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The Efficacy of Mycosorb in Broiler Diets Contaminated with Low Doses of Aflatoxin B1

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ABSTRACT

The objective of the present study was to evaluate the efficacy of Mycosorb in broiler diets containing a low level of aflatoxin B1 (AFB1). A total of 200 male broiler chicks (Lohmann) were randomly distributed into 20 pens (10 birds/pen). The experimental design used was a 2 x 2 factorial completely randomized design with two main factors which were the AFB1 levels (non-detectable level; 2.58 ppb) and mycotoxin binder (MB) (0 and 0.15% Mycosorb), respectively. The treatments were control diet (P1), control diet + MB (P2), 2.58 ppb AFB1 diet (P3), and 2.58 ppb AFB1 diet + MB (P3). The AFB1 diets were formulated by replacing the whole proportion of fresh corn with moldy corn containing 4.22 ppb AFB1. The results showed that except for the digestibility coefficient of crude fat (DCCF), AL x MB interaction was not significant (P>0.05) for the growth performance and DCCP. The AFB1 levels (AL) improved (P<0.001) feed intake (FI), feed conversion ratio (FCR), and reduced the DCCF of broilers. The AFB1 levels enhanced the body weight gain (BWG) of growing broilers, but it did not augmented (P>0.05) the BWG of starter broilers. The digestibility coefficient of crude protein was not influenced (P>0.05) by the AFB1 levels. DCCF of broilers who received AFB1 diets were lower (P<0.05) than that of the control diet. Mycosorb did not affect (P>0.05) all variables measured. In conclusion, 1) except for DCCF, AFB1 levels x MB interaction did not improve growth performance and DCCP; 2) the AFB1 level of 2.58 ppb in the diets increased FI and BWG of broilers, but reduced the feed efficiency and DCCF; and 3) Mycosorb did not improve all variables measured.

Keywords: Aflatoxin, Broilers, Growth performance, Mycotoxin binder, Nutrient digestibility

Introduction

Public awareness about food safety is increasing due to the increased economic status, educational level, and easy access to the internet. Therefore, the safety and healthy animal-derived products must also be the primary objective of the poultry industry. These could be started from the selective use of high-quality feed ingredients, including free from various biological contaminants such as aflatoxins. The maximum aflatoxin content in the pre-starter, starter, and finisher broiler diets is 50 µg/kg (Indonesian National Standardization Board, 2015a, b, c).

Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Coppock *et al.,* 2018). The aflatoxins produced by these two types of poisonous fungus consist of aflatoxin B1 (AFB1, $C_{17}H_{12}O_6$), B2 (AFB2, $C_{17}H_{14}O_6$), G1 (AFG1, $C_{17}H_{12}O_7$), and G2 (AFG2, $C_{17}H_{14}O_7$), with the AFB1 type is the occurrent and the most toxic (Carvajal-Moreno, 2015; Fouad *et al.,* 2019). The AFB1 causes mutagenicity, hepatotoxicity, immunotoxicity (destruction of lymphoid organ tissues), and embryotoxicity in poultry, decrease the nutrient digestibility, metabolisable energy, and bird's productivity of birds, change the gut morphology and histology (Massomo, 2020; Yang *et al.,* 2012; Marchioro *et al.,* 2013; Peng *et al.,* 2015; Monson *et al.,* 2015; Gacem and Hadj-Khelil, 2016; Galarza-Seeber *et al.,* 2016; Sineque *et al.,* 2017; Kurniasih and Prakoso, 2019). Ducks and turkeys are most susceptible to liver cancer caused by AFB1 (Diaz *et al.,* 2010; Monson *et al.,* 2015) because of the cytochrome (CYP) P450 enzyme, which is responsible for the bioactivation of AFB1 to form epoxides (aflatoxin-8,9-exo-epoxide, AFBO) in the liver microsomes. The acute sensitivity to aflatoxicosis in turkey is also because of the combination of the enzyme cytochrome (CYP) P450 and dysfunctional liver GST enzymes (Monson *et al.,* 2015).

The AFB1 residue in animal-derived products which was consumed by humans could lead to cirrhosis, liver and lung cancer (Benkerroum, 2020; Gacem and Hadj-Khelil, 2016). Liver cytochrome CYP1A2, CYP2A6 and CYP3A4 enzymes are responsible for the bio-activation of AFB1 to AFBO (Diaz *et al.,* 2010). Besides, aflatoxins cause low birth weight and semen infertility (Shuaib *et al.,* 2010). The lethal dose of AFB1 in humans varies depending on age, individual health, presence of other diseases, and body condition (Massomo, 2020).

Aflatoxicosis in poultry is influenced by several factors, for example, the strain of birds and the dose of AFB1 (Monson *et al.,* 2015); however, the results of previous studies are still contradictory. For example, Njoki (2019) proved that a feeding diet containing 14.6 and 21.95 ppb AFB1 lowered the feed efficiency of growing broilers. Denli and Okan (2006) and (Dersjant-Li *et al.,* 2003) reported that feeding 20 and 40 ppb AFB1 diets caused a deleterious effect on growth performance in growing broilers. Yunus *et al.* (2011) also reported from their review that the weight/length ratio of the whole intestine of birds decreased after 21d of dietary exposure to 20 ppb AFB1. However, Nalle *et al.* (2019) claimed that the growth performance of birds was depressed after feeding diets containing 10 and 25 ppb AFB1; but at a level of 60 ppb AFB1, the feed efficiency of broilers (14d) decreased. Kan *et al*. (1989) reported that the growth performance of growing broilers (42d) fed 50 ppb AFB1 diet was comparable to those who were fed the control diet.

Regarding the aforementioned deleterious effects caused by the AFB1, the appropriate strategy is needed to prevent or reduce the aflatoxicosis in birds. One strategy that can be done is to implement good management practices, starting from the area of planting forage crops to post-harvest handling. The use mold inhibitors in grains during the storage in silos or the supplementation of mycotoxin binders (i.e. Mycosorb and Toxfin) in a complete diet can also be applied in the feed manufactures to prevent the negative effect of mycotoxins. Mycosorb or glucomannan yeast is a commercial mycotoxin binder that contains crude protein, crude fiber, $CaCO_3$, calcium sodium hydrate aluminosilicate, dried yeast and fermentation soluble brewer yeast. Saki *et al.* (2018) explained that Mycosorb, a glucomannan-containing yeast product from cell wall, can absorb the different types of mycotoxins by forming a stable complex to reduce the harmful effect of mycotoxin in animals. According to Girish and Devegowda (2006), the Mycosorb decreases the relative organ weight by binding the mycotoxin molecule in its glucomannan matrix, which hinders its absorption from the gastrointestinal tract and the following toxin induction. The efficacy of Mycosorb has been evaluated by previous researchers (Girish and Devegowda, 2006; Moran *et al.,* 2013; Njoki, 2019; Nazarizadeh and Pourreza, 2019; Nalle *et al.,* 2019; Nalle *et al.,* 2021); however, the results were still contradictory. For example, Nalle *et al.* (2021) proved that the supplementation of 0.075% Mycosorb in the low-dose AFB1 diets did not improve feed intake, body weight gain, but improved feed efficiency of broilers during 35 d of experiment. However, Girish and Devegowda (2006) reported that Mycosorb was effective to improve the growth performance of birds fed AFB1 diets. Moran *et al.* (2013) claimed that there was a reduction in liver AFB1 residue of birds fed diets supplemented with Mycosorb. Whereas, Nalle *et al.* (2021) showed that there was no difference in AFB1 liver residue of birds between control and Mycosorb treatments. The problem of mycotoxin in birds and humans is still a world problem that requires special attention. The correct strategy to prevent the negative effects of aflatoxicosis should be extensively evaluated. Based on these considerations, a study to evaluate the growth response and nutrient digestibility of broilers diets naturally contaminated with AFB1 and supplemented with a commercial mycotoxin binder (Mycosorb) was conducted. Implementing the low dose of AFB1 in the present experiment was based on the results obtained by Nalle *et al.* (2019; 2021) and Njoki (2019) who found that even at the low level (6 to 60 ppb), AFB1 had any negative effects on the performance and nutrient digestibility traits.

Materials and Methods

Animal ethical approval

The Animal Ethics Commission of the Faculty of Veterinary Medicine-Nusa Cendana University officially accepted the animal handling procedure applied in the present study with a certificate of ethical suitability number 001 / KEH / SK / 08/2020 on August 18th, 2020.

Experimental design

This study was designed using a 2 x 2 factorial completely randomized design with the first main factor was the AFB1 level (non-detectable level or nd and 2.58 ppb) and the second main factor was the mycotoxin binder (0 and 0.15%). Thus there were four treatment combinations altogether, namely P1) control diet (nd); P2) control diet (nd) + Mycosorb, P3) AFB1 diet (2.58 ppb), and P4) AFB1 diet (2.58 ppb) + Mycosorb. The control diets (P1 and P2) were formulated using fresh corn (nd), soybean meal, bone and meat meal, and vegetable oil (Table 1). Meanwhile, the AFB1 diets (2.58 ppb) were formulated by replacing the whole proportion of fresh corn with naturally contaminated AFB1 corn (4.22 ppb). The amount of AFB1 corn was obtained from the dilution procedure Volume1 x Concentration1 = Volume2 x Concentration2 (Aly and Anwer, 2009). All the treatment diets were given to birds in mash form for 28 days. No antimicrobial growth promoters were given to the birds either through drinking water or feed during the experiment. The variables measured in this

study were feed intake (g/bird), body weight gain (g/bird), feed conversion ratio, and total nutrient digestibility coefficient (crude fat and crude protein).

Birds and housing

The present study was conducted at the State Polytechnic of Agriculture Kupang, East Nusa Tenggara Province, Indonesia. A total of 200 one-day-old male broilers (Lohmann strain), provided by PT Japfa Comfeed Tbk (in-kind contribution), were used in this study. The average initial body weight of birds was 42.01 g/bird, measured with a digital scale (max. 2000 g; readability 0.01 g). The birds were then randomly distributed into 20 pens which consisted of 10 birds/pen. In the starter period (0-21 days), the birds were reared in the floor pen covered with rice husk. A gas heater was used during the first week. Each pen also was added with a clear bulb (75 watts) for additional heating. On the 22nd day, the birds were transferred to the metabolic cages (5 birds per cage) for nutrient digestibility assay. The drinking water was available *ad libitum* through two nipple drinkers in each metabolic cage. During the experimental period (35 days), the temperature and relative humidity of housing were monitored with a thermo-hygrometer. The temperature during the 28 days of the experimental period ranged between 27 to 30ºC; and the average humidity was 70%.

Feed ingredients

The major feed ingredients used were yellow corn (fresh and moldy). A commercial mycotoxin binder (Mycosorb) was provided by Alltech Ltd distributor in Indonesia. Mycosorb or glucomannan yeast is a commercial mycotoxin binder which contains brewer's dried yeast, calcium carbonate ($CaCO_3$), dissolved beer fermentation, aluminosilicate calcium sodium hydrate, crude protein (min 18 %) and crude fiber (max 4%). The recommended dosage of Mycosorb is 0.05 to 0.2%. In the present study, the Mycosorb dose used was 0.15%, a curable dose because the corn has been contaminated with AFB1.

The production of AFB1-contaminated corn

Naturally aflatoxin contaminated corn was prepared by the following procedure: The moisture content of the fresh corn was increased by adding 10% clean water and then kept in a polyethylene bag (50 kg capacity) for two months in the feed mill storage room. The addition of clean water was conducted every other day (Mogadam and Azizpour, 2011). Moldy corn was then harvested, ground with a hammer mill (3 mm screen size; KAL-EC.2, Electric Motor 3000 RPM), mixed and sub-sampling with the Cone and Quartering method according to Campos-M and Campos-C (2017) to obtain a sample size of about 1.5 kg. Then the ground sample was reduced with a cone sample divider (Retch PT 100) to get laboratory samples. Then, the laboratory samples were milled with a sample mill (Foss CT 193 CyclotecTM) to produce samples with a particle size of 0.5 mm and then packed, labeled and sent to the SEAMEO Biotrop Bogor laboratory for aflatoxin analysis (B1, B2, G1 and G2) using High Performance Liquid Chromatography (HPLC). The AFB1 content of moldy corn was 4.22 ppb.

Chemical analysis

Dry matter, crude fat and nitrogen. The dry matter content was determined by using the convection oven method according to AOAC Official Method (AOAC, 2005). The crude fat content was determined by using a fat extraction tool (Ankom XT10). The nitrogen content was analyzed using three different stages according to AOAC (2005), which were digestion, distillation, and titration.

Gross energy. Gross energy (GE) level was determined using an Automatic Bomb Calorimeter (IKA C2000). The procedure of gross energy analysis was as follows: 1) weighed one gram of the ground sample and placed in a dish. 2) 10 cm threads were cut and tied to the fuser wire and placed under the sample. 3) The heat bomb was closed, and it was put in the bomb cylinder. Oxygen ($O_{2)}$ was added to the bomb cylinder (30 ATM/birdBAR). Two liter of distilled water was added into the bucket. 4) Bomb was put in the bucket, the ignition fire was connected, the drive ring was attached and stirrer turned on. 5) The digital temperature machine was turned on and left for 5 minutes for the temperature to stabilize. 6) The initial temperature was recorded and then the bombing was carried out by pressing the bomb button and waited for about 5 to 10 seconds for the temperature to rise. The final temperature reading was recorded when the temperature rises and then the drops.

Aflatoxin B1 (AFB1). The aflatoxin content of corn (fresh and moldy) was analyzed according the AOAC Official Method (Latimer, 2012) using High Performance Liquid Chromatography (HPLC, limit of detection: 0.28 ppb) with. The ground sample (0.5 mm screen size) was extracted with methanol: water (70:30), then filtered, diluted and passed through an immuno-affinity column which takes the specific monoclonal antibody of AFB1, AFB2, AFG1 and AFG2. The pure and isolated aflatoxin, concentrated in the column, was released from the antibody and methanol. The aflatoxin concentration was then carried out using liquid chromatography with a fluorescence detector and post column derivatization. Aflatoxin post-column derivatization could increase the detection and or respond selectively to the HPLC detector.

Determination of nutrient digestibility

Nutrient digestibility was determined using a total excreta collection (Nalle *et al.,* 2020). On day 24, a tray was placed under each cage and the excreta collection from each cage was carried out on days 25 to 28, then weighed and immediately stored in the freezer (-20 ºC) to avoid

the fermentation process. Next, the excreta was removed from the freezer, thawed, pooled within each cage, mixed, sub-sampled, oven-dried (60ºC), crushed with mortal and ground using a sample mill (screen size 0.5 mm; CT 193 CyclotecTM laboratory mill). The ground excreta and treatment diets samples were packaged, labeled and then sent to the laboratory together with the treatment rations for analysis of dry matter content (Memmert oven, 105 ºC), crude protein (Kjeldahl method), and crude fat (Fat Extractor-Ankom XT10). The chemical analysis was conducted at Nutrition Laboratory, State Polytechnic of Agriculture Kupang.

Measurements

Growth performance: The birds and feed were weighed using a digital scale on days 0, 7, 14, 21, and 28. The measurement of body weight on day 28 was taken to the birds (5 birds/cage) which were not on the digestibility assay. The feed intake was calculated by the initial quantity of feed given to the birds subtracted the leftover. The body weight gain was obtained by the difference between the final body weight and the initial body weight of birds. The number and the weight of dead birds were recorded daily and these data were used to correct the calculation of the feed conversion ratio (FCR). The calculation of FCR was carried out using the following formula according to Nalle *et al.* (2012).

$$FCR = \frac{feed\ intake}{weight\ gain + dead\ bird's\ weight}$$

The apparent nutrient digestibility coefficient: The apparent nutrient digestibility coefficient was determined using the following formula (McDonald *et al.,* 2002):

$$\text{Apparent nutrient digestibility coefficient} = \frac{(feed\ intake\ x\ nutrient\ diet) - (total\ excreta\ x\ nutrient\ excreta)}{feed\ intake\ x\ nutrient\ diet}$$

Statistical analysis

The data were analyzed using a two-way analysis of variance using SAS software, University Edition of the SAS System. The significant difference between the treatments was determined at P<0.05 and was further tested using the Duncan test.

Results and Discussion

During the experimental period, the birds exposed to AFB1 were dirtier than those fed on control diets. The feathers and cloacae of birds were dirty due to wet, smelly, and black excreta.

Growth performance

Table 2 shows the effect of treatment diets on feed intake of broilers on days 7, 14, 21, and 28. No significant interaction (P>0.05) between AFB1 level x mycotoxin binder was observed in feed intake of broilers during the 28-day experimental period, agreed with Nalle *et al.* (2019). The AFB1 level increased (P<0.001) the feed intake during the starter and grower phases. The feed intake of birds fed a diet containing 2.58 ppb AFB1 was higher (P<0.05) than those who received the control diet (Table 2). The improvement of feed intake during the starter phase (Table 2) was inconsistent with Nalle *et al.* (2019; 2021) and Njoki (2019). Nazarizadeh and

Table 1. Control diets (with and without Mycosorb)

Feed Ingredients	Inclusion Level, %	
Yellow corn	61.12	61.12
Soybean meal	25.00	24.85
Meat and bone meal	5.00	5.00
Fish meal	2.50	2.50
Vegetable oil	4.25	4.25
L-Lysine HCl	0.26	0.26
DL-Methionine	0.30	0.30
Limestone (powder)	0.50	0.50
Dicalcium phosphate	0.40	0.40
Salt	0.25	0.25
Sodium bicarbonate	0.12	0.12
Vitamin - Mineral Premix*	0.30	0.30
Mycosorb	-	0.15
Total	100.00	100.00
Nutrient composition (calculated, g/kg as fed)		
Apparent metabolizable energy) (Kcal/kg)	2,907	2,904
Crude protein	200.7	200.6
Crude fiber	20.0	20.0
Lysine	12.6	12.6
Met + Cys	8.5	8.5
Ca	9.1	9.1
Av. P	4.2	4.2
Aflatoxin B1 (ppb)	-	-

**Top Mix: Every 10 kg contain 12.000.000 IU vitamin A, 2.000.000 IU vitamin D3, 8.000 IU vitamin E, vitamin K3 2.000 mg, vitamin B1 2000 mg, vitamin B2 5.000 mg, vitamin B12 12.000.000 µg, vitamin C 25.000 mg, Calcium-D-panthotenate 6000 mg, choline chloride 10.000 mg, niacin 40.000 mg, methionine 30.000 mg, lysine 30.000 mg, mangan 120.000 mg, Fe 20.000 mg, iodine 200 mg, zink 100.000 mg, cobalt 200 mg, copper 4.000 mg, santoquin (antioxidant) 10.000 mg.*

*** Supplied by Alltech Ltd, Indonesia*

Pourreza (2019) even reported a decrease in feed intake as the increase in AFB1. The difference was probably due to the individual response of birds (strain), and the concentration of AFB1 in the diets. The concentration of AFB1 in the present study was 2.58 ppb; whereas, in the study of Nalle *et al.* (2019) and Njoki (2019) were 10 to 60 ppb, and 6 to 21 ppb, respectively. Yang *et al.* (2012) showed no difference in feed intake of broilers exposed to low-dose AFB1 (up to 60 ppb). This difference was probably due to individual response and the strain of birds used. Khan *et al.* (1990) and Bryden *et al.* (2007) reported that the genetic difference of broilers showed a different response to aflatoxicosis. Monson *et al.* (2015) explained that, for example, the presence of P450 enzymes, encoded by CYP1A5 and CYP3A37 which were found in turkey was responsible for the higher sensitivity of AFB1 compared to other strains. Bryden *et al.* (2007) explained that broiler strains have different capacities to metabolise aflatoxin. According to Diaz *et al.* (2010), the sensitivity of a species to AFB1 was determined by the presence of the specific CYP450 enzymes, as the key metabolizing enzyme, which is capable of bio-transforming AFB1 to AFBO related to poor conjugation with glutathione. The increased feed intake associated with the low dose AFB1 diet demonstrated in the present study was probably due to the difference in the level of the key metabolising enzymes, which determine the capability of each strain to metabolise aflatoxin.

The high feed intake of birds fed AFB1 diets (2.58 ppb) during the starter and grower phases (Table 2) was probably due to the physical factors of feed (colour, texture, and taste) and nutrient intake regulation of birds. The colour of AFB1 diets in the present study was greenish, and the texture was soft due to the high moisture and the color of *Aspergillus spp*. Ferket and Gernat (2006) explained in their review that the physical properties (colour, texture, and taste) of feed influenced feed intake of birds. It was further explained that the young birds prefer green colour compared to orange or blue colour. Neves *et al.* (2014) reported that poultry associated the physical characteristics of feed with its nutritional content, which shows that the perception of contact contributes to feeding identification. Furthermore, Neves *et al.* (2014) explained that when the beaks intake the feed, the birds will decide to accept or reject the feed via tactile cells. This decision is based on reflectivity and taste of feed, even though the number of taste buds in birds is small.

Regarding the nutrient intake regulation, factors associated with the nutrient intake mechanism were the glucostatic theory, the thermostatic theory, distention of the gastrointestinal tract, amino acid circulation, the intake of protein, and the lipostatic mechanism (Ferket and Gernat, 2006). Based on the glucostatic control mechanisms, the birds attempt to consume feed to meet their energy requirement as the first priority, then they will fulfill their amino acid requirement (Ferket and Gernat, 2006). In the present study, the laboratory analysis showed that the crude fat (CF) content of AFB1 diets was lower (4.4% DM) than the CF content of control diets (9.1% DM). The gross energy (GE) content of AFB1 diets was also lower (12.5 MJ/kg) than the GE content of control diets (14.2 MJ/kg). The low crude fat and gross energy contents in AFB1 diets were because of the low quality of moldy corn used in the diets, as the nutrient content of corn being used by *Aspergillus spp* for their growth. Thus, why broilers eat more feed that contains the AFB1 than the control diet to fulfill their energy requirement. Kumar *et al.* (2009) showed that the feed intake of birds went down in the same way as the feed energy level went up. The authors also explained that the central

Table 2. The effect of treatment diets on the feed intake of broilers (g/bird)

AFB1 Level (AL, ppb)	Mycotoxin binder (MB, %)	Feed Intake (g/bird)			
		7d	14d	21d	28d
nd	0	67.30	341.15	615.25	790.39
nd	0.15%	57.44	307.47	554.37	768.88
2.58	0	93.35	540.16	905.63	1332.79
2.58	0.15	103.13	640.81	921.85	1484.85
SEM		10.439	32.382	48.23	123.56
Main Factor I					
AFB1 level (AL, ppb)					
nd		62.37[b]	324.31[b]	584.81[b]	779.64[b]
2.58		98.24[a]	590.49[a]	913.76[a]	1408.82[a]
SEM		7.382	23.216	34.200	87.370
Main Factor II					
MB (%)					
0		80.32	440.66	760.44	779.64
0.15		80.29	474.14	738.13	1408.82
SEM		7.382	23.216	34.20	87.37
Probability, Pr > F					
AFB1 level (AL)		***	***	***	***
Mycotoxin Binder (MB)		NS	NS	NS	NS
AL x MB		NS	NS	NS	NS

[a,b] Different superscripts at the same row indicate significant differences (P<0.05) and highly significant (P<0.01).
* significant different (P<0.05), *** highly significant different (P<0.001).
nd = non-detectable level (Limit of detection with HPLC: AFB1 = 0.43 ppb); AFB2 = 2.02 ppb; AFG1=1.53 ppb; AFG2=0.20 ppb)

nervous system and peripheral tissues of birds make birds being able to sense energy levels and regulate feed intake.

The main effect of the mycotoxin binder did not affect (P>0.05) the feed intake, body weight gain, and feed efficiency of broilers during the trial periods (Table 2, 3, 4), partly agreed with Nalle *et al.* (2019) and Njoki (2019).

The effect of treatment diets on body weight gain (BWG) of broilers is presented in Table 3. During 1 to 21 days of the experiment, The results also proved that there was no significant interaction (P>0.05) between AFB1 level x mycotoxin binder on BWG of broilers over the 7-, 14-, 21- and 28-day feeding periods. The result was consistent with Nalle *et al.* (2019) and Njoki (2019). The ineffectiveness of Mycosorb was probably due to the level of AFB1 applied was too small, so to some extent, the birds were still able to tolerate the toxicity of AFB1. The results were inconsistent with the previous study (Nalle *et al.*, 2019; Fouad *et al.*, 2019). Njoki (2019) reported that feeding diets exposed to the low dose of AFB1 (6.1 and 14.1 ppb) and supplemented with 0.1% Mycosorb was not effective to increase the growth performance of birds; Mycosorb was effective to improve the feed efficiency of broilers when the AFB1 diet was increased to 22.0 ppb. The supplementation of 0.25% and 0.1% Mycosorb in the diets containing 1ppm and 2 to 4 ppm AFB1, respectively, ameliorated the growth performance of broilers (Saki *et al.,* 2018; Nazarizadeh and Pourreza, 2019). These differences indicated that the efficacy of Mycosorb depends on the concentration of AFB1 or Mycosorb applied in the diets. The response individual of birds also affected the efficacy of Mycosorb.

The body weight gain of birds was not affected (P>0.05) by the main effect of AFB1 level, agreed with Nalle *et al.* (2019). The improvement in feed intake of birds fed the AFB1 diet (2.58 ppb) during the starter phase (7, 14, and 21d; Table 2) did not improve the body weight gain (Table 3). This indicated the low ability of young birds to convert feed to body tissue because of AFB1 toxicity.

Over the 28-day experimental period, the AFB1 level influenced (P<0.05) the body weight gain of birds. Broilers fed a diet containing 2.58 ppb AFB1 had a higher BWG (P<0.05) compared to the control diet at 28 days of the experiment (Table 3), disagreed with Nalle *et al.* (2019) and Njoki (2019). The difference was probably due to the individual response of birds, which was related to the strain and age of the broilers used. In addition, the AFB1 level used by Njoki (2019) and Nalle *et al.* (2019) were higher (6.06 to 61.06 ppb) than the AFB1 level used in the present study (2.58 ppb).

The improvement of body weight gain of broilers was due to the cumulative improvement of feed intake during the 28-day experiment (Table 2). This indicated that growing birds were more tolerable to the AFB1 compared to the young birds. Ferket and Gernat (2006) explained that feed intake plays an important role in the improvement of body weight gain. The comparison of the increased body weight of broilers fed low levels of AFB1 diets in this experiment was difficult to be made due to the relevant research was unavailable. However, In a review of Diaz *et al.* (2008), it was reported that the maximum increase in body weight gain of broilers fed AFB1 diet 625 and 1250 ppb was about 3,3 and 1.0 to 7%, respectively. Furthermore, Diaz *et al.* (2008) also reported in their review that the significant decrease in body weight gain of broiler chickens occurred in broilers fed higher levels of AFB1 (2500 and 5000 ppb). According to these authors, the AFB_1 effects on weight gain in broilers could be a reduction at high doses or an improvement at low doses (biphasic nature or hormesis). The supplementation

Table 3. The effect of treatment diets on the body weight gain of broilers (g/bird)

AFB1 Level (AL, ppb)	Mycotoxin Binder (MB, %)	BWG (g/bird)			
		7d	14d	21d	28d
nd	0	66.90	151.98	215.54	316.86
nd	0.15	69.30	141.00	243.24	351.18
2.58	-	66.20	136.66	242.70	390.46
2.58	+	70.00	159.64	247.68	389.12
SEM		2.018	7.549	12.238	24.187
Main Factor I					
AFB1 level (AL, ppb)					
nd		68.10	146.49	229.39	334.02[b]
2.58		68.10	148.15	245.15	389.79[a]
SEM		1.427	5.338	8.654	17.103
Main Factor II					
MB (%)					
0		66.55	144.32	229.12	353.66
0.15		69.65	150.32	245.46	370.15
SEM		1.427	5.338	8.654	17.103
Probability, Pr > F					
AFB1 level (AL)		NS	NS	NS	*
Mycotoxin Binder (MB)		NS	NS	NS	NS
AL x MB		NS	NS	NS	NS

[a,b] Different superscripts at the same row indicate significant differences (P<0.05)

* significant different (P<0.05); NS Not Significant (P>0.05).

nd = non-detectable level (Limit of detection with HPLC: AFB1 = 0.43 ppb); AFB2 = 2.02 ppb; AFG1=1.53 ppb; AFG2=0.20 ppb)

of mycotoxin binder did not significantly (P>0.05) affect BWG of broilers during the experimental period, agreed with Nalle *et al.* (2019) and Njoki (2019).

The effect of treatment diets on the feed conversion ratio (FCR) of broilers during the 28 days of the experiment is summarized in Table 4. The AFB1 level x mycotoxin binder did not affect (P>0.05) the feed efficiency of birds during the experimental period, agreed with Nalle *et al.* (2019). Mycotoxin binder had no effect (P>0.05) on the feed conversion ratio (FCR) of broilers on day 28. AFB1 level x mycotoxin binder interaction was not significant (P>0.05) for the FCR of broilers during the trial period.

The results showed that the main effect of the AFB1 level significantly (P<0.001) affected the FCR of broilers during the starter and grower phases. Broilers fed on a diet containing 2.58 ppb AFB1 had a higher FCR (P<0.05) than those who were fed the control diet. The low feed efficiency of broilers in AFB1 treatment diet group was probably due to the toxicity effect of AFB1 on pancreatic enzymes (Table 5) and the low nutritional quality of AFB1 diets, leading to low nutrient absorption. The present result agreed with Monson *et al.* (2020) and Yunus *et al.* (2011) who reported that a low concentration of AFB1 in the diet reduced feed efficiency. However, it is unclear how much nutrient uptake in the intestine contributes to AFB1 effects on feed conversion in poultry (Monson *et al.,* 2020). The results of the present study partly agreed with Nalle *et al.* (2019) and Nazarizadeh and Pourreza (2019) who reported an increase in FCR in broilers fed on diets containing a low level of AFB1. The difference was probably due to the difference in methodology, especially related to the strain of birds and the level of AFB1 used.

Good quality of feed, from a nutritional perspective and safe from feed contaminants such as aflatoxins, will contribute to good productivity of animals, safety animal-derived products, and achievement of high profit. AFB1, one of four types of aflatoxins, is the most poisonous and widespread compound produced by *Aspergillus spp* (Yunus *et al.,* 2011; Suganthi *et al.,* 2011; Kumar, 2018). The intake of AFB1 by broilers could lead to poor productivity, pancreatic enzyme activity disturbance, morphology and histological changes, illnesses, and death (Massomo, 2020; Yang *et al.,* 2012; Marchioro *et al.,* 2013; Peng *et al.,* 2015; Monson *et al.,* 2015; Gacem and Hadj-Khelil, 2016; Galarza-Seeber *et al.,* 2016; Sineque *et al.,* 2017; Kurniasih and Prakoso, 2019). Thus, the negative impact of AFB1 should be minimized or eliminated with the correct method. The addition of a mycotoxin binder to animal diet is a strategy that can be implemented to decrease the toxicity of AFB1. In the present study, a commercial mycotoxin binder (Mycosorb, 0.15%) had applied in a broiler diet contaminated with low doses of AFB1.

Nutrient digestibility

Nutrients are absorbed in the epithelial cells of the small intestine where these cells also facilitate the absorption of aflatoxins (Monson *et al.,* 2015). It is further explained that almost all AFB1 is transferred directly into the bloodstream so that the negative effects of AFB1 can directly affect the small intestine where digestion and absorption occur. Table 5 shows the effects of feeding diets containing a different level of AFB1 supplemented with mycotoxin binder on nutrient digestibility.

The interaction between AFB1 level and mycotoxin binder was significant (P<0.05) for digestibility coefficient of crude fat (DCCF) but not significant (P > 0.05) for digestibility coefficient of crude protein (DCCP). The DCCF of birds fed 2.58 ppb AFB1 diet supplemented with mycotoxin binder (Mycosorb) did not differ (P>0.05) from the DCCF of birds fed 2.58 ppb AFB1 diet without

Table 4. The effect of treatment diets on the feed conversion ratio of broilers (g/g)

AFB1 Level (AL, ppb)	Mycotoxin binder (MB, %)	Feed Conversion Ratio (FCR)			
		7d	14d	21d	28d
nd	0	0.887	1.958	2.539	2.379
nd	0.15	0.776	1.993	2.346	2.160
2.58	0	1.123	3.209	3.694	3.302
2.58	0.15	1.117	3.268	3.761	3.550
SEM		0.083	0.123	0.143	0.169
Main Factor I AFB1 level (AL, ppb)					
nd		0.831[b]	1.973[b]	2.443[b]	2.269[b]
2.58		1.119[a]	3.239[a]	3.728[a]	3.426[a]
SEM		0.058	0.087	0.101	0.119
Main Factor II MB (%)					
0		1.005	2.583	3.117	2.840
0.15		0.946	2.631	3.053	2.855
SEM		0.058	0.087	0.101	0.119
Probability, Pr > F					
AFB1 level (AL)		***	***	***	***
Mycotoxin Binder (MB)		NS	NS	NS	NS
AL x MB		NS	NS	NS	NS

[a,b] Different superscripts at the same row indicate significant differences (P<0.05) and highly significant (P<0.01).
*** highly significant different (P<0.001); NS Not Significant (P>0.05).
nd = non-detectable level (Limit of detection with HPLC: AFB1 = 0.43 ppb); AFB2 = 2.02 ppb; AFG1=1.53 ppb; AFG2=0.20 ppb).

Mycosorb supplementation. The DCCF of birds fed the control diet with Mycosorb was lower (P<0.05) than the DCCF of birds fed the control diet without Mycosorb (Table 5).

The insignificant interaction between AFB1 level and mycotoxin binder on DCCP was possibly owing to the low AFB1 dose applied in the diet so that the binding effect of Mycosorb was not visible. Also, the insignificant interaction between AFB1 level and mycotoxin binder for the DCCP variable was probably due to the similar crude protein (CP) content of both treatment diets. The laboratory analysis showed that the CP content of diet without Mycosorb was 20.3% DM; while the CP content of diet supplemented with Mycosorb was 19.5%. The insignificant difference in DCCP might be also because of the protease activity, as was not disturbed by AFB1. In a review by Fouad *et al.* (2019), it was reported that broilers fed 300 ppb had a larger pancreas than those who were fed 100 ppb AFB1. Furthermore, Fouad *et al.* (2019) explained that the hyperthropy of the pancreas caused by AFB1 may be owing to the high quantity of mature crystalline granules in the pancreatic cells. The large pancreas size in broilers fed AFB1 may have an effect on its functions, where the amylase, lipase, protease, chymotrypsin, and trypsin activities were elevated which would normally be expected to enhance the digestion of nutrients.

In contrast, the decreased DCCF on AFB1 diets (2.58 ppb) added with 0.15% Mycosorb was an unexpected result since Mycosorb also contains nutrients including minerals such as Ca which were expected to increase the metabolism of nutrients. Mycosorb has the capability to absorb mycotoxins by forming a stable complex in its glucomannan matrix, which inhibits its absorption from a gastrointestinal tract in animals (Saki *et al.,* 2018; Girish and Devegowda, 2006). As a result, the metabolic process will be performed well to produce metabolites. Regarding the role of calcium in Mycosorb on metabolic process, Proszkowiec-Weglarz and Angel (2013) and Bonner and Pansu (1999) explained that calcium also plays an important role in metabolism, blood clotting, activation of the enzyme, neuromuscular function, contraction of muscle, cell adhesion, and intracellular signaling in many system and cells. Intestinal absorption of calcium was regulated by the concentration of calcium In the diet; the active absorption of calcium increased when the calcium diet was low or the requirement of calcium increased (Bronner and Pansu, 1999). The comparison was difficult to make because the relevant research was not available.

The main effect of AFB1 level was not significant (P>0.05) for the digestibility coefficient of crude protein (DCCP), but decreased the digestibility coefficient of crude fat (DCCF). The DCCP and DCCF of birds fed on a diet added with mycotoxin binder was found to be similar (P>0.05) to those who were fed the control diet.

The birds who were given the treatment diet containing 2.58 ppb AFB1 had a significantly (P<0.05) lower DCCF than those who were fed a control diet. This was probably due to the negative effect of AFB1 on the pancreatic enzyme activity, especially lipase (Marchioro *et al.,* 2013; Osborne and Hamilton, 1981). Another factor contributing to low DCCF in 2.58 ppb AFB1 diet was the lower crude fat content of the diet containing 2.58 ppb AFB1 (4.4% DM basis) compared to the control diet (9.1% DM basis).

Based on the present results, the very low level of AFB1 in the diets caused a deleterious effect on feed efficiency and crude fat digestibility. The addition of 0.15% Mycosorb in the AFB1 diets did not improve the growth performance of birds. Further study is needed to evaluate the effect of AFB1 on the pancreatic activity, morphology, and histopathology of digestive organs, and lymphoid organs, and the residue of AFB1 in the organ tissues of broilers. Also, the effect of a high dose of aflatoxin B1 supplemented with synthetic or natural toxin binders, either alone or in combination use needs to be evaluated.

Table 5. The effect of treatment diets on the apparent nutrient digestibility coefficient of broiler diets (g/bird)

AFB1 Level (LA, ppb)	Mycotoxin Binder (MB, %)	Digestibility Coefficient	
		Crude Protein	Crude Fat
nd	0	0.586	0.817[a]
nd	0.15	0.483	0.703[b]
2.58	0	0.547	0.571[c]
2.58	0.15	0.501	0.634[bc]
SEM		0.0361	0.0316
Main Factor IAFB1 level (ppb)			
nd		0.534	0.760[a]
2.58		0.524	0.596[b]
SEM		0.0255	0.0224
Main Factor II MB (%)			
0		0.567	0.687
0.15		0.492	0.669
SEM		0.0255	0.0224
Probability, Pr > F			
AFB1 level (AL)		NS	***
Mycotoxin Binder (MB)		NS	NS
AL x MB		NS	*

[a,b] Different superscripts at the same row indicate significant differences (P<0.05) and highly significant (P<0.01).
* significant different (P<0.05), *** highly significant different (P<0.001); NS Not Significant (P>0.05).
nd = non-detectable level (Limit of detection with HPLC: AFB1 = 0.43 ppb); AFB2 = 2.02 ppb; AFG1=1.53 ppb; AFG2=0.20 ppb).

Conclusions

In conclusion, a low concentration of AFB1 increased the feed intake and body weight gain for birds. Also, the low concentration of AFB1 reduced feed efficiency and crude fat digestibility. Mycosorb did not improve the growth performance and nutrient digestibility. The supplementation of Mycosorb to the treatment diets did not improve the growth performance and digestibility coefficient of crude protein, but it improved the crude fat digestibility.

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