Carcass yield and health status of broilers fed aflatoxin B1 diets added with Mycosorb

Catootjie Lusje Nalle*[1], Max Arthur Julian Supit[2], Andrijanto Hauferson Angi[3], Beatrix Sena Bha[1] and Ni Sri Yuliani[3]

[1]Feed Technology Study Program, Department of Animal Husbandry, State Polytechnic of Agriculture Kupang
[2]Animal Production Study Program, Department of Animal Husbandry, State Polytechnic of Agriculture Kupang
[3]Animal Health Study Program, Department of Animal Husbandry, State Polytechnic of Agriculture Kupang
Jl. Prof. Herman Yohanes, Kelurahan Lasiana 85228, Kupang-Indonesia
Institution or departement

ABSTRACT: The intake of aflatoxin B1 (AFB1) can lead to poor productivity and diseases in birds. Thus, it is important to minimize the toxic effects of AFB1. The objective of this study was to evaluate the carcass yield and haematological profile of broilers fed diets containing AFB1 added with Mycosorb. A total of 240-day old broiler chicks were randomly distributed to 24 pens (10 birds/pen). The experiment was designed using a 4 x 2 factorial arrangement with two different factors, namely aflatoxin level and Mycosorb. The treatments were control diets, control diets added with Mycosorb, diets containing 10.36 ppb AFB1, diets containing 10.36 ppb AFB1 added with Mycosorb, diets containing 26.97 ppb AFB1, diets containing 26.97 ppb AFB1 added with Mycosorb, diets containing 61.06 ppb AFB1, diets containing 61.06 ppb AFB1 added with Mycosorb. There was an interaction ($p<0.05$ to 0.01) between AFB1 level and Mycosorb on the white blood cells (WBC) and litter/excreta score (LES), but not ($p>0.05$) in tcarcass traits (CT), lymphoid organ weight (LOW), and haematological profile (HP) of broilers. Level of AFB1 did not affect ($p>0.05$) all CT, LOW, and HP, but it affected ($p<0.001$) the LES. Mycosorb did not improve ($p>0.05$) CT, LOW and HP of broilers. In conclusions, 1) dietary Mycosorb in afla-treated diets improved litter quality and reduced white blood cell counts of broilers; 2) the AFB1 level up to 61.06 ppb did not impair carcass yield, lymphoid organ weights and other haematological index of broilers.

Keywords: Aflatoxin; Broiler; Mycotoxin binder; Performance; Toxicological effect

*Corresponding Author: nallecatootjie@gmail.com

INTRODUCTION

Productivity of broilers is determined by several factors which are breeding, feeding and management. The quality and quantity of feed will support the optimum production and quality of broiler meat. One of the important factors in feed quality requirement is aflatoxin level. According to Fountain *et al.* (2015), aflatoxins are the secondary metabolite produced by fungi during the infection and growth of *Aspergillus flavus* and *Aspergillus parasiticus* in food and feed ingredients such as corn and peanuts. Iqbal *et al.* (2013) reported that the production of aflatoxin will occur when the moisture level is between 18% and 20%, water activity >0.82, a pH 3.0 to 8.5, and ambient temperature is between 12 and 40 °C with an optimum temperature for growth at 25 to 30 °C. In addition, the availability of nutrients such as carbohydrate, nitrogen, and minerals (i.e., phosphates, zinc) also involved in the production rate of aflatoxin. Aflatoxins are grouped into two types namely (blue) B and (green) G types based on ultraviolet under fluorosence (Benkerroum, 2020; Kumar, 2018; Alhousein and Gurbuz, 2015), these are called AFB or AFG respectively. Fouad *et al.* (2019) and Kumar *et al.* (2017) reported that AFB1 is the most common and hazardous aflatoxin.

The toxicity of aflatoxins varies according to the type of animal species and aflatoxin concentration in the diet. Among all avian species, growing ducklings, goslings, turkey poults and chicks are more susceptible to the aflatoxicosis (Valchev *et al.*, 2018) which are characterised by liver necrosis, haemorrhages, and proliferation of biliary duct epithelium. Published data showed that in most cases, the low level (< 100 ppb) of AFB1 did not cause a negative impact on growth performance and nutrient digestibility of broilers (Kurniasih and Prokoso, 2019; Yang *et al.* 2012). However, some reported that the low concentration (< 100 ppb) of AFB1 in the diets impaired productivity and nutrient digestibility of

broilers (Saminathan *et al.* 2018; Liu *et al.* 2018; Wade *et al.* 2015; Resanovic and Sinovec 2006). The undigested nutrients will be modified by hind gut through fermentation process which results in changes in microbiota composition and reduced water absorption (Hermans *et al.,* 2006; Teirlynk *et al.,* 2011) leading to wet litter. Collet (2012) explained that the undigested nutrients will increase the output of water urinary. In a study by de Jong *et al.* (2014), they found that wet litter induced footpad dermatitis, resulted in dirty birds, hock burn, breast irritation and reduced carcass yield and carcass component parts of broilers. The incidence of hock burn and breast blister will reduce the carcass quality leading to a rejection for commercial purchase. In a review by Murugesan *et al.* (2015), it was reported that poor growth performance and carcass bruising were due to the aflatoxicosis of AFB1.

The harmfulness of aflatoxins in birds are also associated with the changes in morphology and histology of the digestive tract of birds, lymphoid organ weight, the profile of blood, lower egg production and quality, the presence of aflatoxin residue in the tissue, and death (Kurniasih and Prokoso, 2019; Sineque *et al.*2017; Galarsa-Seeber *et al.* 2016; Peng *et al.* 2015; Yang *et al.* 2012; Kumar and Balachandran, 2009; Resanovic and Sinovec, 2006). The toxicity of AFB1 can also cause a hepatocellular carcinoma which is the main form of liver cancer in human, rat, primate, and ducks (Benkerroum 2020; Cai *et al.* 2020; Wu and Santella, 2012; Marchese *et al.*, 2018). In a review by Marchese *et al.* (2018), the AFB1 also caused lung and intestinal cancer in human.

Efforts to prevent the contamination of fungus, or to cure or eliminate the negative impacts of aflatoxins have been conducted by previous researchers (Bhatti *et al.,* 2017; Nazarizdeh and Pourreza, 2019; Fowler *et al.* 2015; Moran *et al.,* 2013 El-Katcha *et al.,* 2017). Nowadays, it is easy to obtain commercial mycotoxin binders

(organic and inorganic) in the world market; however, the efficacy of each type of toxin binder is different. Mysocorb or yeast glucomannan is one of the mycotoxin binders that has been evaluated its efficacy in poultry diets and the results are still contradictive. For example, Fouad *et al.* (2019) reported that the addition of 0.5 kg/ton Mycosorb did not improve the performance or immunity in birds fed aflatoxin diet (40 ppb AFB1/kg). While, Mogadam and Azizpour (2011) claimed that the addition Mycosorb and sodium bentonite into diets containing aflatoxins (250 ppb) improved the performance and immunity towards Newcastle disease on broilers. Another study by Nazarizadeh and Pourreza (2019) also proved that the addition of Formycin, Anzymit and Mycosorb in the compound feed contaminated with 0.2 and 4 µg/g AFB1 enhanced the growth performance, haematology value and serum protein on broilers. The deactivation process of aflatoxins occurs through the binding process between negative polarity of aflatoxins and positive polarity of toxin binder (Kanna *et al.,* 2014).

In consideration of the vulnerability of AFB1 in broilers and human health, thus the effective method for reducing or eliminating the toxicity of AFB1 in broiler feed is necessary to be evaluated. Based on the above explanation, an experiment has been conducted to evaluate the carcass yield, lymphoid organ weight and haematological profile of broilers given a low level AFB1 diet mixed with a commercial toxin adsorbent (Mycosorb). Mycosorb or yeast glucomannan is a feed supplement anticaking agent which contains a few nutrients such as crude protein, crude fiber, calcium carbonates, hydrated sodium calcium aluminosilicate, dried yeast and fermentation soluble brewer yeast.

MATERIALS AND METHODS
Animal Ethic Approval

The experimental procedures of the present study were approved by the Animal Ethic Committee of Faculty of Veterinary Medicine, University of Nusa Cendana Kupang-Indonesia, with Ethical Clearance Number KEH/FKH/NPEH/015/2019 on July 8th, 2019.

Birds and Housing

The study was conducted in State Polytechnic of Agriculture Kupang, East Nusa Tenggara Province, Indonesia. A total of 240 one-day-old Cobb-strain broilers (45.7 ± 0.2 g/bird) were acquired from the local hatchery. The birds were randomly distributed into 24 pens (10 birds/pen). A gasolec was used to heat the chicks during the first seven days. The lighting of the room was provided for 20 hours every day by placing a bulb on the ceiling of the room. Each pen was provided with a bulb (75 watts) for additional heating and lighting. The housing temperature and humidity were monitored twice a day (morning and afternoon) by a digital thermo-hygrometer. On day 22 to 35, the birds were removed to metabolic cages for nutrient digestibility assay (the data were reported in the previous article) and excreta score.

Ingredients

Yellow corn, sago and Mycosorb, as the main ingredients used in the present study, were obtained from the local distributor. Mycosorb product was provided by Alltech Ltd distributor in Indonesia as inkind contribution. The recommended dosage of Mycosorb used in the diet formulation was 0.075%. Yellow corn (fresh and mouldy) and sago were ground with a hammer mill with the screen size of 3 mm prior to use in the assay. Mouldy corn was obtained by naturally growing *Aspergillus flavus* in the fresh corn. Aflatoxin corn was produced as following: the fresh yellow corn (moisture content 14.5%) was put in some plastic sacks (50 kg capacity each) and added with clean water (10% of corn weight) in order to improve the moisture content and grow the *Aspergillus flavus*. The clean water was added every other day for two months (modified method of Mogadam and Azizpour, 2011). The sampling procedure of mouldy corn was conducted according to Campos and

Campos (2017) and continued with a cone sample divider (RETSCH PT 100) to produce laboratory sample. The laboratory sample was then reduced in particle size to 0.5 mm using a sample mill (FOSS CT 193 Cyclotec[TM]). The fresh and mouldy corn samples were packed and sent to the laboratory for aflatoxin analysis. The AFB1 content of mouldy and fresh corn was tested with a Thin-Layer Chromatography (TLC) in SEAMEO Biotrop Laboratory, Bogor, Indonesia. The aflatoxin concentration of mouldy corn obtained from the mouldy corn was 134 ppb.

Experimental Diets

Two basal diets (with and without Mycosorb) were formulated to meet nutrient requirements of broilers (Table 1). The fresh yellow corn was used in those basal diets. Then six aflatoxin B1 (AFB1) diets were developed by substituting the proportion of fresh corn in the control diets with mouldy corn which contained AFB1. The proportion of mouldy corn in each AFB1 diet was determined using a dilution formula (V1 x C1 = V2 x C2) (Aly and Anwer, 2009). The assay diets were pelleted in 4 mm pellet size using a Pellet Mill (1 ton/hour), then crumbled using a crumbling machine (capacity: 100-200 kg/hour; roll tube diameter: 10 inch, screen size: 2 mm) at Mini Feed Mill of State Polytechnic of Agriculture Kupang. The concentration of aflatoxin (B1, B2, G1 and G2) in the sample diets was analysed with HPLC at SEAMEO Biotrop Laboratory, Bogor, Indonesia.

Table 1. The composition (g/100 g as-is) of the control diets

Feed ingredients	Inclusion level	
Maize	51.19	51.19
Putak, CP 3.6%	3.98	3.98
Soybean meal, CP 44%	33.0	33.0
Meat and Bone Meal	6.0	6.0
Vegetable oil	4.0	4.0
DL-Methionine 99%	0.25	0.25
L-Lysine	0.25	0.25
Limestone	0.05	0.05
Dicalcium phosphate	0.60	0.60
Salt	0.25	0.25
Sodium bicarbonate	0.12	0.12
Vitamin-Mineral Premix*	0.30	0.30
Mycosorb**	-	0.075
Total	**100.00**	**100.00**
Nutrient composition (calculated)		
Apparent Metabolisable energy (Kcal/kg DM)	3,100	3,100
Crude Protein (g/kg)	210	210
Lysine (g/kg)	12.7	12.7
Met + Cys (g/kg)	9.7	9.7
AFB1 (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

Experimental Design

The experiment was designed using a 4 x 2 factorial arrangement consisting of two main factors which were the AFB1 level and Mycosorb. So, there were eight treatment combinations altogether, involving T1) the control diets; T2) control diets + Mycosorb, T3) diets containing 10.36 ppb AFB1, T4) diets containing 10.36 ppb AFB1 + Mycosorb, T5) diets containing 26.97 ppb AFB1, T6) diets containing 26.97 ppb AFB1 + Mycosorb, T7) diets containing 61.06 ppb AFB1, T8) diets containing 61.06 ppb AFB1 + Mycosorb. The AFB1 level used in the assay diets was determined by the published data of the previous studies (Fouad *et al.,* 2019; Yang *et al.,* 2012; Resanovic and Sinovec, 2006). Variables measured were carcass yield (%), carcass parts (%), aflatoxin residues in the breast meat.

Chemical Analysis

Determination of dry matter content of corn and experimental diets used convection oven (105 $^{\circ}$C) based on AOAC method (2005). The concentration of aflatoxins (B1, B2, G1 and G2) of moldy corn was determined using a Thin-Layer Chromatography (TLC) with the standard procedure of AOAC Official Method 993.17 (Latimer, 2012). While the aflatoxin concentration of the experimental diets was determined using High Performance Liquid Chromatography (HPLC, with detection limit: 0.43 ppb) using the standard procedure of AOAC Official Method AOAC 49.2.18-993.21).

The principle of analysis using HPLC is as following: sample is extracted with methanol: water (70:30), then filtered, diluted, and passed through immuno-affinity column which take the specific monoclonal antibody of AFB1, AFB2, AFG1 and AFG2. The pure and isolated aflatoxin will be concentrated in the column and released from the antibody and methanol.

Carcass processing

On day 35, two birds from each cage replicate were selected, weighed (live body weight LBW), slaughtered and manually bled by severing the jugular vein and bled

out for two minutes. Next, the birds were scalded (54 - 58 $^{\circ}$C, 30 seconds) and the feathers were manually removed. The carcasses were washed with clean water and drained for 30 seconds. Later, the carcasses were eviscerated and cleaned again with tissue before weighing.

The carcasses were weighed and manually divided into four parts: thigh, breast, wings and back. The wing of carcass was removed by cutting through the humeral insertion. The whole legs were removed, including gluteus muscle and the drumstick. Chicken thigh was removed by cutting through the femur-tibial junction. The thigh, wings, breast, and the back were then weighed in pairs. The individual part weights for wings, thigh, breast meat and back were used to calculate part yields.

Blood and lymphoid organ samples collection

Phlebotomy or blood collection was taken intramuscularly using 3 mL syringe from 48 broiler chickens before they were euthanized (cervical dislocation) on day 35. The whole blood samples were then put into EDTA tubes and then examined for the red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), haemoglobin (HGB).

The whole blood samples were tested according to laboratory standard procedure using Auto Haematology Analyser (Rayto) at UPT Veterinary Laboratory, Kupang, East Nusa Tenggara, Indonesia. The bursa of fabricius and thymus organs were taken and weighed using a digital balance (Camry, 0.01 g).

Measurements:
1. Carcass yield (as % of live weight) were calculated using the following formula (A)
2. Carcass component parts (as % of carcass weight) were calculated using the following formula (B)
3. Absolute weight of lymphoid organ (g/bird): On day 35, forty eight birds with from all treatments were selected, weighed individually and euthanized by

cervical dislocation. Then, the lymphoid organs (bursa of fabricius and thymus) were taken and individually weighed to get the absolute weight.

4. Haematological profile: the red blood cells (x $10^3/\mu L$), white blood cells (x $10^3/\mu L$), mean corpuscular volume (fL), mean corpuscular haemoglobin (pg), mean corpuscular haemoglobin

concentration (g/dL), and haemoglobin (g/L) were measured using an Auto Haematology Analyser.

5. The litter/excreta score: the litter/excreta was scored using the visual examination on day 21 and 35 using the modified method of Kheravii *et al.* (2017) with the scale 1 to 3 (1 = dry; 2= moist and 3 = wet/cakey).

Formula A

$$Carcass\ recovery = \frac{carcass\ weight}{slaughter\ weight}\ x\ 100$$

Formula B

$$Percentage\ of\ carcass\ parts = \frac{weight\ of\ carcass\ parts}{carcass\ weight}\ x\ 100$$

Statistical Analysis

The carcass, lymphoid organ weight, haematological profile data obtained from the present experiment were statistically analysed using the two-way analysis of variance according to the General Linear Model procedure of SAS (University Edition, SAS Institute). Differences between treatments were calculated to be significant at P<0.05, and further calculated using Fisher's Least Significant Difference Test (LSD).

RESULT AND DISCUSSION
Carcass parameter

The live body weight (LBW), carcass yield (in weight and percentage), and commercial carcass component parts (in percentage) of 5-week-old (35d) broiler chickens are depicted in Tables 2 and 3. The average LBW of selected birds fed AFB1 contaminated diets up to 61.06 ppb added with Mycosorb tended to be higher than those fed AFB1 diets without Mycosorb (Table 2).

Table 2. The average of live body weight (g/bird) and carcass weight (g/bird) of selected birds (35 d) for carcass parameter from all dietary treatments

Aflatoxin B1 level (ppb)	Mycosorb	Live body weight	Carcass weight
		……………….(g/bird)…………………	
nd	-	1,220	818.43
nd	+	1,340	886.05
10.36	-	1,295	878.05
10.36	+	1,295	965.80
26.97	-	1,285	860.56
26.97	+	1,407	964.28
61.06	-	1,252	827.06
61.06	+	1,445	1002.53

nd = non detectable level (Limit of detection with HPLC: AFB1 = 0.43 ppb)

A similar trend was also recorded in carcass weight of birds. When expressed on percentage weight basis (Table 3), the statistical analysis showed that carcass yield

and component parts (thigh, breast, wings, and back percentages) were not significantly (p>0.05) different by the level of AFB1. This indicated that the birds could tolerate to

the AFB1 level < 70 ppb in the diets. Regarding the second main factor, the addition of Mycosorb in the diets did not improve (p>0.05) the percentage of carcass yield and commercial component parts. There was no interaction (p>0.05) between AFB1 level and Mycosorb on the carcass yield and parts of broilers fed the treatment diets.

However, there was an increase tendency in carcass yield and parts of broilers fed AFB1 contaminated diets added with Mycosorb (Table 3). This result indicated that Mycosorb had the ability to bind AFB1 in the gastrointestinal tract and excreted into faeces. The range of carcass percentage obtained in the present study was 66.16 to 69.34 %.

Table 3. Carcass yield and parts of broilers fed diets containing low level of aflatoxin B1 and mycotoxin binder (Mycosorb)

Treatment		Variables				
AFB1 Level, ppb	Mycosorb	Carcass yield	Thigh	Breast	Wings	Back
		....................................%......................................				
nd	-	66.2	24.5	30.1	9.07	17.1
nd	+	67.0	31.8	36.6	10.59	20.4
10.36	-	67.0	29.7	38.7	11.93	20.0
10.36	+	68.2	30.4	38.9	10.50	19.6
26.97	-	68.4	30.5	39.4	9.90	19.2
26.97	+	67.6	31.0	37.9	10.27	20.0
61.06	-	69.3	30.8	39.4	10.20	19.0
61.06	+	67.9	31.2	39.3	10.62	21.1
SEM		0.75	2.71	2.70	0.85	1.42
Main effect, AFB1 level (ppb, AL)						
nd		66.6	28.1	33.3	9.83	18.8
10.36		67.6	30.0	38.9	11.21	19.8
26.97		67.9	30.7	38.6	10.08	19.6
61.06		68.6	31.0	37.7	10.41	20.0
SEM		0.53	1.47	1.61	0.60	1.00
Main effect, Mycosorb (M)						
-		67.7	28.9	36.9	10.27	18.8
+		67.7	31.1	37.3	10.49	20.3
SEM		0.38	1.04	1.35	0.42	0.71
Pr > F						
Aflatoxin Level (AL)		NS	NS	NS	NS	NS
Mycosorb (M)		NS	NS	NS	NS	NS
AL x M		NS	NS	NS	NS	NS

[a,b] Means of column with the superscripts significant difference (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)

Published data have shown that long term exposure to aflatoxin in human body causes a number of chronic and acute diseases including carcinogenic disease (i.e., liver cancer), and hepatitis B virus infection (Benkerroum, 2020). Thus, it is crucial to

analyse the AFB1 concentration in edible animal products such as meat, eggs, and milk. The present result shows undetectable aflatoxin (B1, B2, G1 and G2) residue in broiler breast meat which were exposed to low level of AFB1 (≤ 61.06 ppb)-diets

supplemented with Mycosorb for 35 days (not presented in this article, the data has been used for another article).

The result was in agreement with Hussain *et al.*, (2016) who recorded the absence of AFB1 residue in broiler muscle after 28days- feeding of a low level of AFB1 diet (50 ppb and 100 ppb). In addition, Hussain *et al.*, (2016) only found the AFB1 residue above the permissible threshold (> 2 ng/g) in the muscle of birds fed 400 ppb and 800 ppb of AFB1. One contrary study

reported breast muscle residues of 0.015 ppb AFB1 in broilers offered 36.9 ppb and 69.3 ppb for 42 days experimental period (Yang *et al.*, 2012).

Bird's health status

Lymphoid organ weights

Thymus and bursa of fabricius (BOF) are the fundamental lymphoid organs in the chicken which play an important role in producing specific antibodies and become the target organ of aflatoxins (Karimy *et al.*, 2017).

Table 4. Lymphoid organ weight (g/bird) and litter/excreta score of broilers fed diets containing low level of aflatoxin B1 (AFB1) and Mycosorb

Treatments		Variables			
AFB1 Level, ppb	Mycosorb	Bursa of fabricius	Thymus	Litter score (21d)	Excreta score (35d)
		…………(g/bird)...............			
nd	-	1.52	3.08	1.00^c	1.00
nd	+	1.92	3.39	1.00^c	1.33
10.36	-	1.76	3.27	2.00^b	1.67
10.36	+	2.32	3.63	3.00^a	1.33
26.97	-	1.52	3.98	3.00^a	2.00
26.97	+	1.63	3.11	1.67^b	2.00
61.06	-	1.84	3.19	3.00^a	3.00
61.06	+	1.59	3.73	1.67^b	3.00
SEM		0.22	0.49	0.16	0.29
Main effect, AFB1 level (ppb, AL)					
nd		1.72	3.23	1.00^b	1.17^c
10.36		2.04	3.44	2.50^a	1.50^{bc}
26.97		1.58	3.55	2.33^a	2.00^b
61.06		1.72	3.46	2.33^a	3.00^a
SEM		0.16	0.35	0.12	0.20
Main effect, Mycosorb (M)					
-		1.66	3.38	2.25^a	1.91
+		1.87	3.47	1.83^b	1.91
SEM		0.11	0.24	0.01	0.14
Pr > F					
Aflatoxin Level (AL)		NS	NS	***	***
Mycosorb (M)		NS	NS	**	NS
AL x M		NS	NS	***	NS

^{a,b} Means of column with the superscripts significant difference (p<0.05), **: Significant (p<0.01); ***: Significant (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)

Thus, it is crucial to keep these lymphoid tissues to work optimal, so they can support the health and productivity of

chickens. The present study assessed the lymphoid organ weight of birds given different AFB1 level (nd to 61.06 ppb) in the

diets supplemented with 0.075% yeast containing glucomannan (Mycosorb). Tabel 4 depicted the effects of dietary treatments on the absolute weight of lymphoid organs of broilers and litter/excreta score during the 35 days of experiment. The statistical analysis showed that the AFB1 level alone, Mycosorb supplementation, and combination between AFB1 and Mycosorb did not affect (p>0.05) the absolute weight of thymus and bursa of fabricius, agreed with Kurniasih and Prakoso (2019). However, the previous authors found a decrease in the lymphoid organ weights when the AFB1 level > 100 ppb AFB1 diets. A study conducted by Peng *et al.* (2015) and Liu *et al.* (2018) showed a decrease in thymus and BOF of birds fed aflatoxin-contaminated diets.

The differences were probably due to the level and type of aflatoxin applied in the diets. Liu *et al.* (2018) used a pure AFB1 with dose level of 40 ppb. In the study conducted by Peng *et al.* (2015), the diets containing 134 ppb AFB1 and 23.6 ppb AFB2; while, in the present study only used AFB1 (nd o 61.06 ppb). According to Peng *et al.* (2015) the decrease in in thymus and bursa weight was related to the increased debris and reticulocytes in the lymphoid follicles of the BOF which indicated depressed proliferation of B cells and atrophy of the BOF.

Litter/excreta quality

In a review by Bolan *et al.* (2010), it was explained that during 35 days of rearing, a broiler chicken is able to produce about 4 kg of fresh excreta which contains not only a high level of nutrients (such as N, P and K) (van Ryssen *et al.,* 1977; Bolan *et al.*, 2010), bacteria (van der Hoeven-Hangoor *et al.*, 2014), but also water (free and bound water) (Belay and Teeter, 1996). Approximately 54% the excretion of water in broilers occurs via the urine and 46% are excreted through the feces (Belay and Teeter, 1996). The average moisture content of the fresh dropping of birds was reported to be 55% (Hoover, 2015). Published data have shown that the poor litter/excreta quality is related

to high moisture content (> 25%) which leads to wet litter (Collett, 2012; Dunlop *et al.,* 2016). This condition is triggered by several conditions such as management and housing of birds, diseases, nutritional factors (composition and chemical properties), toxin and gut health (Belay and Teeter, 1996; van der Hoeven-Hangoor *et al.*, 2014).

Infectious diseases, gut health, toxin, and dietary factors (composition and chemical properties) can lead to poor nutrient digestibility and absorption in birds (Teirlynck *et al.*, 2011; Liu *et al.*, 2018). Published data showed that AFB1 caused gut damage, macronutrient malabsorption syndrome and impaired digestive enzyme activities (Feng *et al.*2017; Yunus *et al.*, 2011; Han *et al,* 2008; Grenier and Applegate, 2013) which lead to changes in gut histology and lower nutrient digestibility. The undigested nutrients in the proximal small intestine will be fermented by bacteria in the distal ileum and ceca of birds. As a result, there will be changes in the microbiota composition and water reabsorption in the hind gut as well as the amount nutrient excreted in the feces. Thus, the litter/excreta quality is needed to be controlled because it is associated with environmental and animal welfare problems and to reduce productivity losses.

The statistical analysis showed that the AFB1 level significantly affected (p<0.001) the litter and excreta score of broilers (Table 4). The birds fed AFB1-treated diets had higher (p<0.05) litter and excreta scores than those who given a control diet. The litter score of broilers fed afla-treated diets were similar (p>0.05). Broilers fed diets containing 61.06 ppb AFB1 had higher (p<0.05) excreta score compared to those fed a control diets and diets containing 10.36 and 26.97 ppb AFB1. No significant differences (p>0.05) in excreta score were observed in birds fed 10.36 and 26.97 ppb AFB1 and between a control diet and diets contaminated with 10.36 AFB1. Data obtained from the present study showed that the litter/excreta quality

of broilers exposed to AFB1 diets are poor (wet/cakey), especially in diets containing high AFB1 (Tabel 4). During the experiment, the litter/excreta of AFB1-treated birds were not only wet/cakey but also producing a bad smell and colour (black). Sheperd and Fairchild (2010) explained that cakey litter is a compressed layer that forms on the top of litter or bedding materials which contains moisture and fecal materials. Hermans *et al.* (2006) explained that wet litter is a condition of litter or bedding materials that could not hold more moisture.

The poor litter/excreta quality of AFB1-treated birds was probably due to several mechanism of actions namely 1) the AFB1 impaired the morphology of gut which reduced the digestibility and absorption of nutrients; 2) the undigested nutrients in the proximal small intestine were then delivered to the distal ileum and ceca to continue microbial fermentation. 3) This condition will in turn change microbiata composition and the reabsorption condition. The excess nutrients will increase urinary outputs of water in the hind gut (Dunlop *et al.*, 2016). The visual observation during the experiment showed that the birds fed AFB1 diets had dirty feathers (side and back) and cloaca. In addition, the housing produced bad smell. De Jong *et al.* (2014) reported that wet litter could induce footpad dermatitis, reduce the bird's welfare, severe hock burn, breast irritation, performance, and carcass yield.

Moreover, the wet litter caused more rejection of commercial parts at the slaughterhouse. Mycosorb supplementation decreased (P<0.05) the litter score of birds during the 21 day of experiment, but it did not reduce the excreta score at the end of experiment (35 d). The improvement of litter score during the starter period was probably due to the ability of starter birds to metabolise the AFB1 in the liver was low, thus the supplementation of Mycosorb help the starter birds to bind the AFB1 and excreted in feces. Mycosorb also worked better in birds fed 26.97 ppb and 61.06 ppb

AFB1 diets compared to those fed diets containing < 2.02 and 10.36 ppb AFB1. The comparison is difficult to be made because of the limitation of references. The average of litter score of birds given Mycosorb was 1.83, while the litter score of those received no Mycosorb was 2.25 on day 21 of experiment.

The AFB1 level x Mycosorb interaction was found to be significant (p<0.001) in litter score parameter but not significant (p>0.05) in excreta score. The birds fed afla-treated diets added with Mycosorb had lower (p<0.05) litter score than those who fed afla-treated diets without Mycosorb. The litter score of birds fed control diets with the addition of Mycosorb was not different (p>0.05) from those who received a control diet without Mycosorb.

Haematological profile

Published data showed that aflatoxins destroy the blood cell production (haematopoiesis) in bone marrow and lymphatic organs, where blood cells and other blood components are formed (Abdel-Wahhab *et al.*, 2002). The evaluation of blood components such as white blood cells (WBC) count, mean corpuscular haemoglobin concentration (MCHC), the count of the red blood cells (RBC) and haemoglobin (HGB), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) is essential to monitor the toxicity of feed constituents (Etim *et al.*, 2014). The blood profile will provide crucial information regarding the pathological status of a living organism. In the present experiment, the effect of the afla-treated diets supplemented with Mycosorb on haematological profile was studied.

The haematological profile of broilers fed dietary Mycosorb was presented in Table 5. The AFB1 level or Mycosorb alone did not affect (p>0.05) white blood cells (WBC) and red blood cells (RBC) counts, haemaglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin mean corpuscular haemoglobin concentration (MCHC) of

broiler chickens during the experiment. For the first main effect (AFB1 level), the lack of significant change in the WBC counts was inline with Mohaghegh *et al.* (2017). However, it seems that the WBC numbers was slightly higher in AFB1-treated group compared to the control group. In a study carried out by Valchev *et al.* (2018) showed that the WBC of mulard ducks fed aflatoxin contaminated diet was higher than those who were fed with a control diet. According to Safamehr (2008) and Abdel-Wahhab *et al.* (2002) and, the increase in WBC number as a result of aflatoxicosic could be explained by 1) the irritating effect of aflatoxins on the gut mucosa and its inflammation; and 2) the AFB1 changes the function of bone marrow lymphoid organs.

Table 5. Haematological index of broilers fed diets containing low level of aflatoxin B1 (AFB1) and mycotoxin binder (Mycosorb)

Treatment		Variables					
AFB1 Level, ppb	Mycosorb	WBC	RBC	HGB	MCH	MCV	MCHC
		….(x 10^3/µL)…..		g/L	pg	fL	g/dL
nd	-	70.3^c	3.04	9.58	31.3	112	26.4
nd	+	72.2^c	3.11	10.02	32.3	120	26.9
10.36	-	88.4^a	3.03	10.76	32.5	117	27.8
10.36	+	72.0^c	2.77	8.95	32.3	124	26.0
26.97	-	83.3^b	2.85	9.36	32.9	123	26.8
26.97	+	81.6^b	2.92	9.60	32.9	122	27.0
61.06	-	92.4^a	3.08	9.96	32.4	118	28.4
61.06	+	80.7^b	3.16	9.99	31.7	120	26.5
SEM		4.65	0.12	0.38	0.76	1.61	0.70
Main effect, AFB1 level (ppb, AL)							
nd		71.2	3.07	9.80	31.8	119	26.6
10.36		80.2	2.90	9.85	32.4	121	26.9
26.97		82.5	2.88	9.48	32.9	122	26.8
61.06		86.6	3.12	9.98	32.1	118	27.4
SEM		3.29	0.08	0.27	0.55	1.14	0.49
Main effect, Mycosorb (M)							
-		83.6	3.00	9.92	32.3	119	27.3
+		76.6	2.99	9.63	32.3	121	26.6
SEM		2.32	0.06	0.19	0.39	0.81	0.35
Pr > F							
Aflatoxin Level (AL)		NS	NS	NS	NS	NS	NS
Mycosorb (M)		NS	NS	NS	NS	NS	NS
AL x M		*	NS	NS	NS	NS	NS

[a,b] Means of column with the superscripts significant difference (P<0.05), *: Significant (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)
RBC = red blood cells, WBC = white blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin (pg), MCHC = mean corpuscular haemoglobin concentration, and HGB = haemoglobin.

The AFB1 level did not decrease the number of RBC, HGB, MCH and MCV of birds during 35 days of experiment, partly agreed with Kurniasih and Prakoso (2019) and Aravind *et al.* (2003). Especially for RBC, Kurniasih and Prakoso (2019) reported that during the starter period the number of RBC of birds given < 100 ppb

AFB1- diets did not decrease, but when exposed to > 100 ppb AFB1, the RBC number went down.

Except for WBC, no significant interaction (P>0.05) between AFB1 level and Mycosorb was observed in all haematologocal parameters of broilers during the experiment. The WBC count of birds fed the AFB1 diets supplemented with Mycosorb tended to be lower than the WBC number of birds fed a control diet. The present results agreed with Mohaghegh *et al.* (2017) who used esterified glucomannan as adsorbent in afla-treated diets. The ability of yeast glucomannan to decrease the WBC count indicated that this mycotoxin binder has a high ability to bind the AFB1 in the gastrointestinal tract of birds leading to the decrease of AFB1 ansorption, thus reduced the detrimental effects on broilers.

The range of WBC in all dietary treatments was from 70.3 to 92.4 x 10^3/µL. The high WBC number in control diets (with and without Mycosorb) was unexpected result. The present study was not in agreement with Kurniasih and Prakoso (2019) who reported that the WBC number of birds fed control diet and afla-treated diets was between 18.29 and 21.75 x 10^3/µL. In addition, Kurniasih and Prakoso (2019) observed that the number of WBC in broilers decreased when the level of aflatoxin diet > 100 ppb. The variation in the WBC counts and other haematological index may be owing to the difference in the level of aflatoxin, the variation in temperature and geographical location.

Except for white blood cells (WBC) count and mean corpuscular haemoglobin concentration (MCHC), the red blood cells (RBC), haemoglobin (HGB), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) of birds in all dietary treatments were in a normal range.

CONCLUSIONS

Dietary Mycosorb (0.075%) in afla-treated diets improved litter quality and reduced white blood cell counts of broilers. The AFB1 level up to 61.06 ppb did not impair carcass yield, lymphoid organ weights and other haematological index of broilers. Further research is needed to evaluate the efficacy of Mycosorb in poultry diets contaminated with a higher level of aflatoxin.

CONFLICT OF INTEREST

Authors warrant that any financial interests, direct or indirect, that exist or may be perceived to exist for individual contributors in connection with this manuscript have been disclosed in the covering letter. Furthermore, sources of financial support of the project are named in the covering letter as well as the *Acknowledgements*.

ACKNOWLEDGMENT

REFERENCES

Abdel-Wahhab, M. A., Nada, S. A., & Khalil, F. A. (2002). Physiological and toxicological responses in rats fed aflatoxin-contaminated diet with or without sorbent materials. *Animal Feed Science and Technology*, *97*(3–4), 209–219. https://doi.org/10.1016/S0377-8401(01)00342-X

AOAC. (2005). *Official Methods of Analysis of AOAC International* (18th ed.). Assoc. Off. Anal.

Aravind, K., Patil, V., Devegowda, G., Umakantha, B., & Ganpule, S. (2003). Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance and serum biochemical and hematological parameters in broilers.

Poultry Science, *82*(4), 571–576. https://doi.org/10.1093/ps/82.4.571

Belay, T., & Teeter, R. G. (1996). Effects of ambient temperature on broiler mineral balance partitioned into urinary and faecal loss. *British Poultry Science*, *37*(2), 423–433. https://doi.org/10.1080/00071669608417873

Benkerroum, N. (2020). Chronic and acute toxicities of aflatoxins: mechanisms of action. *International Journal of Environmental Research and Public Health*, *17*(2), 423. https://doi.org/10.3390/ijerph17020423

Bhatti, S. A., Khan, M. Z., Saleemi, M. K., Saqib, M., Khan, A., & Ul-Hassan, Z. (2017). Protective role of bentonite against aflatoxin B 1 - and ochratoxin A-induced immunotoxicity in broilers. *Journal of Immunotoxicology*, *14*(1), 66–76. https://doi.org/10.1080/1547691X.2016.1264503

Bolan, N. S., Szogi, A. A., Chuasavathi, T., Seshadri, B., Rothrock, M. J., & Panneerselvam, P. (2010). Uses and management of poultry litter. *World's Poultry Science Journal*, *66*(4), 673–698. https://doi.org/10.1017/S0043933910000656

Cai, P., Zheng, H., She, J., Feng, N., Zou, H., Gu, J., Yuan, Y., Liu, X., Liu, Z., & Bian, J. (2020). Molecular mechanism of aflatoxin-induced hepatocellular carcinoma derived from a bioinformatics analysis. *Toxins*, *12*(3), 203. https://doi.org/10.3390/toxins12030203

Campos-M, M., & Campos-C, R. (2017). Applications of quartering method in soils and foods. *International Journal of Engineering Research and Applications*, *7*(1), 35–39. https://doi.org/10.9790/9622-0701023539

Collett, S. R. (2012). Nutrition and wet litter problems in poultry. *Animal Feed Science and Technology*, *173*(1–2), 65–75. https://doi.org/10.1016/j.anifeedsci.2011.12.013

de Jong, I. C., van Harn, J., Gunnink, H., Hindle, V. A., & Lourens, A. (2012). Footpad dermatitis in Dutch broiler flocks: Prevalence and factors of influence. *Poultry Science*, *91*(7), 1569–1574. https://doi.org/10.3382/ps.2012-02156

Dunlop, M. W., Moss, A. F., Groves, P. J., Wilkinson, S. J., Stuetz, R. M., & Selle, P. H. (2016). The multidimensional causal factors of 'wet litter' in chicken-meat production. *Science of The Total Environment*, *562*, 766–776. https://doi.org/10.1016/j.scitotenv.2016.03.147

Elkatcha, M., Soltan, M., ElShobokshy, S., & Shokry, A. (2017). Protective effect of chemical and biological mycotoxin binder on growth performance, serum biochemistry and carcass traits in broiler chicks fed on aflatoxin contaminated diet. *Alexandria Journal of Veterinary Sciences*, *55*(1), 180–197. https://doi.org/10.5455/ajvs.269041

Etim, N. N. (2014). Haematological parameters and factors affecting their values. *Agricultural Science*, *2*(1), 37–47. https://doi.org/10.12735/as.v2i1p37

Feng, G. D., He, J., Ao, X., & Chen, D. W. (2017). Effects of maize naturally contaminated with aflatoxin B1 on growth performance, intestinal morphology, and digestive physiology in ducks. *Poultry Science*, *96*(6), 1948–1955. https://doi.org/10.3382/ps/pew420

Fouad, A., Ruan, D., El-Senousey, H., Chen, W., Jiang, S., & Zheng, C. (2019). Harmful effects and control strategies of aflatoxin B1 produced by aspergillus flavus and aspergillus parasiticus strains on poultry: review. *Toxins*, *11*(3), 176. https://doi.org/10.3390/toxins11030176

Fountain, J. C., Khera, P., Yang, L., Nayak, S. N., Scully, B. T., Lee, R. D., Chen, Z.-Y., Kemerait, R. C., Varshney, R. K., & Guo, B. (2015). Resistance to Aspergillus flavus in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives. *The Crop Journal*, *3*(3), 229–237. https://doi.org/10.1016/j.cj.

2015.02.003

Fowler, J., Li, W., & Bailey, C. (2015). Effects of a calcium bentonite clay in diets containing aflatoxin when measuring liver residues of aflatoxin B1 in starter broiler chicks. *Toxins*, *7*(9), 3455–3464. https://doi.org/10.3390/toxins7093455

Galarza-Seeber, R., Latorre, J. D., Bielke, L. R., Kuttappan, V. A., Wolfenden, A. D., Hernandez-Velasco, X., Merino-Guzman, R., Vicente, J. L., Donoghue, A., Cross, D., Hargis, B. M., & Tellez, G. (2016). Leaky gut and mycotoxins: aflatoxin B1 does not increase gut permeability in broiler chickens. *Frontiers in Veterinary Science*, *3*. https://doi.org/10.3389/fvets.2016.00010

Grenier, B., & Applegate, T. (2013). Modulation of Intestinal functions following mycotoxin ingestion: meta-analysis of published experiments in animals. *Toxins*, *5*(2), 396–430. https://doi.org/10.3390/toxins5020396

Han, X.-Y., Huang, Q.-C., Li, W.-F., Jiang, J.-F., & Xu, Z.-R. (2008). Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. *Livestock Science*, *119*(1–3), 216–220. https://doi.org/10.1016/j.livsci.2008.04.006

Hermans, P. G., Fradkin, D., Muchnik, I. B., & Morgan, K. L. (2006). Prevalence of wet litter and the associated risk factors in broiler flocks in the United Kingdom. *Veterinary Record*, *158*(18), 615–622. https://doi.org/10.1136/vr.158.18.615

Hoeven-Hangoor. (2014). Broiler excreta composition and its effect on wet litter: aspects of nutrition. In *Broiler excreta composition and its effect on wet litter: aspects of nutrition*. Wangeningen University.

Hoover, N. L., Kanwar, R., Soupir, M. L., & Pederson, C. (2015). Effects of poultry manure application on phosphorus in soil and tile drain water under a corn-soybean rotation. *Water, Air, & Soil Pollution*, *226*(5), 138. https://doi.org/10.1007/s11270-015-2403-9

Hossein, A., & Gürbüz, Y. (2016). Aflatoxins in poultry nutrition. *Kahramanmaraş Sütçü İmam Üniversitesi Doğa Bilimleri Dergisi*, *18*(4), 1. https://doi.org/10.18016/ksujns.98227

Hussain, Z., Rehman, H.-, Manzoor, S., Tahir, S., & Mukhtar, M. (2016). Determination of liver and muscle aflatoxin B1 residues and select serum chemistry variables during chronic aflatoxicosis in broiler chickens. *Veterinary Clinical Pathology*, *45*(2), 330–334. https://doi.org/10.1111/vcp.12336

Iqbal, S. Z., Asi, M. R., & Ariño, A. (2013). Aflatoxin. In Brenner's Encyclopedia of Genetics (Ed.), *Stanley Maloy and Kelly Hughes* (2nd ed., pp. 43–47). Academic Press.

Karimy, M. F., Sutrisno, B., Agus, A., Suryani, A. E., Istiqomah, L., & Damayanti, E. (2017). Aflatoxin effect on erythrocyte profile and histopathology of broilers given different additives. *IOP Conference Series: Earth and Environmental Science*, *101*, 012033. https://doi.org/10.1088/1755-1315/101/1/012033

Khan, M. Z. I., Masum, M., Khan, M. Z. I., Aziz, A. R. Bin, Nasrin, M., Siddique, M. N. H., & Arshad, M. M. Bin. (2014). Histomorphology of the lymphoid tissues of broiler chickens in Kelantan, Malaysia. *Sains Malaysiana*, *43*(8), 1175–1179.

Kheravii, S. K., Swick, R. A., Choct, M., & Wu, S.-B. (2017). Potential of pelleted wheat straw as an alternative bedding material for broilers. *Poultry Science*, *96*(6), 1641–1647. https://doi.org/10.3382/ps/pew473

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., & Kang, S. G. (2017). Aflatoxins: a global concern for food safety, human health and their

management. *Frontiers in Microbiology*, *07*, 2170. https://doi.org/10.3389/fmicb.2016.02170

Kumar, R., & Balachandran, C. (2009). Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid. *Veterinarski Arhiv*, *79*(1), 31–40.

Kumar, V. V. (2018). Aflatoxins: properties, toxicity and detoxification. *Nutrition & Food Science International Journal*, *6*(5). https://doi.org/10.19080/NFSIJ.2018.06.555696

Latimer, G. W. (2005). *AOAC International. Official methods of analysis of AOAC International* (19th ed.). AOAC International.

Liu, N., Wang, J., Deng, Q., Gu, K., & Wang, J. (2018). Detoxification of aflatoxin B 1 by lactic acid bacteria and hydrated sodium calcium aluminosilicate in broiler chickens. *Livestock Science*, *208*, 28–32. https://doi.org/10.1016/j.livsci.2017.12.005

Marchese, S., Polo, A., Ariano, A., Velotto, S., Costantini, S., & Severino, L. (2018). Aflatoxin B1 and M1: biological properties and their involvement in cancer development. *Toxins*, *10*(6), 214. https://doi.org/10.3390/toxins10060214

Mogadam, N. (2011). Ameliorative effect of glucomannan-containing yeast product (Mycosorb) and sodium bentonite on performance and antibody titers against Newcastle disease in broilers during chronic aflatoxicosis. *African Journal Of Biotechnology*, *10*(75), 17372–17378. https://doi.org/10.5897/AJB11.238

Mohaghegh, A., Chamani, M., Shivazad, M., Sadeghi, A. A., & Afzali, N. (2017). Effect of esterified glucomannan on broilers exposed to natural mycotoxin-contaminated diets. *Journal of Applied Animal Research*, *45*(1), 285–291. https://doi.org/10.1080/09712119.2016.1174122

Moran, C. A., Apajalahti, J., Yiannikouris, A., Ojanperä, S., & Kettunen, H. (2013). Effects of low dietary aflatoxin B1 on broiler liver concentration without and with Mycosorb ® toxin binder. *Journal of Applied Animal Nutrition*, *2*, e4. https://doi.org/10.1017/jan.2013.11

Murugesan, G. R., Ledoux, D. R., Naehrer, K., Berthiller, F., Applegate, T. J., Grenier, B., Phillips, T. D., & Schatzmayr, G. (2015). Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. *Poultry Science*, *94*(6), 1298–1315. https://doi.org/10.3382/ps/pev075

Nazarizadeh, H., & Pourreza, J. (2019). Evaluation of three mycotoxin binders to prevent the adverse effects of aflatoxin B 1 in growing broilers. *Journal of Applied Animal Research*, *47*(1), 135–139. https://doi.org/10.1080/09712119.2019.1584106

Peng, X., Bai, S., Ding, X., Zeng, Q., Zhang, K., & Fang, J. (2015). Pathological changes in the immune organs of broiler chickens fed on corn naturally contaminated with aflatoxins B 1 and B 2. *Avian Pathology*, *44*(3), 192–199. https://doi.org/10.1080/03079457.2015.1023179

Prakoso, Y. A., & Kurniasih, K. (2019). Recent update: effects of aflatoxin in broiler chickens. *Journal of World's Poultry Research*, *9*(2), 68–77. https://doi.org/10.36380/jwpr.2019.8

Raphal, K., Ferdinand, N., Herv, T., Dalex, T., Romo, S., Alexis, T., & Benoit, G. (2014). Effect of biocharcoals on hematological, serum biochemical and histological parameters in broiler chickens fed aflatoxin B1-contaminated diets. *Journal of Animal Science Advances*, *4*(7), 939. https://doi.org/10.5455/jasa.20140617040106

Resanovic, R., & Sinovec, Z. (2006). Effects of limited feeding of aflatoxin B1 contaminated feed on the performance of broilers. *Mycotoxin Research*,

22(3), 183–188. https://doi.org/10.1007/BF02959274

Safameher, A. (2008). Effects of Clinoptilolite on performance, biochemical parameters and hepatic lesions in broiler chickens during aflatoxosis. *Journal of Animal and Veterinary Advances*, *7*(4), 381–388.

Salwa, A. A., & Anwer, W. (2009). Effect of naturally contaminated feed with aflatoxins on performance of laying hens and the carryover of aflatoxin B1 residues in table eggs. *Pakistan Journal of Nutrition*, *8*(2), 181–186. https://doi.org/10.3923/pjn.2009.181.186

Saminathan, M., Selamat, J., Abbasi Pirouz, A., Abdullah, N., & Zulkifli, I. (2018). Effects of nano-composite adsorbents on the growth performance, serum biochemistry, and organ weights of broilers fed with aflatoxin-contaminated feed. *Toxins*, *10*(9), 345. https://doi.org/10.3390/toxins10090345

Shepherd, E. M., & Fairchild, B. D. (2010). Footpad dermatitis in poultry. *Poultry Science*, *89*(10), 2043–2051. https://doi.org/10.3382/ps.2010-00770

Sineque, A., Macuamule, C., & Dos Anjos, F. (2017). Aflatoxin B1 Contamination in chicken livers and gizzards from industrial and small abattoirs, measured by ELISA Technique in Maputo, Mozambique. *International Journal of Environmental Research and Public Health*, *14*(9), 951. https://doi.org/10.3390/ijerph14090951

Teirlynck, E., Gussem, M. D. E., Dewulf, J., Haesebrouck, F., Ducatelle, R., & Van Immerseel, F. (2011). Morphometric evaluation of "dysbacteriosis" in broilers. *Avian Pathology*, *40*(2), 139–144. https://doi.org/10.1080/030794

57.2010.543414

Valchev, I., Groseva, N., Kanakov, D., Hristov, T., Lazarov, L., & Binev, R. (2018). Effect of experimentally induced aflatoxicosis on haematological parameters and bone marrow morphology in mulard ducks. *Agricultural Science and Technology*, *10*(3), 208–214. https://doi.org/10.15547/ast.2018.03.040

van Ryssen, J. B. J., Channon, P., & Stielau., W. J. (1977). Minerals and nitrogen in poultry manure. *South African Journal of Animal Science*, *7*, 195–199.

Wade, M. R., Sapcota, D., & Verma, U. (2017). Ameliorating aflatoxicosis in commercial broiler chickens by dietary Mycosorb: Heamato-Biochemical studies. *Indian Journal of Animal Research*, *3182*(00), 1–5. https://doi.org/10.18805/ijar.B-3182

Wu, H.-C., & Santella, R. (2012). The role of aflatoxins in hepatocellular carcinoma. *Hepatitis Monthly*, *12*(10 HCC). https://doi.org/10.5812/hepatmon.7238

Yang, J., Bai, F., Zhang, K., Lv, X., Bai, S., Zhao, L., Peng, X., Ding, X., Li, Y., & Zhang, J. (2012). Effects of feeding corn naturally contaminated with AFB_1 and AFB_2 on performance and aflatoxin residues in broilers. *Czech Journal of Animal Science*, *57*(No. 11), 506–515. https://doi.org/10.17221/6383-CJAS

Yunus, A. W., Razzazi-Fazeli, E., & Bohm, J. (2011). Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. *Toxins*, *3*(6), 566–590. https://doi.org/10.3390/toxins3060566