

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## ARTICLE HIGLIGHTS

- The anti-mold used effectively maintained low aflatoxin levels in 13% moisture corn.
- Synthetic mold inhibitors effectively decreased aflatoxin levels in corn during storage and maintained some nutritional quality
- Mycotoxin binder supplementation did not improve broiler performance.
- Broiler performance declined as aflatoxin concentrations increased.

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## ABSTRACT

The quality of animal feed is determined by high-quality ingredients and appropriate feed additives. This study aimed to assess: 1) the nutrient and aflatoxin total (AT) content of corn treated with an anti-mold (A) and 2) broiler performance fed with aflatoxin B1 (AFB1)-contaminated diets supplemented with a mycotoxin binder (MB). Two experiments were carried out to achieve the objectives. Experiment 1 was set up with a 2 x 2 Factorial Completely Randomized Design (FCRD) with two factors: moisture content (MC at 13 and 15%) and anti-mold (A, -/+). Meanwhile, Experiment 2 was set up with a 3 x 2 Factorial Completely Randomized Design with two factors: the AFB1 (< 100, 165, 222 µg/kg) and MB (-/ +). The MC and A interaction was significant ($P < 0.01$) on the aflatoxin total of corn throughout the 2-month assay. The utilization of the anti-mold in afla-corn with different moisture levels did not influence ($P > 0.05$) the corn's nutrient content. The MC x A interaction was significant ($P < 0.05$) in the valine and glycine content of the stored corn. In the second experiment, no interaction of AFB1 x MB ($P > 0.05$) was observed in the bird's performance during the study. The AFB1 Concentration (AC) of corn decreased significantly ($P < 0.05$ to 0.001) in feed intake, body weight gain, and feed efficiency of birds. Our study concluded that the anti-mold effectively maintained low aflatoxin levels in 13% moisture corn. Also, the anti-mold did not affect the nutrient profile of corn during storage. Our study also showed that mycotoxin binder supplementation did not improve broiler performance and broiler performance declined as aflatoxin concentrations increased.

**Keywords**: caflatoxin, mold inhibitor, nutrient, performance, toxin binder

## INTRODUCTION

Mycotoxins, toxic compounds produced by pathogenic fungi, can infect animal feed and cause various negative effects on livestock. Aflatoxin, Ochratoxin, Zearalenone, and Fumonisin are examples of mycotoxins. Aflatoxin was classified into two strains, namely large (L) and small (S) strains, where the most dangerous strains were the small strains (Norlia *et al.* 2019; Almatakeez 2020). According to Mohammed *et al.* (2021), the L-strain Aflatoxin produced sclerotia with a size of > 400 µm, whereas the S-strain had a sclerotia with a size of < 400 µm.

Grains such as corn and peanuts are favorable media for the growth of *Aspergillus* spp. The presence of *Aspergillus flavus* in corn could be identified by the bright greeny-yellow fluorescence and black light test (Stack & Carlson 2003). The growth of *Aspergillus* spp. in corn kernels depends on several factors, such as water activity, pH, and relative humidity (Shehu & Belo 2011; Norlia *et al.* 2019). According to Shehu and Bello (2011), the favorable temperature and relative humidity for *Aspergillus* spp. to grow well were 30 - 35 oC and 85 - 100%, respectively.

The growth of fungi involves two forms: yeast-like cells and mycelial growth. During their development, fungi need nutrients, such as carbohydrates (simple and complex sugars), proteins (for C, H, O, and N sources), and certain minerals (Liu *et al.* 2016; Barzee *et al.* 2021). All nutrients are taken from the substrates on which the fungi are growing. Grains, such as corn, infested by fungi, such as *Aspergillus* spp., may experience a decrease in nutrient components due to the utilization of corn's chemical components by fungi. According to Liu *et al.* (2016), saccharides and proline are utilized by *Aspergillus flavus* for its mycelium propagation and the making of Aflatoxin B1.

The presence of aflatoxin in corn not only deteriorates corn quality but also triggers diseases and death in animals when being consumed. Aflatoxins are mutagenic, teratogenic, and carcinogenic compounds (Okechukwu *et al.* 2023). The amount of aflatoxin in the diet, the type of birds, and age of bird influence the harmfulness level of aflatoxin. Ducks, turkeys, and chickens are resistant to aflatoxicosis, with ducks being the most resistant species (Wu *et al.* 2021; Murcia & Diaz 2020). According to Diaz and Murcia (2019), ducks produce the highest Aflatoxin $B_1$-dihydrodiol, causing the acute toxic effect of Aflatoxin B1.

Several efforts to control the growth and spread of toxin-producing fungi are by drying the corn immediately after harvest, by storing the corn on top of the pallets in a storage room, by using clean feed materials, and by using fungal inhibitor agents, such as charcoal and organic acids. Charcoal as an inhibitor agent works by binding to mycotoxins and excreting them through chicken excreta, thus reducing the amount of toxin absorbed in the body.

The application of certain commercial fungal inhibitors in feed succeeded in suppressing the *A. flavus* development but changed certain amino acid profiles in corn (Elsamra *et al.* 2012). Supplementation of 0.045% *Mintai Feed Anti-mold* effectively protected the nutritional characteristics of low-moist corn (Nalle *et al.* 2022).

Regarding the use of mycotoxin binder, Nalle *et al.* (2021) proved that Mycosorb, as a toxin binder product, did not augment the productivity of birds given a low-dose AFB1 diet. On the contrary, Fernandes *et al.* (2022) claimed that commercial toxin binders effectively increase the feed efficiency of birds that received aflatoxin diets. This indicated a contradictory result in terms of using mycotoxin binders in feed.

Feed security is still a worldwide issue, so it is important to intensively evaluate the strategy to maintain corn quality, especially using mold inhibitors (synthetic or natural). In Ethiopia, for example, a study found that 94% of poultry feed samples were contaminated with aflatoxins with a range of levels of 18 µg/kg to 190.18 µg/kg, exceeding the FDA's regulatory limit of 20 µg/kg in 72.75% of samples (Kassaw *et al.* 2022). In Northern Pakistan, 92.5% of poultry feed samples were found positive for aflatoxins, with grower feeds exhibiting the highest contamination levels (Naveed *et al.* 2022). These findings highlight the widespread aflatoxin contamination in broiler feed across different regions. The economic impact is considerable, as aflatoxins can impair poultry health, leading to reduced growth rates, lower feed conversion efficiency, and increased mortality, thereby affecting overall productivity and profitability in the poultry industry. Aflatoxins in poultry products pose a risk to human health, potentially leading to aflatoxicosis upon consumption. Therefore, monitoring and controlling aflatoxin levels in poultry feed are crucial to mitigate these adverse effects. Considering the above problems, two experiments were conducted. In the first experiment, mold inhibitors were applied to corn with different moisture content (13% and 15%). Meanwhile, the mycotoxin binder was added to the Aflatoxin B1 diets in the second experiment.

## MATERIALS AND METHODS

### Experiment I: Trial on Mold Inhibitor

#### *Primary Materials*

The primary materials of this experiment were shelled corn and anti-mold. The shelled corn used complies with the quality requirements of corn as feed (Indonesian National Standard Board 2013), as presented in Tables 1 and 2.

Table 1 Initial quality assessment of corn used in Experiment I

| Moisture level | Whole seed | Broken seed | Moldy seed | Aflatoxin total (µg/kg) |
|---|---|---|---|---|
| ……%..... | | | | |
| 13 | 91.98 | 2.09 | 4.85 | 29.5 |
| 15 | 89.90 | 2.20 | 5.00 | 52.4 |

Table 2 Quality standards for corn used as animal feed

| No | Parameter | Unit | Requirements | |
|---|---|---|---|---|
| | | | Grade I | Grade II |
| 1 | Moisture Level (max) | % | 14 | 16 |
| 2 | Crude Protein (min) | % | 8 | 7 |
| 3 | Aflatoxin (max) | µg/kg | 100 | 150 |
| 4 | Damaged seed (max) | % | 3 | 5 |
| 5 | Moldy seed (max) | % | 2 | 5 |
| 6 | Broken seed (max) | % | 2 | 4 |
| 7 | Foreign objects | % | 2 | 2 |

The anti-mold product contains active compounds including propionic acid (57%), lead (0.003%), and arsenic (0.0054%). The anti-mold dose of 45g per 100 kg of feed was applied according to the manufacturer's recommendation. The concentration of lead and arsenic contained in the mold inhibitor used in this experiment were below the maximum permitted levels (2 ppm in feed material) by the EC Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed to minimize the contamination risk of these toxic substances. This regulation is in place to prevent the accumulation of harmful metals in the food chain, which can cause serious health issues, such as organ damage and cancer in animals and humans (Zwolak 2020).

### *Experimental Design and Statistical Analysis*

The experiment was performed in a 2 x 2 Factorial Completely Randomized Design with 2 moisture levels (13% and 15%) and anti-mold (-, +), resulting in four treatments altogether. Every treatment consisted of five replicates, each containing 20 kg of corn.

A two-way analysis of variance (ANOVA) was used to analyze the data using SAS Application software (AS OnDemand). The significant difference between each treatment was further analyzed using Fisher's Least Significant Difference test.

### *Sampling Procedure*

Corn grains (with and without mold inhibitor) were stored from day 1 to day 60. On day 60, the corn grains were sampled using the Quartering method, referring to Gerlach *et al.* (2002), followed by a Seed Sampler to obtain the representative sample. The laboratory samples were then chemically analyzed according to the measured parameters.

### *Chemical Analysis*

The proximate and gross energy content was analyzed using the Official Method of AOAC (AOAC 2005). The Thin Layer Chromatography tool was operated to determine the aflatoxin total contained in the corn samples (Bainton *et al.* 1980). The analysis principle used was that aflatoxins in the sample were extracted with methanol and defatted with n-hexane. The clean-up process was carried out using chloroform, and aflatoxins were identified through the TLC method. The detection limits for TLC were 3.01 ppb for AFB1, 3.50 ppb for AFB2, 0.54 ppb for AFG1, and 1.0 ppb for AFG2. The amino acid concentration was identified and quantified using a chromatogram method.

## Experiment II: Performance Trial using Mycotoxin Binder

### *Agreement Letter on Laboratory Animal Ethics*

The approval from the Animal Ethics Committee of the Veterinary Medicine Faculty at the University of Nusa Cendana, Kupang-Indonesia, became the basis for conducting this study. The approval was stated in a formal agreement letter, number 002 KEH/SK/VIII/2023, signed on 7 August 2023.

### *Animals and Rearing Management*

A total of 216 one-day-old broiler chicks of the Lohmann strain, each with an average body weight of 40.7 ± 0.2 g, were placed in 24 experimental pens, with a density of 9 chicks per pen. The chicks remained in the pens for 28 days, during which the feed and water were provided ad libitum. Each pen measured 70 cm in length, 70 cm in width, and 70 cm in height, with the floor covered by 5 cm of paddy husk litter. A 75-watt electric bulb was provided in each pen for heating, while two 45-watt bulbs were hung on the ceiling for lighting. The chicks were exposed to continuous light for the first five days to acclimate to a temperature range of 32 °C to 34 °C. After this acclimation period, light was provided only at night to help gradually reduce the temperature to the standard range of 22 °C to 24 °C. Thermo-hygrometers were used to monitor temperature and humidity in the housing, with measurements taken in the morning and afternoon.

### *Primary Materials*

The primary materials used in this study were corn contaminated with Aflatoxin B1 (AFB1) and *Alvitox™ Bio* supplied by a local poultry feed mill. *Alvitox™ Bio* works as an inhibiting and toxin-binding agent. Each 100 g of *Alvitox™ Bio* contains Mannan Oligosaccharides 4.0 g, Hydrated Sodium Calcium Aluminosilicate 80 g, Propionic acid 1g, Acetic acid 1g, Benzoic acid 0.80 g, activated charcoal 2g, and calcite. The recommended dosage of *Alvitox™ Bio* for industry is 0.05 to 0.1%. The dose of *Alvitox™ Bio* used in the experimental diet was 0.07%.

The laboratory sample of Aflatoxin-B1-contaminated corn (13% and 15% water content) was obtained through the Quartering sampling procedure, followed by a Seed Divider (RETSCH PT 100). The Quartering sampling procedure started by arranging the corn grains in a cone shape and then dividing them into quarters. Two opposite quarters were discarded, and the remaining two quarters were combined. This process was repeated several times until the desired sample size was achieved.

In the Retch seed divider sampling method, corn grains were placed into the hopper of the divider, and the sampler used a rotating mechanism to evenly distribute the grains into small, uniform portions across eight separate bottle containers. Lastly, the seeds from each container were ground with a disk mill and laboratory sample mill (FOSS CT 193 Cyclotec™, 0.5 mm screen size). Subsequently, the ground corn was analyzed to get the initial concentration of Aflatoxin B1 using Thin Layer Chromatography. The initial AFB1 concentration of the corn was < 60 ppb. After getting the initial AFB1 content (< 60 ppb), the corn grains were stored for one year to increase the AFB1 content naturally. After one year, the corns were sampled, reduced, ground, and analyzed to evaluate the change in the AFB1 content of the corns. The aflatoxin concentration of corn obtained after one year of storage was 132 ppb to 504 ppb.

### *Experimental Diets*

Eight experimental diets were formulated using Aflatoxin-B1-contaminated corn (Table 6). The experimental diets (Table 3) in mash form were supplied to the birds for four weeks. The formula to compute the AFB1 concentration in each experimental diet was as follows:

Volume1 x Concentration1 = Volume2 x Concentration2

Table 3 The experimental diets for assay purposes (g/100 as fed)

| Feed ingredients | Without *Alvitox™ Bio* | With *Alvitox™ Bio* |
|---|---|---|
| Shelled Corn | 60.07 | 60.00 |
| SBM* containing 44% Protein | 25.36 | 25.36 |
| MBM** | 5.00 | 5.00 |
| Local fish meal | 2.50 | 2.50 |
| Palm oil | 5.00 | 5.00 |
| L-Lysine HCl 99% | 0.20 | 0.20 |
| DL-Methionine 98% | 0.30 | 0.30 |
| Limestone powder | 0.50 | 0.50 |
| DCP*** | 0.40 | 0.40 |
| NaCl | 0.25 | 0.25 |
| NaHCO3 | 0.12 | 0.12 |
| Vit-Min supplement | 0.30 | 0.30 |
| *Alvitox™ Bio* | - | + |
| **Total** | **100** | **100** |
| Calculated Analysis | | |
| AME**** (Kcal/kg) | 2950 | 2950 |
| Crude Protein (g/kg) | 207 | 207 |
| Cellulose+Hemicellulose+Lignin | 20.0 | 20.0 |
| Lysine (g/kg) | 12.1 | 12.1 |
| Methionine + Cysteine (g/kg) | 8.5 | 8.5 |
| Calcium (g/kg) | 9.1 | 9.1 |
| Non phytate Phosphorus (g/kg) | 4.3 | 4.3 |
| Ratio of Calcium:Phosphor | 2.1 | 2.1 |

Notes: SBM = Soybean Meal; MBM = Meat and Bone Meal; DCP = Dicalcium Phosphate;
AME= Apparent Metabolizable Energy

### *Experimental Design and Statistical Analysis*

A 3 x 2 Factorial Completely Randomized Design consisting of AFB1 concentration (AC) and Mycotoxin Binder (MB, *Alvitox™ Bio*) factors was applied to this experiment. The AC of < 100, 165, and 222 µg/kg were used in this study. The parameters observed were the intake of feed (g/bird), the gain of body weight (g/bird), and the ratio of feed conversion (g/g). Below are the explanations of each parameter:

1. Feed Intake (g/bird): Feed intake is the difference between the amount of feed provided and the remaining feed, measured weekly. The results are adjusted to account for any spilled feed.
2. Body Weight Gain (g/bird): Body weight gain is determined by subtracting the initial weight from the final weight. The measurements were taken weekly.
3. Feed Conversion Ratio (g/g): The feed conversion ratio (FCR) is calculated by comparing feed intake to body weight gain (BWG), with adjustments made for dead weight.

The detailed treatment combinations are as follows:

| Code | Dietary Treatment |
|---|---|
| A | Aflatoxin B1 < 100 µg/kg |
| B | Aflatoxin B1 < 100 µg/kg + MB (0.07%) |
| C | Aflatoxin B1 165 µg/kg |
| D | Aflatoxin B1 165 µg/kg + MB (0.07%) |
| E | Aflatoxin B1 222 µg/kg |
| F | Aflatoxin B1 222 µg/kg + MB (0.07%) |

All data were arranged and analyzed using two-way analysis of variance (ANOVA) using SAS Application software (SAS OnDemand). The Fisher's Least Significant Difference test was performed when the ANOVA test appeared significant at a P value less than 0.05.

## RESULTS AND DISCUSSION

### Experiment I

#### *Changes of Aflatoxin Total in Corn*

Table 1 describes the initial quality of corn used for the experiment. As shown in Table 1, the broken seeds were observed at ≤ 2.20%. The moldy corn seeds were at ≤ 5%, and the aflatoxin total of 13% Moisture Content (MC) corn grains were 29.5 ppb and 56.6 ppb for 15% MC corn seeds before the trial. The AT of corn increased after 60 days of the test; however, a substantial increase was detected in the group without a mold inhibitor and with the higher MC (Table 4).

The present result indicated that mold inhibitor (MI) decreased the AT of corn with ML 13% and 15%. The percentage of AT reduction was 285.3 ppb and 144.6 ppb in corn, with 13% and 15% ML, respectively. The results suggested that the active compound of the mold inhibitor product worked effectively to constrain the metabolism of pathogenic molds. The AT of corn with 15% ML was higher (2,310 ppb) than that of the AT level of ML 13% corn. The result indicated that the use of MI in 15% ML aflatoxin corn was not valuable since the AT still increased beyond the maximum standard requirement of BSNI (2013, Table 2).

The outcome of this trial did not align with Nalle *et al.* (2022) due to methodological variations, particularly in moisture content and the initial corn condition. The study conducted by Nalle *et al.* (2022) used freshly harvested corn with low moisture content (< 12%), while the current study used Aflatoxin-B1-contaminated corn with a higher moisture content (> 12%).

The corn grains' final aflatoxin total (AT) is presented in Figures 1, 2, and 3. Statistical analysis shows that Moisture Content (MC), Anti-mold (A), and the MC x A interaction affected (P < 0.001) the corn AT for the period of the experiment.

As can be seen in Figure 1 (the interaction between MC and A), the Aflatoxin Total (AT) of 13% and 15% MC corn treated with the anti-mold product was less (P < 0.05) compared to the 13% and 15% MC corn without anti-mold supplementation.

It is interesting to note that the 13% ML corn that had been contaminated with aflatoxin (29.5 ppb, Table 3) before the experiment remained low in aflatoxin total (AT) (38.7 ppb, Table 3) after 60 days of the experiment (Table 4) due to the addition of anti-mold 0.07%. These corn grains still fulfilled the maximum aflatoxin level required by the Indonesian National Standard Board (2013), which was 50 ppb for the first quality and 100 ppb for the second quality of corn as feed.

This low AT level of corn after the experiment suggested that the anti-mold product used in this assay effectively prevented the further growth of *Aspergillus* spp. that had already existed in corn seed before the experiment. As a consequence, the production of aflatoxin was also kept low.

On the other hand, the aflatoxin total of 15% MC corn grains added with MI increased from 56.6 ppb to 237.4 ppb after the experiment, which was beyond the maximum level (100 ppb, second quality of corn as feed) required by the Indonesian National Standard Board (2013). Thus, these corn grains cannot be used as feed ingredients in the feed industry for complete feed making.

Regarding the Moisture Content (MC) effect, the Aflatoxin Total (AT) of 15% MC corn was higher (P < 0.05) than that of the 13% MC corn (Fig. 2). This result contradicted the findings of Nalle *et al.* (2022), likely due to the difference in methodology. The findings suggested that storing corn kernels with high moisture content that have already been contaminated with *Aspergillus* spp. and aflatoxin is risky, as the fungi can rapidly multiply and produce high levels of aflatoxin.

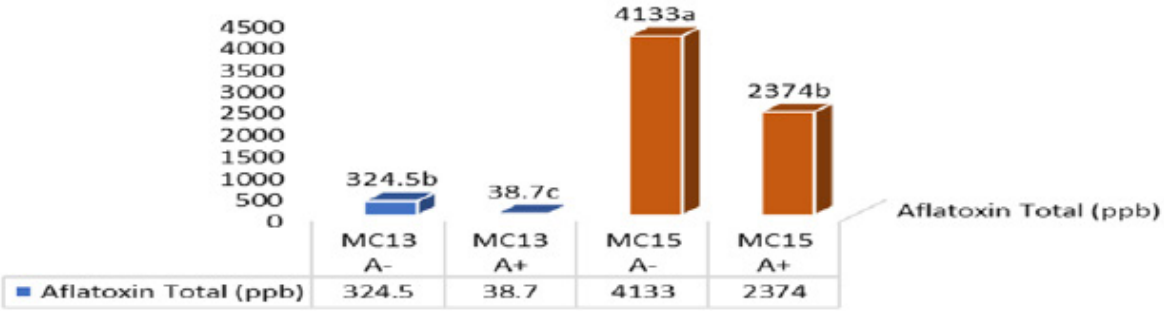


Figure 1 Moisture content (MC, 13% and 15%) and anti-mold (A, -, +) interaction on Aflatoxin Total (ppb); Notes: P < 0.0001; SEM = 65.92.

Regarding the mold inhibitor effect (Fig. 3), anti-mold (A) significantly reduced ($P < 0.05$) the AT of shelled corn, which was in contrast with the findings of Nalle *et al.* (2022). The discrepancies may be attributed to differences in methodology. Nalle *et al.* (2022) used shelled corn with a moisture content of less than 12%, whereas this study used dried shelled corn with a moisture content ranging from 13% to 15%. Additionally, Nalle *et al.* (2022) worked with freshly harvested corn free of aflatoxin contamination at the start, while this experiment used aflatoxin-B1-contaminated corn from the outset (Table 1).

The result indicated that it is important to add mold inhibitors to prevent the physical and chemical deterioration of corn seeds. The reduction in aflatoxin levels due to the addition of mold inhibitors is attributed to the action of active compounds contained in the mold inhibitor, such as propionic acid, lead, and arsenic acid. Amari *et al.* (2017) and Yun and Lee (2016) highlighted that propionic acid eliminates fungal cells through a distinct mechanism that involves apoptosis signaling mediated by mitochondria.

Regarding the effect of lead on fungal cell destruction, Amari *et al.* (2017) explained that lead (Pb) binds to the fungal cell wall or membrane, impairing their plasticity and disrupting processes like cell division and elongation. The plasma membrane, which is the first to encounter lead, is particularly affected, as Pb interferes with membrane function and alters lipid compositions. Arsenic (As) compounds may lower the pH of the corn medium, creating an acidic environment that inhibits fungal growth. According to Ceci *et al.* (2020), arsenic acid can alter the pH of the medium, which in turn affects fungal metabolism and potentially influences pH changes during fungal growth.

The present experiment proved that the amount of aflatoxin in corn grains increased during the 60-day storage compared to the initial AT content before the experiment (Tables 1 and 4). However, the increase of aflatoxin in corn treated with the mold inhibitor was not as high as in the treatment groups without mold inhibitor addition.

Safe and healthy animal feed must be produced so that animals can consume good-quality feed. Therefore, the responsibility of the feed industry is to formulate and produce complete feed using good-quality raw ingredients. One of the quality requirements of complete feed is that the feed must be free from mycotoxin contamination. Fungal inhibitor implementation is one strategy for maintaining the quality of feed materials at some stage in storage. Concerning the aflatoxin, Popescu *et al.* (2022) explained that Aflatoxin B1 is the most harmful aflatoxin due to its carcinogenic effect on humans and animals. The present study indicated an increase in aflatoxin production in corn after storage for 60 days.

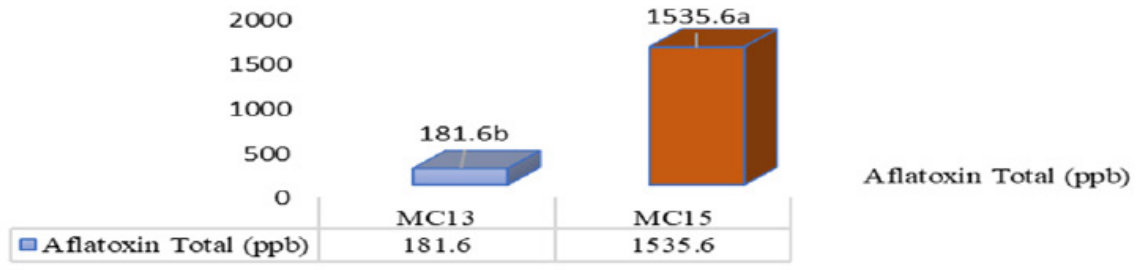


Figure 2 Moisture content (MC, 13% and 15%) effect on Aflatoxin Total (ppb)
Notes: $P < 0.0001$; SEM = 46.98.

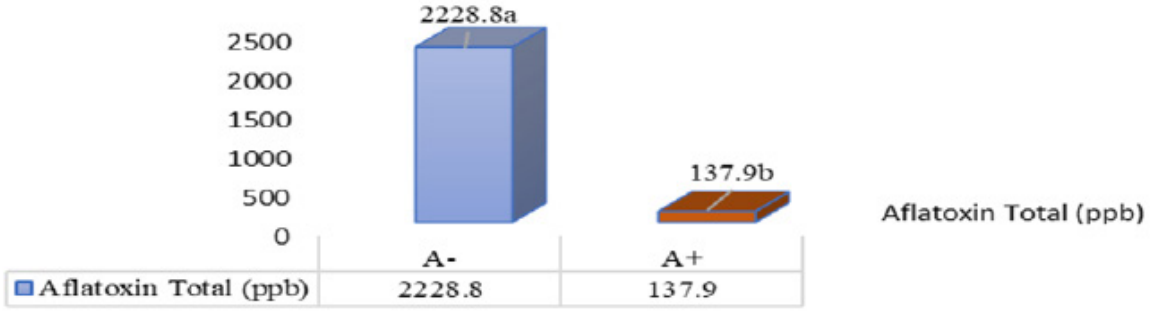


Figure 3 Anti-mold (A, -, +) effect on Aflatoxin Total (ppb)
Notes: $P < 0.0001$; SEM = 46.98.

### *The Proximate and Gross Energy Profile of Corn Grains*

Table 4 describes the chemical content of corn samples after 60 days of storage. The statistical analysis discovered an insignificant interaction (P > 0.05) between Moisture Content (MC) and Anti-mold (A) on the parameters observed.

The main effect of MC was not significant (P = 0.0501 and 0.067) for moisture and dry matter parameters but significant (P < 0.01) for the other proximate parameters and GE. The CP and ash content of corn with 15% MC was superior (P < 0.05) to 13% MC corn. The GE content of corn with 15% MC was poorer (P < 0.05) than the GE concentration of corn with 13% MC. Concerning the anti-mold factor, the anti-mold did not produce any changes (P > 0.05) in the concentration of proximate and GE content of corn.

The increased protein concentration of 15% MC corn after 60 days of storage was probably caused by the decrease of ether extract, leading to protein improvement. The other possibility was the presence of protease and crude protein of mycelial fungi (*Aspergillus* sp.) in corn. Prawira *et al.* (2015) reported that *Aspergillus flavus* could produce alkaline protease. This finding was partly comparable to Nalle *et al.* (2022).

For the anti-mold factor, the insignificant effect of MI on dry matter content was not in line with Nalle *et al.* (2022). This discrepancy was most likely a result of the disparity in methodology, especially the MC and storage duration differences.

Table 4 Proximate composition and gross energy content of corn after 60 days of storage

| Moisture content (MC) | Anti-mold | Moisture content | Dry matter | Crude protein | Ether extract | Ash | Gross energy (GE) (kcal/kg) |
|---|---|---|---|---|---|---|---|
| | | .....% as feed..... | | | | | |
| 13% | - | 11.98 | 87.98 | 7.87 | 5.98 | 2.05 | 3996 |
| | + | 12.00 | 88.00 | 7.65 | 5.69 | 1.99 | 4004 |
| 15% | - | 12.22 | 87.86 | 9.02 | 4.69 | 2.22 | 3950 |
| | + | 12.37 | 88.95 | 8.94 | 5.07 | 2.20 | 3932 |
| SEM | | 0.197 | 0.126 | 0.081 | 0.324 | 0.036 | 14.59 |
| Main Effects | | | | | | | |
| Moisture content (MC) | | | | | | | |
| 13% | | 11.99 | 87.99 | 7.76b | 5.83a | 2.02b | 4000a |
| 15% | | 12.29 | 88.40 | 8.98a | 4.88b | 2.21a | 3941b |
| SEM | | 0.114 | 0.089 | 0.057 | 0.229 | 0.025 | 10.32 |
| Anti-mold (A) | - | 12.10 | 87.92 | 8.44 | 5.33 | 2.13 | 3973 |
| | + | 12.18 | 88.47 | 8.29 | 5.38 | 2.09 | 3968 |
| SEM | | 0.114 | 0.089 | 0.057 | 0.229 | 0.025 | 10.32 |
| Probability P > F | | | | | | | |
| MC | | 0.0501 | 0.067 | <.0001 | 0.0036 | 0.0021 | 0.0033 |
| A | | 0.3501 | 0.3021 | 0.3920 | 0.3686 | 0.4563 | 0.7563 |
| MC × A | | 0.0890 | 0.0912 | 0.1362 | 0.1563 | 0.1833 | 0.09754 |

Notes: Different superscripts specify a significant difference at a P value less than 0.05;
MC = Moisture Content; A = Anti-mold.

The main effect of MC was not significant (P = 0.0501 and 0.067) for moisture and dry matter parameters but significant (P < 0.01) for the other proximate parameters and GE. The CP and ash content of corn with 15% MC was superior (P < 0.05) to 13% MC corn. The GE content of corn with 15% MC was poorer (P < 0.05) than the GE concentration of corn with 13% MC. Concerning the anti-mold factor, the anti-mold did not produce any changes (P > 0.05) in the concentration of proximate and GE content of corn.

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For the anti-mold factor, the insignificant effect of MI on dry matter content was not in line with Nalle *et al.* (2022). This discrepancy was most likely a result of the disparity in methodology, especially the MC and storage duration differences.

### *The Amino Acid Content of Corn*

Table 5 represents the amino acid profile of corn with different Moisture Content (MC) added with Mold Inhibitor (MI). The result showed that the MC x A interaction was significant (P < 0.05) only in valine and glycine (Table 5). The valine content of corn (15% ML without MI) was inferior (P < 0.05) to the other treatments. The glycine content of corn (ML 13% and 15% added with MI) was greater (P < 0.05) compared to that of 13% and 15% MC corn without MI addition. These results were not consistent with Nalle *et al.* (2022). The discrepancies were likely caused by differences in research methodology, particularly the initial quality and moisture content of the dried-shelled corn used.

Regarding the main effect of MC, the result showed that the leucine content of corn was affected (P < 0.05) by the MC. The leucine of 13% MC corn was greater (P < 0.05) than that of 15% MC corn. The present result was in contrast with Nalle *et al.* (2022), who found that moisture content did not affect the leucine content. The difference was probably due to the different research methodology.

For the anti-mold effect, it was revealed that except for valine, the amino acid content of corn was not affected by the anti-mold used, which partly agreed with Elsamra *et al.* (2012) and Nalle *et al.* (2022). Elsamra *et al.* (2012) found that anti-mold produced lower threonine, aspartic acid, cysteine, valine, isoleucine, and leucine content in corn. The disparity between the present result and the results of Elsamra *et al.* (2012) and Nalle *et al.* (2022) was perhaps attributable to the differentiation in methodology, particularly the initial condition of corn, type, and dosage of mold inhibitor.

Amino acids are organic compounds composed of amino and acid groups (Wu 2013) and play a crucial role in the animal and human body for protein synthesis. Payne & Hagler (1983) reported from their experiment that some amino acids, especially asparagine, proline, methionine, and tryptophan, stimulated aflatoxin production. Proline and asparagine were responsible for *A. flavus* development and aflatoxin production. The mechanism(s) by which proline and asparagine stimulate toxin production in culture is unknown. The present experiment indicated that the amino acids of corn that were responsible for *A. flavus* development and aflatoxin production remained stable after the experiment.

Overall, synthetic mold inhibitors effectively decreased aflatoxin levels in corn during storage and maintained some nutritional quality. However, due to the potential residue of harmful chemicals, such as arsenic acid and lead in animal products, it is preferable to use natural mold inhibitors. Consequently, it is necessary to develop natural anti-mold feed additives to avoid those health risk problems in animals and humans.

Table 5 Profile of indispensable amino acids (% as feed) in shelled corn following dietary treatments

| Moisture content | Anti-mold | Leu | Lys | Meth | Thre | Valine | Cys | Gly |
|---|---|---|---|---|---|---|---|---|
| 13% | - | 1.260 | 0.525 | 0.305 | 0.170 | 0.335a | 0.135 | 0.780b |
| | + | 1.270 | 0.535 | 0.255 | 0.170 | 0.325a | 0.145 | 0.810c |
| 15% | - | 1.185 | 0.520 | 0.340 | 0.135 | 0.280b | 0.160 | 0.795b |
| | + | 1.200 | 0.525 | 0.355 | 0.170 | 0.300a | 0.145 | 0.855a |
| SEM | | 0.014 | 0.006 | 0.027 | 0.009 | 0.019 | 0.007 | 0.011 |
| Moisture content (MC) | | | | | | | | |
| 13% | | 1.265a | 0.530 | 0.280 | 0.170 | 0.330 | 0.140 | 0.795 |
| 15% | | 1.192b | 0.522 | 0.347 | 0.152 | 0.290 | 0.152 | 0.825 |
| SEM | | 0.011 | 0.005 | 0.019 | 0.006 | 0.016 | 0.005 | 0.007 |
| Anti-mold (A) | | | | | | | | |
| | - | 1.222 | 0.522 | 0.322 | 0.152 | 0.307 | 0.147 | 0.787 |
| | + | 1.235 | 0.530 | 0.305 | 0.170 | 0.312 | 0.145 | 0.832 |
| SEM | | 0.011 | 0.005 | 0.019 | 0.006 | 0.016 | 0.005 | 0.007 |
| P value | | | | | | | | |
| MC | | 0.0044 | 0.2729 | 0.0771 | 0.2338 | 0.1011 | 0.1106 | 0.0559 |
| A | | 0.1908 | 0.5060 | 0.8864 | 0.5546 | 0.0299 | 1.0000 | 0.0554 |
| MC × A | | 0.8621 | 0.4640 | 0.3902 | 0.0773 | 0.0303 | 0.2519 | 0.0057 |

Notes: Different superscripts specify a significant difference at a P value less than 0.05;
Leu = Leucine; Lys = Lysine; Meth = Methionine; Thre = Threonine; Cys = Cysteine;
Gly = Glycine; MC = Moisture Content; A = Anti-mold.

## Experiment II

### *Feed Intake*

Table 6 shows the feed consumption of bird fed dietary treatments during 28 days of the experiment. The AC x MB interaction was significant (P < 0.05) on FI of broilers only in the fourth week of trial, but not in the first to the third week, partly coherent with Prahara *et al.* (2023), who found that toxin binder addition did not affect the feed intake of broilers. The LSD test indicated that the FI of bird fed diets containing corn 15% MC (with and without MI) was lower than other treatment combination diets. The low FI in broilers could be due to the moldy smell of diets containing high *Aspergillus* spp. According to Hell and Roth (2019), the moldy smell of grains occurred due to fungal contamination when the moisture content of grains was high. On the other hand, aflatoxins did not produce a bad taste and odor, but some grains, such as groundnut kernels, had a bitter taste when contaminated with aflatoxins (Hell & Roth 2019; Mijena & Ijara 2024).

The mycotoxin binder supplementation in the aflatoxin diets did not appear to improve the FI, as approved by Prahara *et al.* (2023). However, other researchers revealed the contrary results (Fernandes *et al.* 2022). The lack of consistency may be owing to the differences in factors, such as bird strain and age, the dosage and active compounds of mycotoxin binder used, exposure time of aflatoxin, and the aflatoxin level exposure.

Kolossova *et al.* (2011) reported that the effectiveness of mycotoxin binders varies based on the chemical properties of the mycotoxin binder and mycotoxin. Furthermore, it was explained that the total charge and charge distribution, the pore size, the accessible surface area, divergence, appearance, and solubility play a notable role in determining the effectiveness of the adsorbent.

For the first main factor, the AC had a highly significant effect (P < 0.01 – 0.001) on the FI of broiler diets on days 7, 14, 21, and 28. The Least Significant Different test showed that on days 7 and 21, consumption of broiler rations in the treatment < 100 ppb and 222 ppb was higher (P < 0.05) than in the treatment group 165 ppb. On day 14, the FI of birds in the 165 ppb diets was lower (P < 0.05) than in the < 100 ppb and 222 ppb treatment groups. The present result was similar to those observed by Zhu *et al.* (2023),

who found that even at the lower AFB1 content (< 80 ppb), the feed intake of laying hens decreased significantly.

The tendency of birds to eat less in the aflatoxin diet group was possibly due to several factors, such as: 1) the change in the unpleasant flavor and odor of feed by aflatoxin-producing mold (Hell & Roth 2019; Mijena & Ijara 2024); and 2) the change in morphology and histology of the hemorrhaging condition of the bird's intestine disturbing the nutrients digestibility. As a result, the nutrient digestion and emptiness of the intestine will take longer than usual, leading to a lower feed intake. Monson *et al.* (2015) stated that the response and capability of birds to metabolize the aflatoxin in their diets depend on the strain of birds, the individual bird, age, and the dosage of aflatoxin.

Regarding the second main factor, the non-significant effect ($P > 0.05$) of mycotoxin binder in feed intake did not have the same result as Mendoza *et al.* (2022) and Alayande *et al.* (2023). The discrepancies were caused by methodological dissimilarity, particularly in the type of mycotoxin binder used. The present study used *Alvitox™ Bio* (powder form), while Mendoza *et al.* (2022) used a liquid toxin binder solution

### *Body Weight Gain*

The AFB1 levels x mycotoxin binder interaction were comparable ($P > .05$) on body weight gain (BWG) of broilers during the study, disagreeing with previous researchers (Fernandes *et al.* 2019; Zabiulla *et al.* 2021). The insignificant effect might be because of the non-significant effect of the mycotoxin binder on feed intake. It is generally accepted that feed intake has a linear correlation to body weight gain.

Nazarizadeh & Pourreza (2019) revealed the effectiveness of a commercial mycotoxin binder (Mycosorb) in improving the BWG of birds exposed to high dosages of aflatoxin B1 (2 ppm and 4 ppm). Moreover, Fernandes *et al.* (2019) and Zabiulla *et al.* (2021) concluded that the mycotoxin binder added to the aflatoxin diet ameliorated the BWG of broilers. The dissimilar result was possibly because of the dissimilar in mycotoxin binder product and dosage used and the level of aflatoxin in the diet.

Regarding the main effect of Aflatoxin B1 concentration (AC), the statistical analysis proved that except for the seventh-day trial, the AFB1 level significantly affected ($P < 0.01 – 0.001$) the Body Weight Gain (BWG) of birds (Table 6). The results of the Least Significant difference (LSD) test showed that the BWG of broiler chickens in the 165 µg/kg and 222 µg/kg AFB1 treatment group was lower ($P < 0.05$) than in the < 100 µg/kg treatment. The decrease in BWG was related to the reduced feed intake of birds. On the contrary, Al-Shawabkeh *et al.* (2009) reported that as the aflatoxin diet increased, the feed consumption would increase, but the BWG of birds decreased. The present result also did not agree with Nalle *et al.* (2021), who reported that the level of AFB1 did not change the BWG of birds. The differences were probably due to the differences in methodology, especially the level of AFB1 and the type and level of mycotoxin binder product used.

### *Feed Efficiency*

Table 6 presents the feed conversion ratio (FCR) of birds that received dietary treatment. It can be seen from Table 6 that, except for the 28$^{th}$ day, the AFB1 level was significant ($P < 0.05$ to $P < 0.001$) on the FCR of birds, which partly concurred with Nalle *et al.* (2021). This experiment showed that the FCR of birds fed a diet containing a higher AFB1 tended to have higher FCR. The high FCR indicated that there might be a reduction in the nutritional quality of high aflatoxin feed. Studies have shown that fungi use feed nutrients to multiply and produce toxins (Liu *et al.* 2016; Barzee *et al.* 2021).

Mycotoxin binder addition did not improve the FCR of birds ($P > 0.05$), as opposed to Fernandes *et al.* (2022) and Zabiulla *et al.* (2021) who reported an improvement in feed efficiency of broilers fed aflatoxin diets added with mycotoxin binder commercial. The difference in efficacy of mycotoxin was probably caused by the difference in mycotoxin binder type and dosage, as well as the aflatoxin level in the experimental diets. The interaction between AFB1 levels and mycotoxin binder was insignificant ($P > 0.05$) on the FCR of broilers.

Table 6 Growth response of broilers fed aflatoxin-contaminated diets supplemented with a commercial feed additive binder

| AFB1 concentration (AC, µg/kg, as feed) | Mycotoxin binder (MB) | FI (g/bird) | | | | BWG (g/bird) | | | | FCR (g/g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7 days | 14 days | 21 days | 28 days | 7 days | 14 days | 21 days | 28 days | 7 days | 14 days | 21 days | 28 days |
| <100 | - | 138 | 436 | 894 | 1298a | 78.1 | 230 | 364 | 488 | 1.767a | 1.985 | 2.516 | 2.690 |
| | + | 132 | 439 | 871 | 1229ab | 80.3 | 217 | 374 | 525 | 1.655ab | 2.038 | 2.268 | 2.345 |
| 165 | - | 119 | 365 | 703 | 1003c | 75.5 | 195 | 311 | 423 | 1.586b | 1.880 | 2.260 | 2.427 |
| | + | 127 | 404 | 734 | 1068c | 77.2 | 209 | 344 | 471 | 1.654ab | 1.937 | 2.133 | 2.282 |
| 222 | - | 139 | 439 | 824 | 1179b | 78.0 | 204 | 334 | 451 | 1.797a | 2.155 | 2.415 | 2.576 |
| | + | 146 | 462 | 857 | 1213ab | 79.4 | 202 | 327 | 433 | 1.834a | 1.744 | 2.622 | 2.651 |
| SEM | | 4.767 | 17.1 | 36.2 | 35.3 | 2.178 | 7.12 | 11.4 | 20.2 | 0.0613 | 0.099 | 0.134 | 0.141 |
| Main effects | | | | | | | | | | | | | |
| AFB1 concentration (AC, µg/kg) | | | | | | | | | | | | | |
| < 100 | | 135a | 438a | 882a | 1263a | 79.2 | 223a | 369a | 507a | 1.711ab | 2.012b | 2.392ab | 2.518 |
| 165 | | 123b | 385b | 718b | 1036b | 79.4 | 202b | 328b | 407b | 1.620b | 1.908b | 2.196b | 2.354 |
| 222 | | 143a | 451a | 840a | 1196a | 78.7 | 203b | 331b | 442b | 1.815a | 2.218a | 2.519a | 2.614 |
| SEM | | 3.042 | 12.1 | 25.6 | 24.9 | 1.540 | 5.03 | 8.05 | 14.3 | 0.043 | 0.070 | 0.095 | 0.099 |
| Mycotoxin binder (MB) | | | | | | | | | | | | | |
| - | | 132 | 413 | 807 | 1160 | 77.2 | 210 | 336 | 454 | 1.717 | 1.993 | 2.397 | 2.564 |
| + | | 135 | 435 | 821 | 1170 | 78.9 | 209 | 348 | 476 | 1.714 | 1.906 | 2.341 | 2.426 |
| SEM | | 2.151 | 8.56 | 18.3 | 17.6 | 1.089 | 3.559 | 5.69 | 10.1 | 0.031 | 0.049 | 0.067 | 0.070 |
| P value | | | | | | | | | | | | | |
| AC | | 0.0008 | 0.0030 | 0.0004 | <.0001 | 0.5750 | 0.0086 | 0.0005 | 0.0008 | 0.0037 | 0.0062 | 0.0129 | 0.0537 |
| MB | | 0.7227 | 0.5612 | 0.2396 | 0.2354 | 0.0908 | 0.8654 | 0.4337 | 0.6199 | 0.0978 | 0.9528 | 0.2448 | 0.1447 |
| AC × MB | | 0.0880 | 0.1579 | 0.1083 | 0.0190 | 0.7698 | 0.3140 | 0.2560 | 0.1101 | 0.0282 | 0.3378 | 0.2682 | 0.5301 |

Notes: Different superscripts in the similar column denote significant differences at a P value < 0.05; FI = Feed Intake; BWG = Body Weight Gain; FCR = Feed Conversion Ratio; AC = AFB1 Concentration; MB = Mycotoxin Binder.

## CONCLUSIONS

The anti-mold effectively maintained low aflatoxin levels in 13% moisture in corn without affecting the nutrient profile of corn during storage. Mycotoxin binder supplementation did not improve broiler performance. Broiler performance declined as aflatoxin concentrations increased.

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