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"Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck"

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[JITAA] Submission Acknowledgement
1 message

Edy Kurnianto <kurniantoedy17@gmail.com> — Sun, Mar 24, 2019 at 1:37 PM
To: Mr Ika SUMANTRI <isumantri@ulm.ac.id>

Mr Ika SUMANTRI:

Thank you for submitting the manuscript, "Effect of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck" to Journal of the Indonesian Tropical Animal Agriculture. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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2. Telaah oleh reviewer tanggal 12 Juni 2019

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Hasil Telaah Manuskrip #22495
3 messages

JITAA JPPT <jppt.fpundip@gmail.com> — Wed, Jun 12, 2019 at 11:40 PM
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Sdr Ika Sumantri

Kami kirimkan hasil telaah manuskrip oleh 2 reviewer. Kami memberikan waktu 2 minggu untuk proses perbaikan. Bila dalam waktu 2 minggu tidak ada respon, kami beranggapan bahwa Sdr tidak melanjutkan proses publikasi.

Salam,

2 attachments

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Effect of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck

ABSTRAK

Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) P1: pakan komersial; (2) P2: pakan terkontaminasi AFB1 70 ppb; (3) P3: P1 + 2% zeolit; (4) P4: P2 + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil memperlihatkan paparan AFB1 70 pbb menyebabkan penurunan bobot badan sekitar 1,12% (*P*<0,05). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat dengan naiknya bobot badan sekitar 2,95% pada P4. Perlakuan tidak berpengaruh terhadap produksi telur (*P*>0,05). Paparan AFB1 cenderung menurunkan bobot telur dan imbuhan zeolit cenderung mengurangi penurunan bobot telur. Paparan AFB1 menghasilkan bobot potong yang lebih rendah (*P*<0,05), namun dengan imbuhan zeolit, itik pada P4 menghasilkan bobot potong yang paling tinggi. Paparan AFB1 cenderung menghasilkan bobot relatif hati yang lebih besar (16,62%), namun dampak ini menurun dengan imbuhan zeolit (15,4%). Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan bobot badan.

Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit

ABSTRACT

The research was objected to studying the effect of zeolite inclusion in aflatoxin B1 (AFB1) contaminated diet on the performance of laying duck. A completely randomized design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1) P1: commercial feed; (2) P2: AFB1-contaminated feed 70 ppb; (3) P1 + 2% zeolite; and (4) P4: P2 + 2% zeolite. Each treatment had 4 replications with 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data was analyzed according to the general linear model of SPSS 21.0 statistical software. Results indicated AFB1 exposure significantly (P<0.05) decreases body weight of laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that increased by 2.95% in P4. Treatments had no significant effect on egg production (P>0.05). The lowest egg weight was found in P2 and zeolite inclusion seemed to increase egg weight, but this was not statistically significant (P>0.05). Zeolite inclusion resulted in the highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight (P<0.05). Ducks received AFB1 contaminated had heavier liver weight (16.62%), and liver weight was decreased by zeolite inclusion (15.4%). In conclusion, 2% zeolite inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying duck.

Key words: Aflatoxin B1, laying duck, perfomans, zeolite

INTRODUCTION

Aflatoxin B1 (AFB1) is highly carcinogenic and genotoxic compounds produced by fungi, especially toxigenic species of *Aspergillus flavus* and *A. parasiticus*. The consumption of feed containing AFB1 by the animal can result in excretion of a hydroxylated metabolite of aflatoxin, namely aflatoxin M1 (AFM1), in the animal products, such as milk, meat, and eggs (Voelkel *et al*., 2011; van der Fels-Klerx and Camenzuli, 2016). In order to avoid AFB1 exposure on livestock and ingestion of its residues by consumers, the Indonesian government has established the maximum limit of AFB1 contamination in feed for the industry (SNI). However, tropical climate causes high occurrences and levels of AFB1 contamination in feed for ruminant and poultry in Indonesia (Agus *et al*., 2013; Sumantri *et al*., 2017).

Several strategies have been developed to minimize the toxic effects of aflatoxins on animal and the transfer of its residues into animal products, such as physical, chemical, and biological methods. However, in recent years the use of aflatoxin adsorbent is the most studied method because it is considered as an effective, safe, economical and applicable method (Kutz *et al*., 2009). One of the aflatoxin adsorbents is zeolite, a silicate mineral that has the ability to bind aflatoxin so that it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al* ., 2010).

Compared to chickens, duck is highly sensitive to aflatoxin exposure because of differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz and Murcia, 2011). Therefore, consumption of AFB1 contaminated diet will adversely affect duck's performance. This research aims to investigate the effects of zeolite inclusion in AFB1 contaminated diet on the performance of laying duck.

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Please provide the "gap" between the existing data/literatures and actual condition!

How is the possible mechanism by which zeolite bind AFB1 in laying duck?? Please explain

MATERIALS AND METHODS

Experimental Diet

AFB1-contaminated diet (AFC) was produced as follow: commercial feed for laying duck (IP333, PT. Wonokoyo) is inoculated with *A. flavus* FNCC 612 with the moisture of 30% then incubated in temperature 35^{o}C for 10 days. The concentration of AFB1 in AFC was analyzed to calculate the dilution factor of AFC in the experimental diet. AFC then mixed with commercial feed based on the dilution factor to obtain AFB1 levels of 70 ppb.

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The zeolite that was used in the experiment was a natural zeolite which is mined and purchased in Central Java. Zeolite was ground using a mortar and sieved through 100 mesh.

In Vivo Experiment

Seven months, sixty-four female Alabio ducks (*Anas platyrinchos* Borneo) were used in the experiment. Ducks were weighed and randomly assigned to 4 dietary treatments with 4 replicates of 4 ducks in each experimental unit. The treatment diets were: commercial feed as a control diet (P1); commercial feed contaminated with AFB1 70 ppb (P2); P1 with 2% of zeolite inclusion (P3); and P2 with 2% of zeolite inclusion (P4).

Dietary treatment was started when the egg production, Duck Day Average (DDA), reaches 70%. The experiment was carried out for 4 weeks. Experimental diet was provided twice a day and restricted, namely 150 g/head/day, to ensure the level of AFB1 exposure on the animal is controlled. Water was provided *ad libitum*.

Egg production was recorded and weighed daily, starting at 15th day until the 28th day of treatment. Body weight of duck was measured individually at two weeks before treatment (D-14), the beginning of treatment (D0), the second week of treatment (D14), and the fourth week of treatment (D28). At the end of the experiment (D28), ducks were sacrificed, then carcass and giblet were collected and weighed. The observed variables were changes in body weight, egg production (egg weight and DDA), the weight percentages of carcass, liver, and intestine, and histopathology of liver.

Liver histopathology was diagnosed as follow: representative liver samples were fixed in 10% buffered neutral formalin. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris according to Manual Standard of Patologi Diagnose of Veterinary Laboratory.

Analysis

Feed samples were analysed for AFB1 concentrations by ELISA method using ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of live weight changes, egg weight, DDA percentage, carcass percentage, liver percentage, and intestine weight were analysed by the general linear model procedure using the IBM SPSS 21.0 statistical program. Significant differences between treatment means were separated using the Duncan's multiple range test with a 5% probability.

RESULTS AND DISCUSSIONS

Live Weight Changes

Treatments had a significant effect on live weight changes ($p < 0.05$). Table 1. showed that AFB1 exposure at 70 ppb would cause a decrease in duck weight, as seen in P2, which experienced an average weight loss of -1.12% after four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be reduced by zeolite inclusion in the diet. This was indicated by the average of live weight of ducks in P4 that increased by 2.95% while receiving a diet containing AFB1 70 ppb and zeolite. Zeolite also significantly improved duck performance, as seen in P3, that had the highest body weight gain, namely 4.56%. This gain was higher than the control feed (P1) which only increased by 2.1%.

The adverse effects of AFB1 on growth performance is related with a decrease in the efficiency of protein and energy utilizations due to the deterioration of the digestive system of the birds (Denly *et al*., 2009). Figure 1. clearly shows that the presence of AFB1 in diet (P2) decreases growth perfomance of the duck.

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This study indicated the positive effect of zeolite inclusion in diet for laying duck. Addition of 2% zeolite in control diet resulted the highest final live weight (P3). By zeolite inclusion, the growth performance of duck receiving AFB1 contaminated diet (P4) was still higher than the control diet (P1).

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Study on the effects of AFB1 on the performance of laying ducks is still very limited. In 1-day-old ducklings which received a feed containing AFB1 at levels up to 100 ppb for 21 days, an increase in AFB1 level caused a decrease in weight gain (Wan *et al*., 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al* ., 2001). Yunus *et al.* (2011)

concluded that in chickens, consumption of aflatoxin caused weight loss, decreased feed consumption, and increased feed conversion. The percentage of weight loss reported varies depending on the dose and duration of exposure, such as 5% weight loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15% weight loss at a dose of 1,000 ppb for 21 days.

This study shows the use of zeolite can increase weight gain and reduce the impact of exposure to AFB1. Chemically, zeolite is a clay group of aluminosilicate minerals wich has a three-dimensional structure consisting of skeletons of SiO_4 and AlO_4 which form interconnected channels where in the channel cavity there are weak bonds of H_2O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO_4 (Mallek *et al* ., 2012).

In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of AFB1 exposure (Wan *et al* ., 2013). In broiler, the dietary use of natural or synthetic zeolites has been reported to improve feed efficiency, thus resulting to a better growth performance of broilers (Mallek *et al*., 2012). Zeolites (montmorillonites) are a class of the smectite clay group, which has 3-layer structures that allow to adsorb heavy metals, bacteria, and toxic antinutritive agents (Fowler *et al* ., 2015; Sulzberger *et al* ., 2017). The binding between aflatoxin and the adsorbent forms an inert and stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract (Huwig *et al* ., 2001).

Egg Production

In this study, the treatments had no effect on egg production and weight ($p >$ 0.05). However, as shown in Table 2., the presence of AFB1 contamination tends to

Commented [T7]: Please provide the p-value reduce egg weight (P2), and the addition of adsorbent tends to increase egg weight (P3) despite the presence of AFB1 contamination in the feed (P4) ($P > 0.05$).

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Aflatoxin-contaminated feed causes a decrease in egg production, as shown in the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40% in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and Awer (2009) showed that the production and egg weight of white leghorn laying hens were not affected by feed contaminated with aflatoxin at the levels of 100 ppb for 60 days, although feeding with aflatoxin contamination caused a decrease in feed consumption. Research Zaghini *et al*. (2005) showed a decrease in egg weight of laying hens receiving AFB1 2,500 ppb for 4 weeks, this was due to a decrease in the percentage of eggshell weight and thinner eggshells due to the AFB1 exprosure through contaminated feed consumption.

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Weights of Carcass, Liver, and Intestine

This experiment showed the treatments have a significant effect (P <0.05) on final body weight. Nevertheless the treatments did not have significant effects ($P >$ 0.05) on carcass percentage, relative weight of giblet, and relative weight of liver (Table 3. and Table 4.).

Duck carcass is mainly distributed in breast and thigh, therefore the weights of breast and thigh muscles is the main factor of carcass yield of duck. Study of Chang *et al.* (2016) showed diet containing aflatoxin at 62-65 ppb significantly reduces live weight, breast muscle weight, and thigh muscle weight of meat male ducks at various age.

Although the statistical test did not show any difference in the relative weight of all variables, it was seen that in AFB1 contaminated diet group (P2 and P4) there was an enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier than the control (13.54%). This results were also found in the relative weights of the intestines for P2 and P4, namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

This study applied a relatively low of AFB1 contamination level in the diet, but this is a common level of AFB1 contamination that found in feed and feedstuffs for duck in Indonesia according to previous study of Sumantri *et al*. (2017). At low dose of aflatoxin exposure, the performance of birds are relatively unchanged, but changes in liver size and pathology can be detected (Magnoli *et al* ., 2011). Study of Denli *et al* . (2009) found broiler liver enlargement after receiving feed containing AFB1 at 1,000 ppb.

Liver is the target organ of AFB1 because most of the AFB1 absorbed will undergo bioactivation to form a compound 8.9-epoxide which then binds to protein and DNA (Pasha *et al*., 2007). Our study indicated mild acute degeneration of vacuoles in the liver of ducks received P1 diet, but this degeneration was severe in P2 diet. In zeolite groups, mild vacuoles degeneration was found in P3 and medium degeneration was in P4 (Figure 2).

Hepatic lesions correlated with aflatoxicosis is described as a vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver (Espada *et al*., 1992). Study of Leenadevi *et al*. (1995) revealed that ducks are a very sensitive species for aflatoxin injury and it would appear that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed zeolite inclusion seems to reduce the adverse effects of AFB1 exposure as indicated in the result of liver histopathology study of P3 and P4 groups. Similarly, Magnolli *et al*. (2011) found that in low levels of AFB1 (50 to 100 ppb), all livers samples of broilers showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet.

CONCLUSION

Zeolite inclusions in AFB1-contaminated diet for laying ducks could reduce the adverse effects of AFB1 exposure, especially on body weight and liver histopatology. Nevertheless, the use of zeolite did not appear to increase the egg production of laying duck.

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Table 1. Effects of Treatment Diet on Live Weight Change of Laying Duck

Treatment Diet	Initial Weight (g)	Final Weight (g)	Live weight Change	
			gram	%
Commercial Feed (P1)	1382	1411	29.06[ab]	2.10[ab]
P1 + AFB1 70 ppb (P2)	1447	1431	-16.25[a]	-1.12[a]
P1 + 2% zeolite (P3)	1405	1469	64.06[b]	4.56[b]
P2 + 2% zeolite (P4)	1386	1427	40.94[ab]	2.95[ab]

[a, b] means in same column with different superscripts differ significantly ($P < 0.05$)

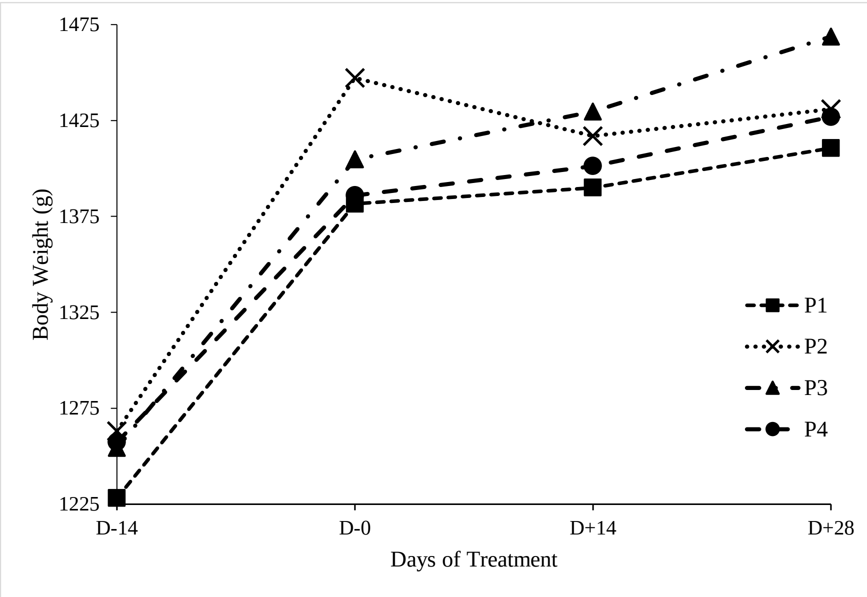

Figure 1. Live weight curves of laying ducks treated with control diet (P1); P1 containing AFB1 70 ppb (P2); P1 + 2% zeolite (P3); and P2 + 2% zeolite (P4)

Tabel 2. Effects of Treatment Diet on Egg Production of Laying Duck

Treatment Diet	DDA (%) ^{ns}	Egg Production (g) ^{ns}	Egg Weight (g) ^{ns}
Commercial Feed (P1)	54.40	4147.25	70.70
P1 + AFB1 70 ppb (P2)	64.58	4842.75	69.23
P1 + 2% zeolite (P3)	56.71	4417.00	72.10
P2 + 2% zeolite (P4)	58.56	4488.25	70.81

^{ns} means in the same column are not significantly different (*P* > 0.05)

Tabel 3. Effects of Treatment Diet on Final Body Weight, Carcass Weight, Giblet Weight, Liver Weight, and Small Intestinum Weight of Laying Duck

Treatment Diet	Final Body Weight (g)	Carcass Weight (g)^{ns}	Giblet Weight (g)^{ns}	Liver Weight (g)^{ns}
Commercial Feed (P1)	1460.00^{ab}	832.5	351.75	43.75
P1 + AFB1 70 ppb (P2)	1426.25^{a}	846.5	289.25	47.50
P1 + 2% zeolite (P3)	1406.25^{a}	835.0	300.00	43.00
P2 + 2% zeolite (P4)	1576.25^{b}	951.0	323.50	49.50

$^{a, b}$ means in same column with different superscripts differ significantly (*P* < 0.05)
^{ns} means in the same column are not significantly different (*P* > 0.05)

Tabel 4. Effects of Treatment Diet on the Percentages of Carcass, Giblet, and Liver of Laying Duck

Treatment Diet	Percentages		
	Carcass (%)^{ns}	Giblet (%)^{ns}	Liver (%)^{ns}
Commercial Feed (P1)	57.28	42.40	13.54
P1 + AFB1 70 ppb (P2)	59.22	35.91	16.62
P1 + 2% zeolite (P3)	59.19	37.24	14.65
P2 + 2% zeolite (P4)	60.30	34.08	15.40

^{ns} means in the same column are not significantly different (*P* > 0.05)

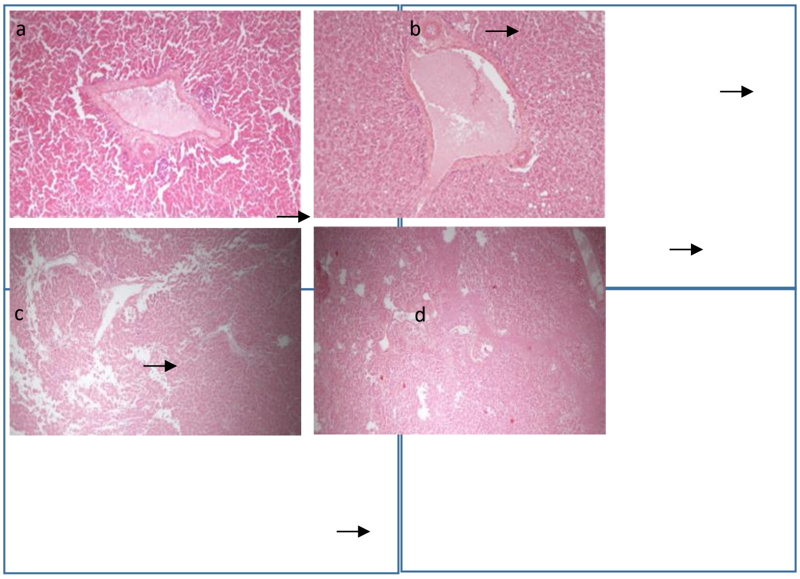

Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial feed); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

Effect of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck

ABSTRAK

Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) P1: pakan komersial; (2) P2: pakan terkontaminasi AFB1 70 ppb; (3) P3: P1 + 2% zeolit; (4) P4: P2 + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil memperlihatkan paparan AFB1 70 pbb menyebabkan penurunan bobot badan sekitar 1,12% (*P*<0,05). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat dengan naiknya bobot badan sekitar 2,95% pada P4. Perlakuan tidak berpengaruh terhadap produksi telur (*P*>0,05). Paparan AFB1 cenderung menurunkan bobot telur dan imbuhan zeolit cenderung mengurangi penurunan bobot telur. Paparan AFB1 menghasilkan bobot potong yang lebih rendah (*P*<0,05), namun dengan imbuhan zeolit, itik pada P4 menghasilkan bobot potong yang paling tinggi. Paparan AFB1 cenderung menghasilkan bobot relatif hati yang lebih besar (16,62%), namun dampak ini menurun dengan imbuhan zeolit (15,4%). Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan bobot badan.

Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit

Commented [SST1]: Jika secara statistik tidak signifikan berarti adalah sama alias tidak ada efek, maka tidak bisa dikatakan menurun. Nyatakan saja fakta nya bobot hati dari 16,62% jadi 15,4%.

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ABSTRACT

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The research was objected to studying the effect of zeolite inclusion in aflatoxin B1 (AFB1) contaminated diet on the performance of laying duck. A completely randomized design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1) P1: commercial feed; (2) P2: AFB1-contaminated feed 70 ppb; (3) P1 + 2% zeolite; and (4) P4: P2 + 2% zeolite. Each treatment had 4 replications with 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data was analyzed according to the general linear model of SPSS 21.0 statistical software. Results indicated AFB1 exposure significantly (P<0.05) decreases body weight of laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that increased by 2.95% in P4. Treatments had no significant effect on egg production (P>0.05). The lowest egg weight was found in P2 and zeolite inclusion seemed to increase egg weight, but this was not statistically significant (P>0.05). Zeolite inclusion resulted in the highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight (P<0.05). Ducks received AFB1 contaminated had heavier liver weight (16.62%), and liver weight was decreased by zeolite inclusion (15.4%). In conclusion, 2% zeolite inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying duck.

Key words: Aflatoxin B1, laying duck, perfomans, zeolite

INTRODUCTION

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Aflatoxin B1 (AFB1) is highly carcinogenic and genotoxic compounds produced by fungi, especially toxigenic species of *Aspergillus flavus* and *A. parasiticus*. The consumption of feed containing AFB1 by the animal can result in excretion of a hydroxylated metabolite of aflatoxin, namely aflatoxin M1 (AFM1), in the animal products, such as milk, meat, and eggs (Voelkel *et al*., 2011; van der Fels-Klerx and Camenzuli, 2016). In order to avoid AFB1 exposure on livestock and ingestion of its residues by consumers, the Indonesian government has established the maximum limit of AFB1 contamination in feed for the industry (SNI). However, tropical climate causes high occurrences and levels of AFB1 contamination in feed for ruminant and poultry in Indonesia (Agus *et al*., 2013; Sumantri *et al*., 2017).

Several strategies have been developed to minimize the toxic effects of aflatoxins on animal and the transfer of its residues into animal products, such as physical, chemical, and biological methods. However, in recent years the use of aflatoxin adsorbent is the most studied method because it is considered as an effective, safe, economical and applicable method (Kutz *et al*., 2009). One of the aflatoxin adsorbents is zeolite, a silicate mineral that has the ability to bind aflatoxin so that it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al* ., 2010).

Compared to chickens, duck is highly sensitive to aflatoxin exposure because of differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz and Murcia, 2011). Therefore, consumption of AFB1 contaminated diet will adversely affect duck's performance. This research aims to investigate the effects of zeolite inclusion in AFB1 contaminated diet on the performance of laying duck.

MATERIALS AND METHODS

Experimental Diet

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AFB1-contaminated diet (AFC) was produced as follow: commercial feed for laying duck (IP333, PT. Wonokoyo) is inoculated with *A. flavus* FNCC 612 with the moisture of 30% then incubated in temperature 35ºC for 10 days. The concentration of AFB1 in AFC was analyzed to calculate the dilution factor of AFC in the experimental diet. AFC then mixed with commercial feed based on the dilution factor to obtain AFB1 levels of 70 ppb.

The zeolite that was used in the experiment was a natural zeolite which is mined and purchased in Central Java. Zeolite was ground using a mortar and sieved through 100 mesh.

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In Vivo Experiment

Seven months, sixty-four female Alabio ducks (*Anas platyrinchos* Borneo) were used in the experiment. Ducks were weighed and randomly assigned to 4 dietary treatments with 4 replicates of 4 ducks in each experimental unit. The treatment diets were: commercial feed as a control diet (P1); commercial feed contaminated with

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AFB1 70 ppb (P2); P1 with 2% of zeolite inclusion (P3); and P2 with 2% of zeolite inclusion (P4).

Dietary treatment was started when the egg production, Duck Day Average (DDA), reaches 70%. The experiment was carried out for 4 weeks. Experimental diet was provided twice a day and restricted, namely 150 g/head/day, to ensure the level of AFB1 exposure on the animal is controlled. Water was provided *ad libitum*.

Egg production was recorded and weighed daily, starting at 15th day until the 28th day of treatment. Body weight of duck was measured individually at two weeks before treatment (D-14), the beginning of treatment (D0), the second week of treatment (D14), and the fourth week of treatment (D28). At the end of the experiment (D28), ducks were sacrificed, then carcass and giblet were collected and weighed. The observed variables were changes in body weight, egg production (egg weight and DDA), the weight percentages of carcass, liver, and intestine, and histopathology of liver.

Liver histopathology was diagnosed as follow: representative liver samples were fixed in 10% buffered neutral formalin. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris according to Manual Standard of Patologi Diagnose of Veterinary Laboratory.

Analysis

Feed samples were analysed for AFB1 concentrations by ELISA method using ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of live weight changes, egg weight, DDA percentage, carcass percentage, liver percentage, and intestine weight were analysed by the general linear model procedure using the IBM SPSS 21.0 statistical program. Significant differences between treatment means were separated using the Duncan's multiple range test with a 5% probability.

RESULTS AND DISCUSSIONS

Live Weight Changes

Treatments had a significant effect on live weight changes (p <0.05). Table 1. showed that AFB1 exposure at 70 ppb would cause a decrease in duck weight, as seen in P2, which experienced an average weight loss of -1.12% after four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be reduced by zeolite inclusion in the diet. This was indicated by the average of live weight of ducks in P4 that increased by 2.95% while receiving a diet containing AFB1 70 ppb and zeolite. Zeolite also significantly improved duck performance, as seen in P3, that had the highest body weight gain, namely 4.56%. This gain was higher than the control feed (P1) which only increased by 2.1%.

The adverse effects of AFB1 on growth performance is related with a decrease in the efficiency of protein and energy utilizations due to the deterioration of the digestive system of the birds (Denly *et al.*, 2009). Figure 1. clearly shows that the presence of AFB1 in diet (P2) decreases growth perfomance of the duck.

This study indicated the positive effect of zeolite inclusion in diet for laying duck. Addition of 2% zeolite in control diet resulted the highest final live weight (P3). By zeolite inclusion, the growth performance of duck receiving AFB1 contaminated diet (P4) was still higher than the control diet (P1).

Study on the effects of AFB1 on the performance of laying ducks is still very limited. In 1-day-old ducklings which received a feed containing AFB1 at levels up to

100 ppb for 21 days, an increase in AFB1 level caused a decrease in weight gain (Wan *et al*., 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al* ., 2001). Yunus *et al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss, decreased feed consumption, and increased feed conversion. The percentage of weight loss reported varies depending on the dose and duration of exposure, such as 5% weight loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15% weight loss at a dose of 1,000 ppb for 21 days.

This study shows the use of zeolite can increase weight gain and reduce the impact of exposure to AFB1. Chemically, zeolite is a clay group of aluminosilicate minerals wich has a three-dimensional structure consisting of skeletons of SiO_4 and AlO_4 which form interconnected channels where in the channel cavity there are weak bonds of H_2O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO_4 (Mallek *et al* ., 2012).

In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of AFB1 exposure (Wan *et al* ., 2013). In broiler, the dietary use of natural or synthetic zeolites has been reported to improve feed efficiency, thus resulting to a better growth performance of broilers (Mallek *et al*., 2012). Zeolites (montmorillonites) are a class of the smectite clay group, which has 3-layer structures that allow to adsorb heavy metals, bacteria, and toxic antinutritive agents (Fowler *et al* ., 2015; Sulzberger *et al* ., 2017). The binding between aflatoxin and the adsorbent forms an inert and stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract (Huwig *et al* ., 2001).

Egg Production

In this study, the treatments had no effect on egg production and weight ($p > 0.05$). However, as shown in Table 2., the presence of AFB1 contamination tends to reduce egg weight (P2), and the addition of adsorbent tends to increase egg weight (P3) despite the presence of AFB1 contamination in the feed (P4) ($P > 0.05$).

Aflatoxin-contaminated feed causes a decrease in egg production, as shown in the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40% in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and Awer (2009) showed that the production and egg weight of white leghorn laying hens were not affected by feed contaminated with aflatoxin at the levels of 100 ppb for 60 days, although feeding with aflatoxin contamination caused a decrease in feed consumption. Research Zaghini *et al*. (2005) showed a decrease in egg weight of laying hens receiving AFB1 2,500 ppb for 4 weeks, this was due to a decrease in the percentage of eggshell weight and thinner eggshells due to the AFB1 exprosure through contaminated feed consumption.

Weights of Carcass, Liver, and Intestine

This experiment showed the treatments have a significant effect ($P <0.05$) on final body weight. Nevertheless the treatments did not have significant effects ($P > 0.05$) on carcass percentage, relative weight of giblet, and relative weight of liver (Table 3. and Table 4.).

Duck carcass is mainly distributed in breast and thigh, therefore the weights of breast and thigh muscles is the main factor of carcass yield of duck. Study of Chang *et al.* (2016) showed diet containing aflatoxin at 62-65 ppb significantly reduces live weight, breast muscle weight, and thigh muscle weight of meat male ducks at various age.

Although the statistical test did not show any difference in the relative weight of all variables, it was seen that in AFB1 contaminated diet group (P2 and P4) there was an enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier than the control (13.54%). This results were also found in the relative weights of the intestines for P2 and P4, namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

This study applied a relatively low of AFB1 contamination level in the diet, but this is a common level of AFB1 contamination that found in feed and feedstuffs for duck in Indonesia according to previous study of Sumantri *et al*. (2017). At low dose of aflatoxin exposure, the performance of birds are relatively unchanged, but changes in liver size and pathology can be detected (Magnoli *et al* ., 2011). Study of Denli *et al* . (2009) found broiler liver enlargement after receiving feed containing AFB1 at 1,000 ppb.

Liver is the target organ of AFB1 because most of the AFB1 absorbed will undergo bioactivation to form a compound 8.9-epoxide which then binds to protein and DNA (Pasha *et al*., 2007). Our study indicated mild acute degeneration of vacuoles in the liver of ducks received P1 diet, but this degeneration was severe in P2 diet. In zeolite groups, mild vacuoles degeneration was found in P3 and medium degeneration was in P4 (Figure 2).

Hepatic lesions correlated with aflatoxicosis is described as a vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver (Espada *et al*., 1992). Study of Leenadevi *et al*. (1995)

revealed that ducks are a very sensitive species for aflatoxin injury and it would appear that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed zeolite inclusion seems to reduce the adverse effects of AFB1 exposure as indicated in the result of liver histopathology study of P3 and P4 groups. Similarly, Magnolli *et al*. (2011) found that in low levels of AFB1 (50 to 100 ppb), all livers samples of broilers showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet.

CONCLUSION

Zeolite inclusions in AFB1-contaminated diet for laying ducks could reduce the adverse effects of AFB1 exposure, especially on body weight and liver histopatology. Nevertheless, the use of zeolite did not appear to increase the egg production of laying duck.

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ACKNOWLEDGEMENT

The author would like to thank the Directorate General of Research and Community Services KEMENRISTEKDIKTI for funding this research activity through the Higher Education Applied Research Scheme with contract No. 119/UN8.2/PL/2017.

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Table 1. Effects of Treatment Diet on Live Weight Change of Laying Duck

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Treatment Diet	Initial Weight (g)	Final Weight (g)	Live weight Change	
			gram	%
Commercial Feed (P1)	1382	1411	29.06[ab]	2.10[ab]
P1 + AFB1 70 ppb (P2)	1447	1431	-16.25[a]	-1.12[a]
P1 + 2% zeolite (P3)	1405	1469	64.06[b]	4.56[b]
P2 + 2% zeolite (P4)	1386	1427	40.94[ab]	2.95[ab]

[a, b] means in same column with different superscripts differ significantly ($P < 0.05$)

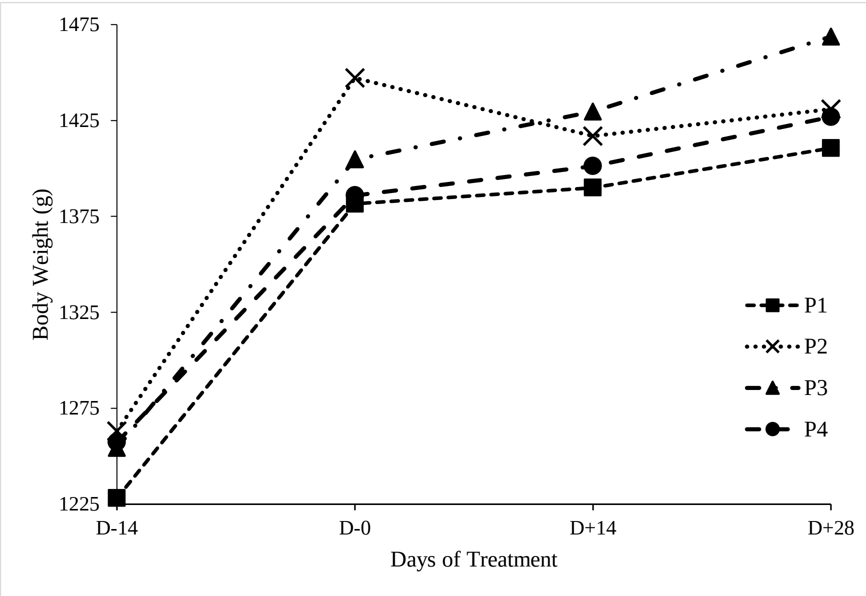

Figure 1. Live weight curves of laying ducks treated with control diet (P1); P1 containing AFB1 70 ppb (P2); P1 + 2% zeolite (P3); and P2 + 2% zeolite (P4)

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Tabel 2. Effects of Treatment Diet on Egg Production of Laying Duck

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Treatment Diet	DDA (%) ^{ns}	Egg Production (g) ^{ns}	Egg Weight (g) ^{ns}
Commercial Feed (P1)	54.40	4147.25	70.70
P1 + AFB1 70 ppb (P2)	64.58	4842.75	69.23
P1 + 2% zeolite (P3)	56.71	4417.00	72.10
P2 + 2% zeolite (P4)	58.56	4488.25	70.81

^{ns} means in the same column are not significantly different (P > 0.05)

Tabel 3. Effects of Treatment Diet on Final Body Weight, Carcass Weight, Giblet Weight, Liver Weight, and Small Intestinum Weight of Laying Duck

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Treatment Diet	Final Body Weight (g)	Carcass Weight (g)^{ns}	Giblet Weight (g)^{ns}	Liver Weight (g)^{ns}
Commercial Feed (P1)	1460.00^{ab}	832.5	351.75	43.75
P1 + AFB1 70 ppb (P2)	1426.25^{a}	846.5	289.25	47.50
P1 + 2% zeolite (P3)	1406.25^{a}	835.0	300.00	43.00
P2 + 2% zeolite (P4)	1576.25^{b}	951.0	323.50	49.50

$^{a, b}$ means in same column with different superscripts differ significantly (P < 0.05)
^{ns} means in the same column are not significantly different (P > 0.05)

Tabel 4. Effects of Treatment Diet on the Percentages of Carcass, Giblet, and Liver of Laying Duck

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If the data showed the non-significant, you no need to mention it by initial ns. Many readers already know, data without superscripts means the ns results statistically.

Treatment Diet	Percentages		
	Carcass (%)^{ns}	Giblet (%)^{ns}	Liver (%)^{ns}
Commercial Feed (P1)	57.28	42.40	13.54
P1 + AFB1 70 ppb (P2)	59.22	35.91	16.62
P1 + 2% zeolite (P3)	59.19	37.24	14.65
P2 + 2% zeolite (P4)	60.30	34.08	15.40

^{ns} means in the same column are not significantly different (P > 0.05)

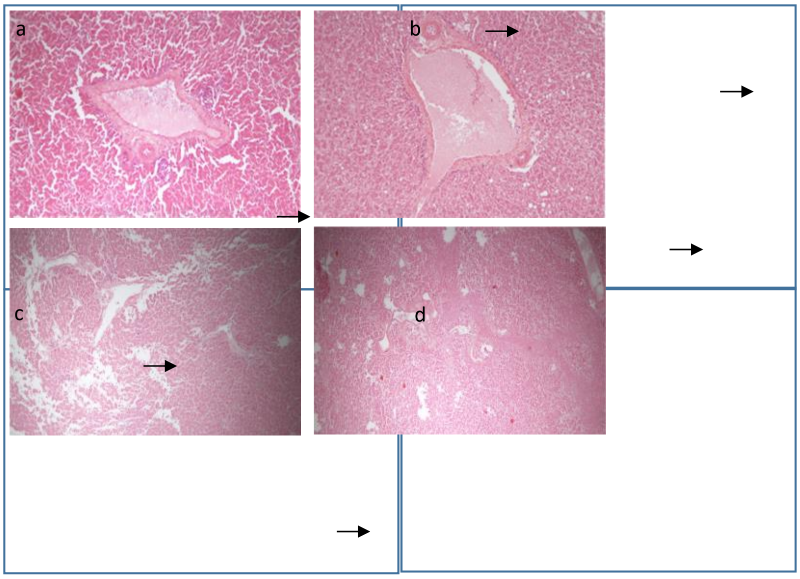

Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial feed); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

3. Submission revisi artikel tanggal 26 Juni 2019

Ika Sumantri <isumantri@ulm.ac.id> Wed, Jun 26, 2019 at 3:37 PM
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Yth. Editor JITAA
Terlampir revisi paper saya dengan judul "Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck"
Perbaikan sebagaimana saran kedua reviewer telah saya lakukan meliputi abstract, penambahan pustaka pada introduction, penambahan keterangan dan tabel pada materi dan metode, penambahan pustaka pada discussion, perbaikan conclusion , serta perbaikan Table dan Figure.
Perbaikan yang saya lakukan dalam isi naskah diberi warna merah.
Demikian atas kerja samanya diucapkan terima kasih.

Salam,
Dr. Ika Sumantri
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ABSTRAK

Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2% zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil memperlihatkan paparan AFB1 70 pbb menyebabkan penurunan bobot badan sekitar 1,12% (P<0,05). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak berpengaruh terhadap produksi telur dan bobot telur (p>0,05). Paparan AFB1 menghasilkan bobot potong yang lebih rendah (P<0,05), namun dengan imbuhan zeolit, itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4% dengan imbuhan zeolit. Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan bobot badan.

Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit

ABSTRACT

The research was objected to studying the effect of zeolite inclusion in aflatoxin B1 (AFB1) contaminated diet on the performance of laying duck. A completely randomized design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1) commercial feed (Control); (2) AFB1-contaminated feed 70 ppb (AFC); (3) Control + 2% zeolite; and (4) AFC + 2% zeolite. Each treatment had 4 replications with 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data were analyzed according to the general linear model of SPSS 21.0 statistical software. Results indicated AFB1 exposure significantly (p<0.05) decreases the body weight of laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that increased by 2.95% in AFC+2% zeolite. Treatments had no significant effect on egg production and egg weight (p>0.05). Zeolite inclusion resulted in the highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight (p<0.05). Relative liver weight of AFC diet was 16.62% and to be 15.4%

by zeolite addition in the diet. In conclusion, 2% of zeolite inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying duck.

Key words: Aflatoxin B1, laying duck, performance, zeolite

INTRODUCTION

Aflatoxin B1 (AFB1) is highly carcinogenic and genotoxic compounds produced by fungi, especially toxigenic species of *Aspergillus flavus* and *A. parasiticus*. The consumption of feed containing AFB1 by the animal can result in excretion of a hydroxylated metabolite of aflatoxin, namely aflatoxin M1 (AFM1), in the animal products, such as milk, meat, and eggs (Voelkel *et al*., 2011; van der Fels-Klerx and Camenzuli, 2016). In order to avoid AFB1 exposure on livestock and ingestion of its residues by consumers, the Indonesian government has established the maximum limit of AFB1 contamination in feed for the industry. However, tropical climate causes high occurrences and levels of AFB1 contamination in feed for ruminant and poultry in Indonesia (Agus *et al*., 2013; Sumantri *et al*., 2017).

Several strategies have been developed to minimize the toxic effects of aflatoxins on animal and the transfer of its residues into animal products, such as physical, chemical, and biological methods. However, in recent years the use of aflatoxin adsorbent is the most studied method because it is considered as an effective, safe, economical and applicable method (Kutz *et al*., 2009). One of the aflatoxin adsorbents is zeolite, a tectosilicates mineral that has the ability to bind aflatoxin so that it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al* ., 2010). Zeolites have a microporous structure that forming a large internal surface. This is associated with their high cation exchange capacity (Ca2+) that making zeolites efficiently adsorbing polar molecules such as AFB1 (Di Gregorio *et al*., 2014)

Many studies have been conducted to determine the efficacy of processed clays, including zeolite, in response to aflatoxin challenge to dairy cow, broiler and meat duck (Sulzberger *et al*., 2017; Fowler *et al*., 2015; Mallek *et al*., 2012; Chang *et al*., 2016). However, little information on the use of natural zeolite dealing with laying duck fed AFB1-contaminated diet has been found. Duck is highly sensitive to aflatoxin exposure because of differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz and Murcia, 2011). Consumption of AFB1 contaminated diet will not only adversely affect on duck's performance but may result in residues in the products (Zhang *et al*., 2016). Therefore, this research aims to investigate the ameliorate effects of natural zeolite inclusion in AFB1 contaminated diet on the performance and health of laying duck.

MATERIALS AND METHODS

Experimental Diet

AFB1-contaminated diet (AFC) was produced as follow: commercial feed for laying duck (IP333, PT. Wonokoyo) was used as a production medium. The medium was added with aquadest to reach moisture of production medium be 30%. The medium was inoculated with *A. flavus* FNCC 612 then incubated in temperature 35^{o}C for 10 days. The concentration of AFB1 in the medium was analyzed by ELISA test to calculate the dilution factor of the medium in the experimental diet. Indonesian National Standard of Industry (SNI) has established the threshold level of AFB1 in complete commercial feed, namely 20 ppb (Kementan RI, 2009). Previous studies showed the detrimental effects of AFB1 on the performance of duck are a dose-dependent response that might be observed in the level of 50 ppb or more (Ostrowski-Meisnerr, 1983;

Sumantri *et al*., 2017). Therefore, this experiment applied the level of contamination at 70 ppb. Production medium then mixed with commercial feed based on the dilution factor to obtain AFB1 levels of AFC at 70 ppb.

The zeolite that was used in the experiment was a natural zeolite which is mined and purchased in Central Java (PT. Brataco Chemika). Zeolite was ground using a mortar and sieved through 100 mesh. Experimental diet composition is shown in Table 1. below:

In Vivo Experiment

Seven months, sixty-four female Alabio ducks (*Anas platyrinchos* Borneo) were used in the experiment. Ducks were weighed and randomly assigned to 4 dietary treatments with 4 replicates of 4 ducks in each experimental unit. The mean of the duck's body weight when randomized into dietary treatment was 1,247±145 g. The treatment diets were: commercial feed as a control diet (Control); AFB1-contaminated diet 70 ppb (AFC); Control + 2% zeolite; and AFC + 2% zeolite.

Dietary treatment was started when the egg production, Duck Day Average (DDA), reaches 70%. The experiment was carried out for 4 weeks. The experimental diet was provided twice a day and restricted, namely 150 g/head/day, to ensure the level of AFB1 exposure on the animal is controlled. Water was provided *ad libitum*.

Egg production was recorded and weighed daily, starting at 15th day until the 28th day of treatment. Body weight of duck was measured individually at two weeks before treatment (D-14), the beginning of treatment (D0), the second week of treatment (D14), and the fourth week of treatment (D28). At the end of the experiment (D28), ducks were sacrificed, then carcass and giblet were collected and weighed. The observed variables were changes in body weight, egg production (egg weight and DDA), the weight percentages of carcass, liver, and intestine, and histopathology of the liver.

Liver histopathology was diagnosed as follow: representative liver samples were fixed in 10% buffered neutral formalin. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris according to Manual Standard of Patologi Diagnose of Veterinary Laboratory.

Analysis

Feed samples were analysed for AFB1 concentrations by ELISA method using ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of live weight changes, egg weight, DDA percentage, carcass percentage, liver percentage, and intestine weight were analysed by the general linear model procedure using the IBM SPSS 21.0 statistical program. Significant differences between treatment means were separated using Duncan's multiple range test with a 5% probability.

RESULTS AND DISCUSSIONS

Live Weight Changes

Treatments had a significant effect on live weight changes (p <0.05). Table 2. showed that AFB1 exposure at 70 ppb would cause a decrease in duck weight, as seen in AFC treatment, which experienced an average weight loss of -0.87% after four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be reduced by zeolite inclusion in the diet. This was indicated by the average of live weight of ducks in AFC + 2% zeolite diet that increased by 3.20%. Zeolite also significantly improved duck performance, as seen in control diet +2% zeolite, that had the highest body weight gain, namely 4.86%. This gain was higher than the control feed which only increased by 2.57%.

The adverse effects of AFB1 on growth performance is related with a decrease in the efficiency of protein and energy utilization due to the deterioration of the digestive system of the birds (Denly *et al*., 2009). Recent studies in broilers suggested that absorptive surface of small intestine would deteriorate during chronic exposure to low levels of AFB1, thus declines absorption of nutrient in the intestine (Galarza-Seeber *et al*., 2016). Figure 1. clearly shows that the presence of AFB1 in the diet (AFC) decreases the growth performance of the duck.

Study on the effects of AFB1 on the performance of laying ducks is still very limited. In 1-day-old ducklings which received a feed containing AFB1 at levels up to 100 ppb for 21 days, an increase in AFB1 level caused a decrease in weight gain (Wan *et al*., 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al* ., 2001). Yunus *et al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss, decreased feed consumption, and increased feed conversion. The percentage of weight loss reported varies depending on the dose and duration of exposure, such as 5% weight loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15% weight loss at a dose of 1,000 ppb for 21 days.

This study indicated the positive effect of zeolite inclusion in the diet for laying duck. Addition of 2% zeolite in the control diet resulted in the highest final live weight (Control + 2% zeolite). By zeolite inclusion, the growth performance of duck receiving AFB1 contaminated diet (AFC + 2% zeolite) was still higher than the control diet. This finding suggests that the use of zeolite can reduce the impact of exposure to AFB1 on body weight. Chemically, zeolite is a clay group of aluminosilicate minerals which has a three-dimensional structure consisting of skeletons of SiO_4 and AlO_4 which form interconnected channels wherein the channel cavity there are weak bonds of H_2O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO_4 (Mallek *et al*., 2012).

In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of AFB1 exposure (Wan *et al* ., 2013). In broiler, the dietary use of natural or synthetic zeolites has been reported to improve feed efficiency, thus resulting in better growth performance of broilers (Mallek *et al*., 2012). Zeolites (montmorillonites) are a class of the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals, bacteria, and toxic antinutritive agents (Fowler *et al* ., 2015; Sulzberger *et al* ., 2017). The binding between aflatoxin and the adsorbent forms an inert and stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract (Huwig *et al* ., 2001).

Egg Production

In this study, the treatments had no effect on egg production and weight ($p >$ 0.05). However, as shown in Table 3., AFB1 contamination in the diet tends to reduce egg weight, and the addition of 2% zeolite tends to increase egg weight despite the presence of AFB1 contamination in the feed ($p = 0.433$).

Aflatoxin-contaminated feed causes a decrease in egg production, as shown in the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40% in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and

Awer (2009) showed that the production and egg weight of white leghorn laying hens were not affected by feed contaminated with aflatoxin at the levels of 100 ppb for 60 days, although feeding with aflatoxin contamination caused a decrease in feed consumption. Research Zaghini *et al*. (2005) showed a decrease in egg weight of laying hens receiving AFB1 2,500 ppb for 4 weeks, this was due to a decrease in the percentage of eggshell weight and thinner eggshells due to the AFB1 exposure through contaminated feed consumption. EvidenceS suggest that AFB1 causes induction or inhibition of liver mixed-function-oxygenase activities that affect the metabolism of exogenous and endogenous substrates in the liver.

Weights of Carcass, Liver, and Intestine

This experiment showed the treatments have a significant effect (p <0.05) on final body weight. Nevertheless, the treatments did not have significant effects (p > 0.05) on carcass percentage, the relative weight of giblet, and relative weight of the liver (Table 4. and Table 5.).

Duck carcass is mainly distributed in breast and thigh, therefore the weights of breast and thigh muscles are the main factor of carcass yield of duck. Study of Chang *et al.* (2016) showed diet containing aflatoxin at 62-65 ppb significantly reduces live weight, breast muscle weight, and thigh muscle weight of meat male ducks at various age.

Although the statistical test did not show any difference in the relative weight of all variables, it was seen that in AFB1 contaminated diet groups (AFC and AFC+2% zeolite) there was an enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier than the control (13.54%). These results were also found in the relative weights of the intestines for AFC and AFC+2% zeolite groups, namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

This study applied a relatively low of AFB1 contamination level in the diet, but this is a common level of AFB1 contamination that found in feed and feedstuffs for a duck in Indonesia according to the previous study of Sumantri *et al*. (2017). At a low dose of aflatoxin exposure, the performance of birds are relatively unchanged, but changes in liver size and pathology can be detected (Magnoli *et al* ., 2011). Study of Denli *et al*. (2009) found broiler liver enlargement after receiving feed containing AFB1 at 1,000 ppb.

Liver is the target organ of AFB1 because most of the AFB1 absorbed will undergo bioactivation to form a compound 8.9-epoxide which then binds to protein and DNA (Pasha *et al*., 2007). Our study indicated mild acute degeneration of vacuoles in the liver of ducks received control diet, but this degeneration was severe in AFC diet. In zeolite groups, mild vacuoles degeneration was found in Control + 2% zeolite and medium degeneration was in AFC + 2% zeolite (Figure 2).

Hepatic lesions correlated with aflatoxicosis is described as vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver (Espada *et al*., 1992). Study of Leenadevi *et al*. (1995) revealed that ducks are a very sensitive species for aflatoxin injury and it would appear that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed zeolite inclusion seems to reduce the adverse effects of AFB1 exposure as indicated in the result of liver histopathology study of P3 and P4 groups. Similarly, Magnolli *et al*. (2011) found that in low levels of AFB1 (50 to 100 ppb), all livers samples of broilers showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet.

CONCLUSION

Zeolite inclusions in AFB1-contaminated diet for laying ducks could reduce the adverse effects of AFB1 exposure, especially on body weight and liver histopathology, however it did not appear to increase the egg production.

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Table 1. Composition of Experimental Diet

Ingredient	Treatment			
	Control diet	AFC**	Control+2% zeolite	AFC+2% zeolite
Commercial complete feed (%)*	100	90	100	90
AFB1-production medium (%)	0	10	0	10
Natural zeolite (%)	0	0	2	2
Analysis				
Dry matter (%)	88	88	88	88
Crude protein (%)	18	18	18	18
Crude fat (%)	7	7	7	7
Crude fiber (%)	6	6	6	6
Ash (%)	14	14	14	14
Calcium (%)	3.3	3.3	3.3	3.3
Phosphorous	0.8	0.8	0.8	0.8
Metabolizable energy (kkal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.
**AFC= Aflatoxin B1 contaminated diet

Table 2. Effects of Treatment Diet on Live Weight Change of Laying Duck

Treatment Diet	Initial (g)	Final (g)	Live weight Change	
			gram	%
Control diet*	1,382±139	1,411±110	29.06±102^{ab}	2.57±7.8^{ab}
AFC**	1,447±94	1,431±92	-16.25±101^{a}	-0.87±7.4^{a}
Control + 2% zeolite	1,405±139	1,469±134	64.06±99^{b}	4.86±7.3^{b}
AFC + 2% zeolite	1,386±142	1,427±130	40.94±66^{ab}	3.20±5.5^{ab}

*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb
$^{a, b}$ means in the same column with different superscripts differ significantly ($P < 0.05$)

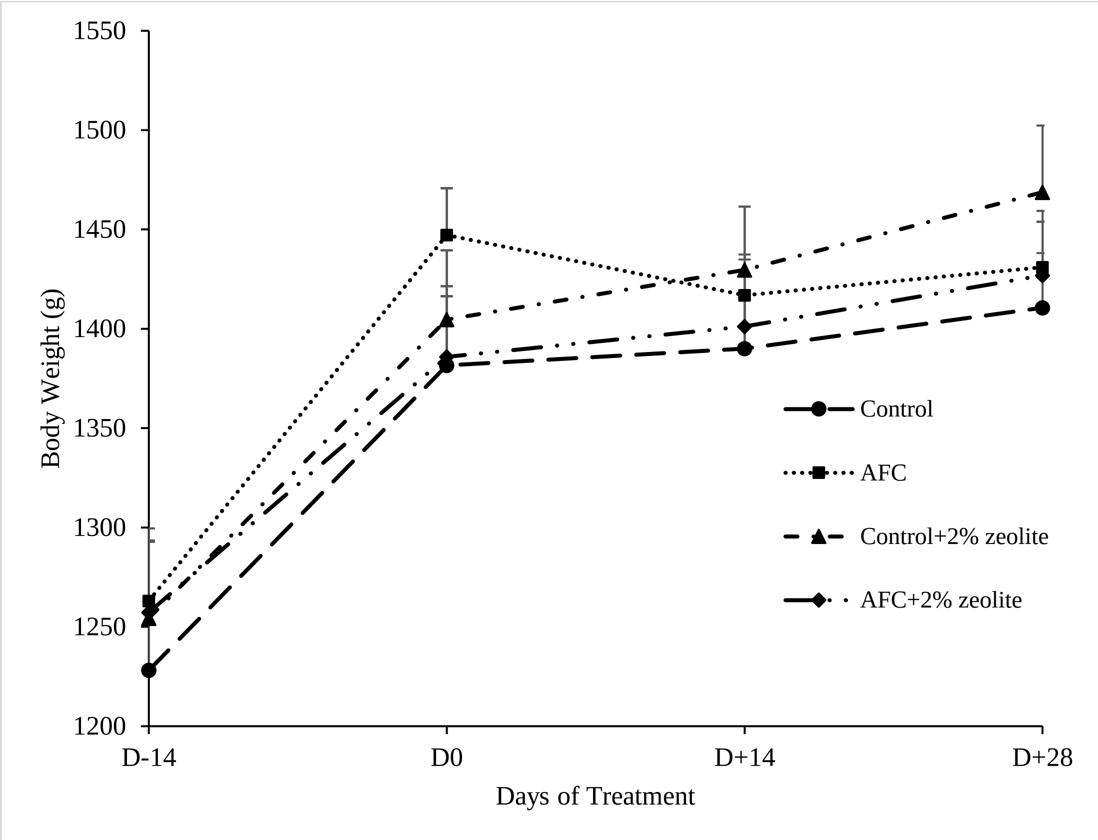

Figure 1. Live weight curves of laying ducks treated with control diet; AFB1-contaminated diet (AFC) 70 ppb (P2); Control+2% zeolite; and AFC+2% zeolite

Tabel 3. Effects of Treatment Diet on Egg Production of Laying Duck^{ns}

Treatment Diet	DDA (%)	Egg Production (g)	Egg Weight (g)
Control diet*	54.40±3.7	4,147±217	70.70±3.7
AFC**	64.58±9.2	4,842±736	69.23±3.4
Control + 2% zeolite	56.71±8.4	4,417±590	72.10±1.5
AFC + 2% zeolite	58.56±12.6	4,488±886	70.81±1.1

^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

Tabel 4. Effects of Treatment Diet on Final Body Weight, Carcass Weight, Giblet Weight, Liver Weight, and Small Intestinum Weight of Laying Duck

Treatment Diet	Final Body Weight (g)	Carcass Weight (g)^{ns}	Giblet Weight (g)^{ns}	Liver Weight (g)^{ns}
Control diet*	1,460±110^{ab}	833±29	352±123	43.8±8.4
AFC**	1,426±55^{a}	847±128	289±105	47.5±15.9
Control + 2% zeolite	1,406±90^{a}	835±121	300±80	43.0±9.6
AFC + 2% zeolite	1,576±104^{b}	951±76	324±33	49.5±5.1

$^{a, b}$ means in the same column with different superscripts differ significantly ($p < 0.05$)
^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

Tabel 5. Effects of Treatment Diet on the Percentages of Carcass, Giblet, and Liver of Laying Duck^{ns}

Treatment Diet	Percentages		
	Carcass (%)	Giblet (%)	Liver (%)
Control diet*	57±5.0	42±15.6	14±4.8
AFC**	59±7.3	36±15.9	17±1.3
Control + 2% zeolite	59±5.5	37±13.2	15±2.2
AFC + 2% zeolite	60±1.5	34±3.2	15±1.9

^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

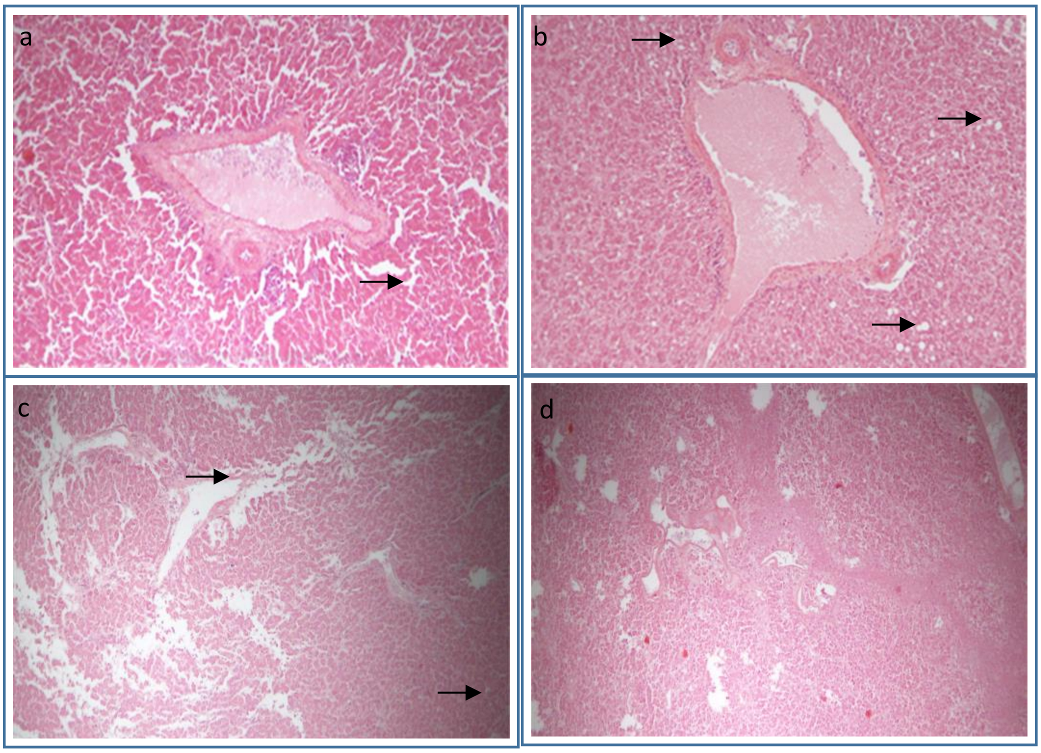

Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial feed); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

4. Submission revisi ditanggapi oleh editor

JITAA JPPT <jppt.fpundip@gmail.com> Sun, Jun 30, 2019 at 10:10 PM
To: Ika Sumantri <isumantri@ulm.ac.id>

Dear Ika,

Terima kasih atas kiriman manuskrip hasil perbaikan
[Quoted text hidden]

5. Accepted artikel oleh editor pada 7 Juli 2019

UNIVERSITAS LAMBUNG MANGKURAT

Ika Sumantri <isumantri@ulm.ac.id>

Letter of Acceptance
2 messages

JITAA JPPT <jppt.fpundip@gmail.com> Sun, Jul 7, 2019 at 5:42 PM
To: Ika Sumantri <isumantri@ulm.ac.id>

Dear Ika Sumantri,

I am pleased to inform you that your manuscript entitled as "Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck" is accepted for publication in JITAA.

Best regards,

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3789K

6. Permintaan perbaikan Bahasa Inggris tulisan oleh editor 1 Agustus 2019

UNIVERSITAS LAMBUNG MANGKURAT

Ika Sumantri <isumantri@ulm.ac.id>

Manuskrip-cek bhs Inggris
6 messages

JITAA JPPT <jppt.fpundip@gmail.com> Thu, Aug 1, 2019 at 11:46 AM
To: Ika Sumantri <isumantri@ulm.ac.id>

Dear Sdr Ika Sumantri,

Bersama ini kami kirimkan file hasil pemeriksaan bahasa Inggris. Kami persilakan Sdr memperbaikinya agar file yang kami terima dalam bersih. Kami memberikan waktu paling lama 5 hari TMT sejak file ini dikirimkan ke Sdr.

Salam,

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Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck

ABSTRAK

Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2% zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil memperlihatkan paparan AFB1 70 pbb menyebabkan penurunan bobot badan sekitar 1,12% (P<0,05). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak berpengaruh terhadap produksi telur dan bobot telur (p>0,05). Paparan AFB1 menghasilkan bobot potong yang lebih rendah (P<0,05), namun dengan imbuhan zeolit, itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4% dengan imbuhan zeolit. Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan bobot badan.

Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit

ABSTRACT

The research was objected to ~~studying~~ study the effect of zeolite inclusion in aflatoxin B1 (AFB1) contaminated diet on the performance of laying duck. A completely randomized design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1) commercial feed (Control); (2) AFB1-contaminated feed 70 ppb (AFC); (3) Control + 2% zeolite; and (4) AFC + 2% zeolite. Each treatment had 4 replications with 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data were analyzed according to the general linear model of SPSS 21.0 statistical software. Results indicated that AFB1 exposure significantly (p<0.05) ~~decreases~~ decreased the body weight of laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that increased by 2.95% in AFC+2% zeolite. Treatments had no significant effect on egg production and egg weight (p>0.05). Zeolite inclusion resulted in the highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight (p<0.05). Relative liver weight of duck fed AFC diet was

16.62% and to be 15.4% by zeolite addition in the diet. In conclusion, 2% of zeolite inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying duck.

Key words: Aflatoxin B1, laying duck, performance, zeolite

INTRODUCTION

Aflatoxin B1 (AFB1) is highly carcinogenic and genotoxic compounds produced by fungi, especially toxigenic species of *Aspergillus flavus* and *A. parasiticus*. The consumption of feed containing AFB1 by the animal can result in excretion of a hydroxylated metabolite of aflatoxin, namely aflatoxin M1 (AFM1), in the animal products, such as milk, meat, and eggs (Voelkel *et al*., 2011; van der Fels-Klerx and Camenzuli, 2016). In order to avoid AFB1 exposure on livestock and ingestion of its residues by consumers, the Indonesian government has established the maximum limit of AFB1 contamination in feed for the industry. However, tropical climate causes high occurrences and levels of AFB1 contamination in feed for ruminant and poultry in Indonesia (Agus *et al*., 2013; Sumantri *et al*., 2017).

Several strategies have been developed to minimize the toxic effects of aflatoxins on animal and the transfer of its residues into animal products, such as physical, chemical, and biological methods. However, in recent years the use of aflatoxin adsorbent is the most studied method ?? → best study methods because it is considered as an effective, safe, economical and applicable method (Kutz *et al*., 2009). One of the aflatoxin adsorbents is zeolite, a tectosilicates mineral that has the ability to bind aflatoxin so that it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al* ., 2010). Zeolites have a microporous structure that forming a large internal surface. This is associated with their high cation exchange capacity (Ca2+) that

~~making~~ makes zeolites efficiently adsorbing polar molecules such as AFB1 (Di Gregorio *et al*., 2014)

Many studies have been conducted to determine the efficacy of processed clays, including zeolite, in response to aflatoxin challenge to dairy cow, broiler and meat duck (Sulzberger *et al*., 2017; Fowler *et al*., 2015; Mallek *et al*., 2012; Chang *et al*., 2016). However, little information on the use of natural zeolite dealing with laying duck fed AFB1-contaminated diet has been found. Duck is highly sensitive to aflatoxin exposure because of differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz and Murcia, 2011). Consumption of AFB1 contaminated diet will not only adversely affect on duck's performance but may result in residues in the products (Zhang *et al*., 2016). Therefore, this research ~~aims~~ aimed to investigate the ameliorate effects of natural zeolite inclusion in AFB1 contaminated diet on the performance and health of laying duck.

MATERIALS AND METHODS

Experimental Diet

AFB1-contaminated diet (AFC) was produced as follow: commercial feed for laying duck (IP333, PT. Wonokoyo) was used as a ~~production~~ medium. The medium was added with aquadest to reach 30% of moisture content ~~of production medium be 30%~~. The medium was inoculated with *A. flavus* FNCC 612 then it incubated in temperature 35^{o}C for 10 days. The concentration of AFB1 in the medium was analyzed by ELISA test to calculate the dilution factor of the medium in the experimental diet. Indonesian National Standard of Industry (SNI) has established the threshold level of AFB1 in complete commercial feed~~, namely~~ that was 20 ppb (Kementan RI, 2009).

Previous studies showed the detrimental effects of AFB1 on the performance of duck ~~are a dose-dependent response that might be~~ was observed in the level of 50 ppb or more (Ostrowski-Meisnerr, 1983; Sumantri *et al*., 2017). Therefore, this experiment was applied the level of contamination at 70 ppb. The ~~Production~~ medium then were mixed with commercial feed based on the dilution factor to obtain AFB1 levels of AFC at 70 ppb.

The zeolite ~~that was~~ used in the experiment was a natural zeolite which is mined and purchased in Central Java (PT. Brataco Chemika). Zeolite was ground using a mortar and sieved through 100 mesh. Experimental diet composition is shown in Table 1. ~~below:~~

In Vivo Experiment

Seven months, sixty-four female Alabio ducks (*Anas platyrinchos* Borneo) were used in the experiment. Ducks were weighed and randomly assigned to 4 dietary treatments with 4 replicates of 4 ducks in each experimental unit. The mean of the duck's body weight when randomized into dietary treatment was 1,247±145 g. The treatments ~~diets~~ were: commercial feed as a control diet (Control); AFB1-contaminated diet 70 ppb (AFC); Control + 2% zeolite (..P3???...); and AFC + 2% zeolite (..P4???...).

Dietary treatment was ~~started~~ given when the egg production, Duck Day Average (DDA), ~~reaches~~ reached 70%. The experiment was carried out for 4 weeks. The experimental diet was provided twice a day and restricted~~, namely~~ about 150 g/head/day, to ensure the level of AFB1 exposure on the animal ~~is~~ was controlled. Water was provided *ad libitum*.

Egg production was recorded and weighed daily, starting at 15th day until the 28th day of treatment. Body weight of duck was measured individually at two weeks before treatment (D-14), the beginning of treatment (D0), the second week of treatment (D14), and the fourth week of treatment (D28). At the end of the experiment (D28), ducks were ~~sacrificed~~ slaughtered, then carcass and giblet were collected and weighed. The observed variables were changes in body weight, egg production (egg weight and DDA), the weight percentages of carcass, liver, and intestine, and histopathology of the liver.

Commented [a1]: the average daily gain ??

Commented [a2]: the percentages of carcass weight ??

Liver histopathology was diagnosed as follow: representative liver samples were fixed in 10% buffered neutral formalin. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris (year?? → reference) according to Manual Standard of Patologi Diagnose of Veterinary Laboratory.

Analysis

Feed samples were analysed for AFB1 concentrations by ELISA method using ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of live weight ~~changes~~, egg weight, DDA percentage, carcass percentage, liver percentage, and intestine weight were analysed by the general linear model procedure using the IBM SPSS 21.0 statistical program. Significant differences between treatment means were separated using Duncan's multiple range test with a 5% probability.

Commented [a3]: body weight??

RESULTS AND DISCUSSIONS

Live Weight Changes → Body weight

Treatments had a significant effect on live weight changes (*p* <0.05). Table 2~~.~~ showed that AFB1 exposure at 70 ppb ~~would cause~~ caused a decrease in duck weight, as seen in AFC treatment, which experienced an average weight loss of -0.87% after four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be reduced by zeolite inclusion in the diet. This was indicated by the average of live weight of ducks in AFC + 2% zeolite diet that increased by 3.20%. Zeolite also significantly improved duck performance, as seen in control diet +2% zeolite, that had the highest body weight gain~~, namely~~ (4.86%). This gain was higher than the control ~~feed~~ ducks which only increased by 2.57%.

The adverse effects of AFB1 on growth performance ~~is~~ was related with a decrease in the efficiency of protein and energy utilization due to the deterioration of the digestive system of the birds (Denly *et al*., 2009). Recent studies in broilers suggested that absorptive surface of small intestine would deteriorate during chronic exposure to low levels of AFB1, thus declines absorption of nutrient in the intestine (Galarza-Seeber *et al*., 2016). Figure 1~~.~~ clearly shows that the presence of AFB1 in the diet (AFC) ~~decreases~~ decreased the growth performance of the duck.

Study on the effects of AFB1 on the performance of laying ducks is still ~~very~~ limited. In 1st-day-old ducklings which received a feed containing AFB1 at levels up to 100 ppb for 21 days~~,~~ showed that an increase in AFB1 level caused a decrease in weight gain (Wan *et al*., 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al* ., 2001). Yunus *et al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss, decreased feed consumption, and increased feed conversion. The percentage of weight loss reported varies depending on the dose and duration of exposure, such as 5% weight loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15% weight loss at a dose of 1,000 ppb for 21 days (reference).

This study indicated the positive effect of zeolite inclusion in the diet for laying duck. The addition of 2% zeolite in the control diet resulted in the highest final live weight (Control + 2% zeolite). By zeolite inclusion, the growth performance of duck receiving AFB1 contaminated diet (AFC + 2% zeolite) was still higher than those of the control diet ducks. This finding suggests that the use of zeolite can reduce the impact of exposure to AFB1 on body weight. Chemically, zeolite is a clay group of aluminosilicate minerals which has a three-dimensional structure consisting of skeletons of SiO_4 and AlO_4 which form interconnected channels wherein the channel cavity there are weak bonds of H_2O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO_4 (Mallek *et al*., 2012).

In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of AFB1 exposure (Wan *et al* ., 2013). In broiler, the dietary use of natural or synthetic zeolites has been reported to improve feed efficiency, thus resulting in better growth performance of broilers (Mallek *et al*., 2012). Zeolites (montmorillonites) are a class of the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals, bacteria, and toxic antinutritive agents (Fowler *et al* ., 2015; Sulzberger *et al* ., 2017). The binding between aflatoxin and the adsorbent forms an inert and stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract (Huwig *et al* ., 2001).

Egg Production

In this study, the treatments had no effect on egg production and egg weight (*p* > 0.05). However, as shown in Table 3~~.~~, AFB1 contamination in the diet ~~tends~~ tended to reduce egg weight, and the addition of 2% zeolite ~~tends~~ tended to increase egg weight despite the presence of AFB1 contamination in the feed (*p* = 0.433).

Aflatoxin-contaminated feed causes a decrease in egg production, as shown in the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40% in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and Awer (2009) showed that the production and egg weight of white leghorn laying hens were not affected by feed contaminated with aflatoxin at the levels of 100 ppb for 60 days, although feeding with aflatoxin contamination caused a decrease in feed consumption. Research of Zaghini *et al.* (2005) showed a decrease in egg weight of laying hens receiving AFB1 2,500 ppb for 4 weeks, this was due to a decrease in the percentage of eggshell weight and thinner eggshells due to the AFB1 exposure through contaminated feed consumption. Evidences suggest that AFB1 causes induction or inhibition of liver mixed-function-oxygenase activities that affect the metabolism of exogenous and endogenous substrates in the liver.

Weights of Carcass, Liver, and Intestine

This experiment showed that the treatments have a significant effect (*p* <0.05) on final body weight. Nevertheless, the treatments did not have significant effects (*p* > 0.05) on carcass percentage, the relative weight of giblet, and relative weight of the liver (Table 4~~.~~ and Table 5~~.~~).

Duck carcass meat is mainly distributed in breast and thigh, therefore the weights of breast and thigh muscles are the main factor of carcass yield ~~of~~ in duck.

Commented [a4]: mostly located

Study of Chang *et al.* (2016) showed that diet containing aflatoxin at 62-65 ppb significantly ~~reduces~~ reduced live weight, breast muscle weight, and thigh muscle weight of meat male ducks at various age.

Although the statistical test did not show any difference in the relative weight of all variables, it was seen that in AFB1 contaminated diet groups (AFC and AFC+2% zeolite) ~~there was~~ was found an enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier than the control (13.54%). These results were also found in the relative weights of the intestines for AFC and AFC+2% zeolite groups, namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

This study applied a relatively low of AFB1 contamination level in the diet, but this is a common level of AFB1 contamination that found in feed and feedstuffs for a duck in Indonesia according to ~~the previous study of~~ Sumantri *et al.* (2017). At a low dose of aflatoxin exposure, the performance of birds ~~are~~ were relatively unchanged, but the changes in liver size and pathology can be detected (Magnoli *et al* ., 2011). ~~Study of~~ Denli *et al.* (2009) found broiler liver enlargement after receiving feed containing AFB1 at 1,000 ppb.

Commented [a5]: the same or similar

Liver is the target organ of AFB1 because most of the AFB1 absorbed will undergo bioactivation to form a compound 8.9-epoxide which then binds to protein and DNA (Pasha *et al.*, 2007). Our study indicated mild acute degeneration of vacuoles in the liver of ducks received control diet, but this degeneration was severe in AFC diet. In zeolite groups, mild vacuoles degeneration was found in Control + 2% zeolite and medium degeneration was in AFC + 2% zeolite (Figure 2).

Hepatic lesions correlated with aflatoxicosis is described as vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver (Espada *et al*., 1992). Study of Leenadevi *et al*. (1995) revealed that ducks are a very sensitive species for aflatoxin injury and it would appear that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically, they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed that zeolite inclusion seems to reduce the adverse effects of AFB1 exposure as indicated in the result of liver histopathology study of P3 and P4 groups. Similarly, Magnolli *et al*. (2011) found that in low levels of AFB1 (50 to 100 ppb), all livers samples of broilers showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet.

Commented [a6]: you did not mention before → in the material and method

CONCLUSION

Zeolite inclusions in AFB1-contaminated diet for laying ducks could reduce the adverse effects of AFB1 exposure, especially on body weight and liver histopathology, however it did not appear to increase the egg production.

ACKNOWLEDGEMENT

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Table 1. Composition of Experimental Diets

Ingredients	Treatments			
	Control diet	AFC**	Control+2% zeolite	AFC+2% zeolite
Commercial complete feed (%)*	100	90	100	90
AFB1-production medium (%)	0	10	0	10
Natural zeolite (%)	0	0	2	2
Analysis				
Dry matter (%)	88	88	88	88
Crude protein (%)	18	18	18	18
Crude fat (%)	7	7	7	7
Crude fiber (%)	6	6	6	6
Ash (%)	14	14	14	14
Calcium (%)	3.3	3.3	3.3	3.3
Phosphorous	0.8	0.8	0.8	0.8
Metabolizable energy (kkal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.
**AFC= Aflatoxin B1 contaminated diet

Table 2. Effects of Treatment Diet on Live Weight Change of Laying Duck

Treatment Diets	Initial weight (g)	Final weight (g)	Live weight Change	
			gram	%
Control diet*	1,382±139	1,411±110	29.06±102^{ab}	2.57±7.8^{ab}
AFC**	1,447±94	1,431±92	-16.25±101^{a}	-0.87±7.4^{a}
Control + 2% zeolite	1,405±139	1,469±134	64.06±99^{b}	4.86±7.3^{b}
AFC + 2% zeolite	1,386±142	1,427±130	40.94±66^{ab}	3.20±5.5^{ab}

*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb
$^{a, b}$ means in the same column with different superscripts differ significantly ($P < 0.05$)

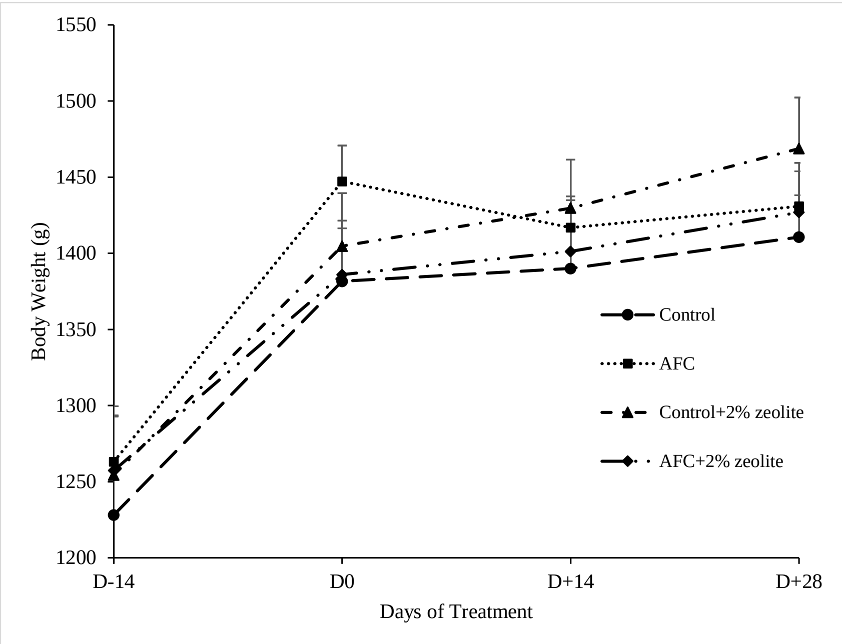

Figure 1. Live weight curves of laying ducks treated with control diet; AFB1-contaminated diet (AFC) 70 ppb (P2); Control+2% zeolite; and AFC+2% zeolite

Tabel 3. Effects of Treatment Diets on Egg Production of Laying Ducks^{ns}

Treatment Diets	DDA (%)	Egg Production (g)	Egg Weight (g)
Control diet*	54.40±3.7	4,147±217	70.70±3.7
AFC**	64.58±9.2	4,842±736	69.23±3.4
Control + 2% zeolite	56.71±8.4	4,417±590	72.10±1.5
AFC + 2% zeolite	58.56±12.6	4,488±886	70.81±1.1

^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

Tabel 4. Effects of Treatment Diets on Final Body Weight, Carcass Weight, Giblet Weight, Liver Weight, and Small Intestinum Weight of Laying Ducks

Treatment Diets	Final Body Weight (g)	Carcass Weight (g)^{ns}	Giblet Weight (g)^{ns}	Liver Weight (g)^{ns}
Control diet*	1,460±110^{ab}	833±29	352±123	43.8±8.4
AFC**	1,426±55^{a}	847±128	289±105	47.5±15.9
Control + 2% zeolite	1,406±90^{a}	835±121	300±80	43.0±9.6
AFC + 2% zeolite	1,576±104^{b}	951±76	324±33	49.5±5.1

$^{a, b}$ means in the same column with different superscripts differ significantly ($p < 0.05$)
^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

Tabel 5. Effects of Treatment Diets on the Percentages of Carcass, Giblet, and Liver of Laying Ducks^{ns}

Treatment Diets	Percentages		
	Carcass (%)	Giblet (%)	Liver (%)
Control diet*	57±5.0	42±15.6	14±4.8
AFC**	59±7.3	36±15.9	17±1.3
Control + 2% zeolite	59±5.5	37±13.2	15±2.2
AFC + 2% zeolite	60±1.5	34±3.2	15±1.9

^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

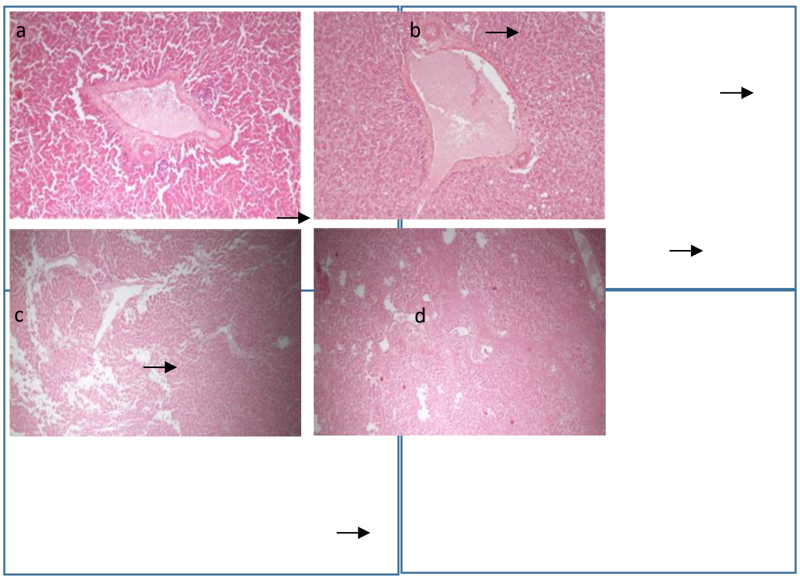

Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial feed); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

7. Submission revisi Bahasa Inggris tanggal 3 Agustus 2019

Ika Sumantri <isumantri@ulm.ac.id> Sat, Aug 3, 2019 at 4:00 PM
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Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck

ABSTRAK

Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2% zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil memperlihatkan paparan AFB1 70 pbb menyebabkan penurunan bobot badan sekitar 1,12% ($P<0{,}05$). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak berpengaruh terhadap produksi telur dan bobot telur ($p>0{,}05$). Paparan AFB1 menghasilkan bobot potong yang lebih rendah ($P<0{,}05$), namun dengan imbuhan zeolit, itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4% dengan imbuhan zeolit. Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan bobot badan.

Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit

ABSTRACT

The research was objected to study the effect of zeolite inclusion in aflatoxin B1 (AFB1) contaminated diet on the performance of laying duck. A completely randomized design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1) commercial feed (Control); (2) AFB1-contaminated feed 70 ppb (AFC); (3) Control + 2% zeolite; and (4) AFC + 2% zeolite. Each treatment had 4 replications with 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data were analyzed according to the general linear model of SPSS 21.0 statistical software. Results indicated that AFB1 exposure significantly ($p<0.05$) decreased the body weight of laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that increased by 2.95% in AFC+2% zeolite. Treatments had no significant effect on egg production and egg weight ($p>0.05$). Zeolite inclusion resulted in the highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight ($p<0.05$). Relative liver weight of duck fed_AFC diet was 16.62% and to be 15.4% by zeolite addition in the diet. In conclusion, 2% of zeolite inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying duck.

Key words: Aflatoxin B1, laying duck, performance, zeolite

INTRODUCTION

Aflatoxin B1 (AFB1) is highly carcinogenic and genotoxic compounds produced by fungi, especially toxigenic species of *Aspergillus flavus* and *A. parasiticus*. The consumption of feed containing AFB1 by the animal can result in excretion of a hydroxylated metabolite of aflatoxin, namely aflatoxin M1 (AFM1), in the animal products, such as milk, meat, and eggs (Voelkel *et al*., 2011; van der Fels-Klerx and Camenzuli, 2016). In order to avoid AFB1 exposure on livestock and ingestion of its residues by consumers, the Indonesian government has established the maximum limit of AFB1 contamination in feed for the industry. However, tropical climate causes high occurrences and levels of AFB1 contamination in feed for ruminant and poultry in Indonesia (Agus *et al*., 2013; Sumantri *et al*., 2017).

Several strategies have been developed to minimize the toxic effects of aflatoxins on animal and the transfer of its residues into animal products, such as physical, chemical, and biological methods. However, in recent years the use of aflatoxin adsorbent is the most frequently studied method because it is considered as an effective, safe, economical and applicable method (Kutz *et al.,* 2009). One of the aflatoxin adsorbents is zeolite, a tectosilicates mineral that has the ability to bind aflatoxin so that it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al* ., 2010). Zeolites have a microporous structure that forming a large internal surface. This is associated with their high cation exchange capacity (Ca2+) that makes zeolites efficiently adsorbing polar molecules such as AFB1 (Di Gregorio *et al*., 2014).

Many studies have been conducted to determine the efficacy of processed clays, including zeolite, in response to aflatoxin challenge to dairy cow, broiler and meat duck (Sulzberger *et al*., 2017; Fowler *et al*., 2015; Mallek *et al*., 2012; Chang *et al*., 2016). However, little information on the use of natural zeolite dealing with laying duck fed AFB1-contaminated diet has been found. Duck is highly sensitive to aflatoxin exposure because of differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz and Murcia, 2011). Consumption of AFB1 contaminated diet will not only adversely affect on duck's performance but may result in residues in the products (Zhang *et al*., 2016). Therefore, this research aimed to investigate the ameliorate effects of natural zeolite inclusion in AFB1 contaminated diet on the performance and health of laying duck.

MATERIALS AND METHODS

Experimental Diet

AFB1-contaminated diet (AFC) was produced as follow: commercial feed for laying duck (IP333, PT. Wonokoyo) was used as a medium. The medium was added with aquadest to reach 30% of moisture content. The medium was inoculated with *A. flavus* FNCC 612 then it was incubated in temperature 35^{o}C for 10 days. The concentration of AFB1 in the medium was analyzed by ELISA test to calculate the dilution factor of the medium in the experimental diet. Indonesian National Standard of Industry (SNI) has established the threshold level of AFB1 in complete commercial feed that was 20 ppb (Kementan RI, 2009). Previous studies showed the detrimental effects of AFB1 on the performance of duck was observed in the level of 50 ppb or more (Ostrowski-Meisnerr, 1983; Sumantri *et al*., 2017). Therefore, this experiment was applied the level of contamination at 70 ppb. The medium then were mixed with commercial feed based on the dilution factor to obtain AFB1 levels of AFC at 70 ppb.

The zeolite used in the experiment was a natural zeolite which is mined and purchased in Central Java (PT. Brataco Chemika). Zeolite was ground using a mortar and sieved through 100 mesh. Experimental diet composition is shown in Table 1.

In Vivo Experiment

Seven months, sixty-four female Alabio ducks (*Anas platyrinchos* Borneo) were used in the experiment. Ducks were weighed and randomly assigned to 4 dietary treatments with 4 replicates of 4 ducks in each experimental unit. The mean of the duck's body weight when randomized into dietary treatment was 1,247±145 g. The treatments were: commercial feed as a control diet (P1); AFB1-contaminated diet 70 ppb (P1); Control + 2% zeolite (P2); and AFC + 2% zeolite (P3).

Dietary treatment was given_when the egg production, Duck Day Average (DDA), reached 70%. The experiment was carried out for 4 weeks. The experimental diet was provided twice a day and restricted, about 150 g/head/day, to ensure the level of AFB1 exposure on the animal was_controlled. Water was provided *ad libitum*.

Egg production was recorded and weighed daily, starting at 15th day until the 28th day of treatment. Body weight of duck was measured individually at two weeks before treatment (D-14), the beginning of treatment (D0), the second week of treatment (D14), and the fourth week of treatment (D28). At the end of the experiment (D28), ducks were slaughtered, then carcass and giblet were collected and weighed. The observed variables were body weight changes, egg production (egg weight and

DDA), the percentages of carcass, liver, and intestine weights, and histopathology of the liver.

Liver histopathology was diagnosed as follow: representative liver samples were fixed in 10% buffered neutral formalin. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris (Bancroft and Gamble, 2008).

Analysis

Feed samples were analysed for AFB1 concentrations by ELISA method using ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of body weight, egg weight, DDA percentage, carcass percentage, liver percentage, and intestine weight were analysed by the general linear model procedure using the IBM SPSS 21.0 statistical program. Significant differences between treatment means were separated using Duncan's multiple range test with a 5% probability.

RESULTS AND DISCUSSIONS

Body Weight

Treatments had a significant effect on live weight changes ($p < 0.05$). Table 2 showed that AFB1 exposure at 70 ppb caused a decrease in duck weight, as seen in AFC treatment, which experienced an average weight loss of -0.87% after four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be reduced by zeolite inclusion in the diet. This was indicated by the average of live weight of ducks in AFC + 2% zeolite diet that increased by 3.20%. Zeolite also significantly improved duck performance, as seen in control diet +2% zeolite, that had the highest body weight gain (4.86%). This gain was higher than the control ducks (P1) which only increased by 2.57%.

The adverse effects of AFB1 on growth performance was related with a decrease in the efficiency of protein and energy utilization due to the deterioration of the digestive system of the birds (Denly *et al*., 2009). Recent studies in broilers suggested that absorptive surface of small intestine would deteriorate during chronic exposure to low levels of AFB1, thus declines absorption of nutrient in the intestine (Galarza-Seeber *et al*., 2016). Figure 1 clearly shows that the presence of AFB1 in the diet (P2) decreased the growth performance of the duck.

Study on the effects of AFB1 on the performance of laying ducks is still ~~very~~ limited. In 1st-day-old ducklings which received a feed containing AFB1 at levels up to 100 ppb for 21 days showed that an increase in AFB1 level caused a decrease in weight gain (Wan *et al*., 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al*., 2001). Yunus *et al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss, decreased feed consumption, and increased feed conversion. The percentage of weight loss reported varies depending on the dose and duration of exposure, such as 5% weight loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15% weight loss at a dose of 1,000 ppb for 21 days (Yunus *et al*., 2011).

This study indicated the positive effect of zeolite inclusion in the diet for laying duck. The addition of 2% zeolite in the control diet (P3) resulted in the highest final live weight. By zeolite inclusion, the growth performance of duck receiving AFB1 contaminated diet (P4) was still higher than those of the control ducks. This finding suggests that the use of zeolite can reduce the impact of exposure to AFB1 on body weight. Chemically, zeolite is a clay group of aluminosilicate minerals which has a three-dimensional structure consisting of skeletons of SiO_4 and AlO_4 which form interconnected channels wherein the channel cavity there are weak bonds of H_2O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO_4 (Mallek *et al*., 2012).

In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of AFB1 exposure (Wan *et al* ., 2013). In broiler, the dietary use of natural or synthetic zeolites has been reported to improve feed efficiency, thus resulting in better growth performance of broilers (Mallek *et al*., 2012). Zeolites (montmorillonites) are a class of the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals, bacteria, and toxic antinutritive agents (Fowler *et al* ., 2015; Sulzberger *et al* ., 2017). The binding between aflatoxin and the adsorbent forms an inert and stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract (Huwig *et al* ., 2001).

Egg Production

In this study, the treatments had no effect on egg production and egg weight ($p > 0.05$). However, as shown in Table 3, AFB1 contamination in the diet tended to reduce egg weight, and the addition of 2% zeolite tended to increase egg weight despite the presence of AFB1 contamination in the feed ($p = 0.433$).

Aflatoxin-contaminated feed causes a decrease in egg production, as shown in the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40% in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and Awer (2009) showed that the production and egg weight of white leghorn laying hens were not affected by feed contaminated with aflatoxin at the levels of 100 ppb for 60 days, although feeding with aflatoxin contamination caused a decrease in feed consumption. Research of Zaghini *et al*. (2005) showed a decrease in egg weight of laying hens receiving AFB1 2,500 ppb for 4 weeks, this was due to a decrease in the percentage of eggshell weight and thinner eggshells due to the AFB1 exposure through contaminated feed consumption. Evidences suggest that AFB1 causes induction or inhibition of liver mixed-function-oxygenase activities that affect the metabolism of exogenous and endogenous substrates in the liver.

Weights of Carcass, Liver, and Intestine

This experiment showed that the treatments have a significant effect ($p < 0.05$) on final body weight. Nevertheless, the treatments did not have significant effects ($p > 0.05$) on carcass percentage, the relative weight of giblet, and relative weight of the liver (Table 4 and Table 5).

Duck carcass meat is mostly located in breast and thigh, therefore the weights of breast and thigh muscles are the main factor of carcass yield in duck. Study of Chang *et al.* (2016) showed that diet containing aflatoxin at 62-65 ppb significantly reduced live weight, breast muscle weight, and thigh muscle weight of meat male ducks at various age.

Although the statistical test did not show any difference in the relative weight of all variables, it was seen that in AFB1 contaminated diet groups (P2 and P4) was found an enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier than the control (13.54%). These results were also found in the relative weights of the intestines for P2 and P4 groups, namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

This study applied a relatively low of AFB1 contamination level in the diet, but this is a common level of AFB1 contamination that found in feed and feedstuffs for a duck in Indonesia according to Sumantri *et al*. (2017). At a low dose of aflatoxin exposure, the performance of birds were relatively similar, but the changes in liver size and pathology can be detected (Magnoli *et al* ., 2011). Denli *et al*. (2009) found broiler liver enlargement after receiving feed containing AFB1 at 1,000 ppb.

Liver is the target organ of AFB1 because most of the AFB1 absorbed will undergo bioactivation to form a compound 8.9-epoxide which then binds to protein and DNA (Pasha *et al*., 2007). Our study indicated mild acute degeneration of vacuoles in the liver of ducks received control diet, but this degeneration was severe in P2 diet. In zeolite groups, mild vacuoles degeneration was found in P3 ducks and medium degeneration was in P4 ducks (Figure 2).

Hepatic lesions correlated with aflatoxicosis is described as vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver (Espada *et al*., 1992). Study of Leenadevi *et al*. (1995) revealed that ducks are a very sensitive species for aflatoxin injury and it would appear that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically, they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed that zeolite inclusion seems to reduce the adverse effects of

AFB1 exposure as indicated in the result of liver histopathology study of P3 and P4 groups. Similarly, Magnolli *et al*. (2011) found that in low levels of AFB1 (50 to 100 ppb), all livers samples of broilers showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet.

CONCLUSION

Zeolite inclusions in AFB1-contaminated diet for laying ducks could reduce the adverse effects of AFB1 exposure, especially on body weight and liver histopathology, however it did not appear to increase the egg production.

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Table 1. Composition of Experimental Diets

Ingredients	Treatments			
	Control diet (P1)	AFC** (P2)	P1+2% zeolite (P3)	P2+2% zeolite (P4)
Commercial complete feed (%)*	100	90	100	90
AFB1-production medium (%)	0	10	0	10
Natural zeolite (%)	0	0	2	2
Analysis				
Dry matter (%)	88	88	88	88
Crude protein (%)	18	18	18	18
Crude fat (%)	7	7	7	7
Crude fiber (%)	6	6	6	6
Ash (%)	14	14	14	14
Calcium (%)	3.3	3.3	3.3	3.3
Phosphorous	0.8	0.8	0.8	0.8
Metabolizable energy (kkal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.
**AFC= Aflatoxin B1 contaminated diet

Table 2. Effects of Treatment Diets on Live Weight Change of Laying Ducks

Treatment Diets	Initial weight (g)	Final weight (g)	Body weight Change	
			gram	%
Control diet (P1)*	1,382±139	1,411±110	29.06±102^{ab}	2.57±7.8^{ab}
AFC (P2)**	1,447±94	1,431±92	-16.25±101^{a}	-0.87±7.4^{a}
P1+2% zeolite (P3)	1,405±139	1,469±134	64.06±99^{b}	4.86±7.3^{b}
P2+2% zeolite (P4)	1,386±142	1,427±130	40.94±66^{ab}	3.20±5.5^{ab}

*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb
$^{a, b}$ means in the same column with different superscripts differ significantly ($p < 0.05$)

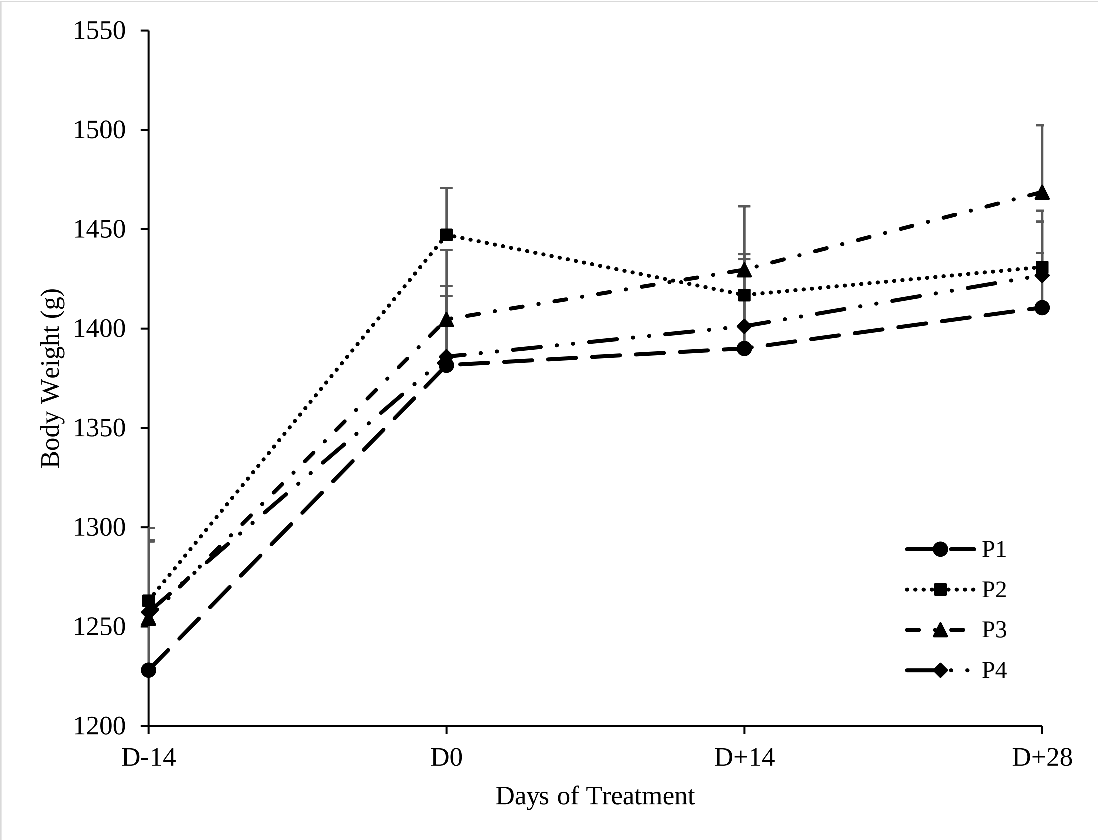

Figure 1. Live weight curves of laying ducks treated with control diet (P1); AFB1-contaminated diet 70 ppb (P2); P1+2% zeolite (P3); and P2+2% zeolite (P4)

Tabel 3. Effects of Treatment Diets on Egg Production of Laying Ducks^{ns}

Treatment Diets	DDA (%)	Egg Production (g)	Egg Weight (g)
Control diet (P1)*	54.40±3.7	4,147±217	70.70±3.7
AFC (P2)**	64.58±9.2	4,842±736	69.23±3.4
P1+2% zeolite (P3)	56.71±8.4	4,417±590	72.10±1.5
P2+2% zeolite (P4)	58.56±12.6	4,488±886	70.81±1.1

^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

Tabel 4. Effects of Treatment Diets on Final Body Weight, Carcass Weight, Giblet Weight, Liver Weight, and Small Intestinum Weight of Laying Ducks

Treatment Diets	Final Body Weight (g)	Carcass Weight (g)^{ns}	Giblet Weight (g)^{ns}	Liver Weight (g)^{ns}
Control diet (P1)*	1,460±110^{ab}	833±29	352±123	43.8±8.4
AFC (P2)**	1,426±55^{a}	847±128	289±105	47.5±15.9
P1+2% zeolite (P3)	1,406±90^{a}	835±121	300±80	43.0±9.6
P2+2% zeolite (P4)	1,576±104^{b}	951±76	324±33	49.5±5.1

$^{a,\ b}$ means in the same column with different superscripts differ significantly ($p < 0.05$)
^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

Tabel 5. Effects of Treatment Diets on the Percentages of Carcass, Giblet, and Liver of Laying Ducks^{ns}

Treatment Diets	Percentages		
	Carcass (%)	Giblet (%)	Liver (%)
Control diet (P1)*	57±5.0	42±15.6	14±4.8
AFC (P2)**	59±7.3	36±15.9	17±1.3
P1+2% zeolite (P3)	59±5.5	37±13.2	15±2.2
P2+2% zeolite (P4)	60±1.5	34±3.2	15±1.9

^{ns} means in the same column are not significantly different ($p > 0.05$)
*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

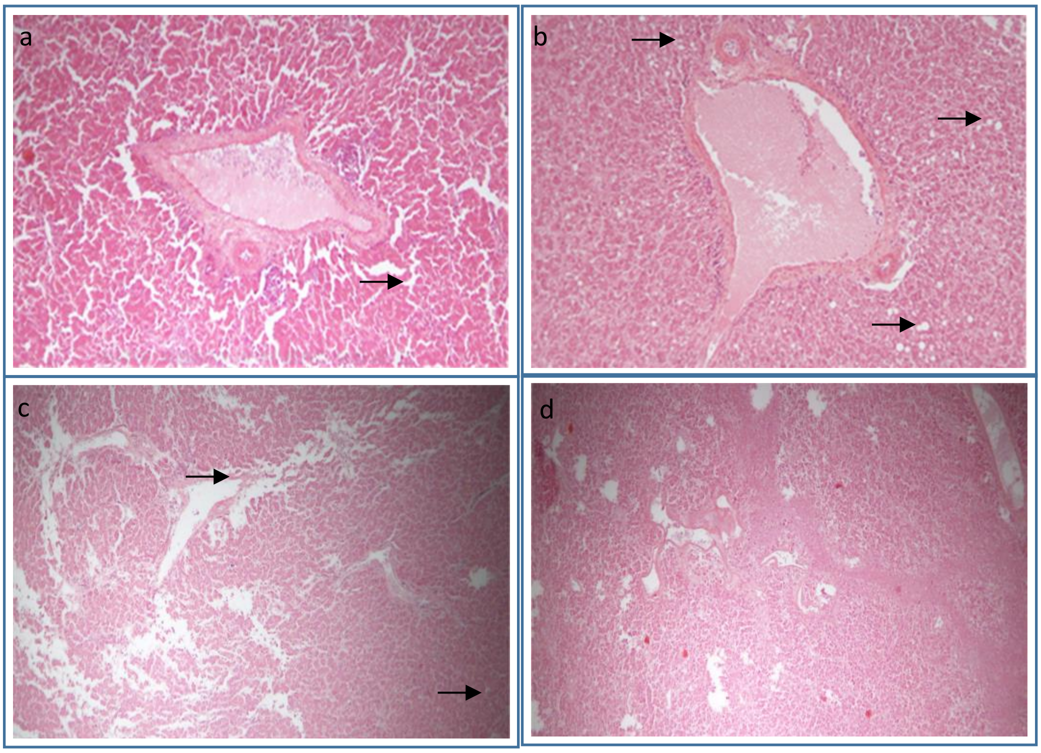

Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: control diet); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

8. Permintaan proof sheet checking dari editor tanggal 9 September 2019

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Ika Sumantri <isumantri@ulm.ac.id>

Proof sheet dan biaya publikasi

5 messages

JITAA JPPT <jppt.fpundip@gmail.com> Mon, Sep 9, 2019 at 11:21 PM
To: Ika Sumantri <isumantri@ulm.ac.id>

Dear Sdr Ika Sumantri,

Bersama ini kami kirimkan proof sheet untuk Sdr periksa. Hasil pemeriksaan harap dirangkum pada lembar terpisah (halaman....kolom...., baris ..., tertulis...., seharusnya....). Kami memberikan waktu untuk pemeriksaan paling lama 5 hari. Kami juga mengirimkan surat berisi penagihan biaya publikasi untuk Sdr respon segera.

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Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck

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ABSTRAK

Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2% zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil memperlihatkan paparan AFB1 70 pbb menyebabkan penurunan bobot badan sekitar 1,12% (P<0,05). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak berpengaruh terhadap produksi telur dan bobot telur (p>0,05). Paparan AFB1 menghasilkan bobot potong yang lebih rendah (P<0,05), namun dengan imbuhan zeolit, itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4% dengan imbuhan zeolit. Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan bobot badan.

Kata kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit

ABSTRACT

The research was objected to study the effect of zeolite inclusion in aflatoxin B1 (AFB1) contaminated diet on the performance of laying duck. A completely randomized design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1) commercial feed (Control); (2) AFB1-contaminated feed 70 ppb (AFC); (3) Control + 2% zeolite; and (4) AFC + 2% zeolite. Each treatment had 4 replications with 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data were analyzed according to the general linear model of SPSS 21.0 statistical software. Results indicated that AFB1 exposure significantly ($P<0.05$) decreased the body weight of laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that increased by 2.95% in AFC+2% zeolite. Treatments had no significant effect on egg production and egg weight ($P>0.05$). Zeolite inclusion resulted in the highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight ($P<0.05$). Relative liver weight of duck fed_AFC diet was 16.62% and to be 15.4% by zeolite addition in the diet. In conclusion, 2% of zeolite inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying duck.

Keywords:Aflatoxin B1, laying duck, performance, zeolite

INTRODUCTION

Aflatoxin B1 (AFB1) is highly carcinogenic and genotoxic compounds produced by fungi, especially toxigenic species of *Aspergillus flavus* and *A. parasiticus*. The consumption of feed containing AFB1 by the animal can result in excretion of a hydroxylated metabolite of aflatoxin, namely aflatoxin M1 (AFM1), in the animal products, such as milk, meat, and eggs (Voelkel *et al*., 2011; van der Fels-Klerx and Camenzuli, 2016). In order to avoid AFB1 exposure on livestock and ingestion of its residues by consumers, the Indonesian government has established the maximum limit of AFB1 contamination in feed for the industry. However, tropical climate causes high occurrences and levels of AFB1 contamination in feed for ruminant and poultry in Indonesia (Agus *et al*., 2013; Sumantri *et al*., 2017).

Several strategies have been developed to minimize the toxic effects of aflatoxins on animal and the transfer of its residues into animal products, such as physical, chemical, and biological methods. However, in recent years the use of aflatoxin adsorbent is the most frequently studied method because it is considered as an effective, safe, economical and applicable method (Kutz *et al*., 2009). One of the aflatoxin adsorbents is zeolite, a tectosilicates mineral that has the ability to bind aflatoxin so that it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al* ., 2010). Zeolites have a microporous structure that forming a large internal surface. This is associated with their high cation exchange capacity (Ca2+) that makes zeolites efficiently adsorbing polar molecules such as AFB1 (Di Gregorio *et al*., 2014).

Many studies have been conducted to determine the efficacy of processed clays, including zeolite, in response to aflatoxin challenge to dairy cow, broiler and meat duck (Sulzberger *et al*., 2017; Fowler *et al*., 2015; Mallek *et al*., 2012; Chang *et al*., 2016). However, little information on the use of natural zeolite dealing with laying duck fed AFB1-contaminated diet has been found. Duck is highly sensitive to aflatoxin exposure because of differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz and Murcia, 2011). Consumption of AFB1 contaminated diet will not only adversely affect on duck's performance but may result in residues in the products (Zhang *et al*., 2016). Therefore, this research aimed to investigate the ameliorate effects of natural zeolite inclusion in AFB1 contaminated diet on the performance and health of laying duck.

MATERIALS AND METHODS

Experimental Diet

AFB1-contaminated diet (AFC) was produced as follow: commercial feed for laying duck (IP333, PT. Wonokoyo) was used as a medium. The medium was added with aquadest to reach 30% of moisture content. The medium was inoculated with *A. flavus* FNCC 612 then it was incubated in temperature 35^{o}C for 10 days. The concentration of AFB1 in the medium was analyzed by ELISA test to calculate the dilution factor of the medium in the experimental diet. Indonesian National Standard of Industry (SNI) has established the threshold level of AFB1 in complete commercial feed that was 20 ppb (Kementan RI, 2009). Previous studies showed the detrimental effects of AFB1 on the performance of duck was observed in the level of 50 ppb or more (Ostrowski-Meisnerr, 1983; Sumantri *et al*., 2017). Therefore, this experiment was applied the level of contamination at 70 ppb. The medium then were mixed with commercial feed based on the dilution factor to obtain AFB1 levels of AFC at 70 ppb.

The zeolite used in the experiment was a natural zeolite which is mined and purchased in Central Java (PT. Brataco Chemika). Zeolite was ground using a mortar and sieved through 100 mesh. Experimental diet composition is presented in Table 1.

In Vivo Experiment

Seven months, sixty-four female Alabio ducks (*Anas platyrinchos* Borneo) were used in the experiment. Ducks were weighed and randomly assigned to 4 dietary treatments with 4 replicates of 4 ducks in each experimental unit. The mean of the duck's body weight when randomized into dietary treatment was 1,247±145 g. The treatments were: commercial feed as a control diet (P1); AFB1-contaminated diet 70 ppb (P1); Control + 2% zeolite (P2); and AFC + 2% zeolite (P3).

Dietary treatment was given_when the egg production, Duck Day Average (DDA), reached 70%. The experiment was carried out for 4 weeks. The experimental diet was provided twice a day and restricted, about 150 g/head/day, to ensure the level of AFB1 exposure on the animal was controlled. Water was provided *ad libitum*.

Table 1. Composition of Experimental Diets

Ingredients	Control diet (P1)	AFC** (P2)	P1+2% zeolite (P3)	P2+2% zeolite (P4)
Commercial complete feed (%)*	100	90	100	90
AFB1-production medium (%)	0	10	0	10
Natural zeolite (%)	0	0	2	2
Analysis				
Dry matter (%)	88	88	88	88
Crude protein (%)	18	18	18	18
Crude fat (%)	7	7	7	7
Crude fiber (%)	6	6	6	6
Ash (%)	14	14	14	14
Calcium (%)	3.3	3.3	3.3	3.3
Phosphorous	0.8	0.8	0.8	0.8
Metabolizable energy (kkal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.
**AFC= Aflatoxin B1 contaminated diet

Egg production was recorded and weighed daily, starting at 15th day until the 28th day of treatment. Body weight of duck was measured individually at two weeks before treatment (D-14), the beginning of treatment (D0), the second week of treatment (D14), and the fourth week of treatment (D28). At the end of the experiment (D28), ducks were slaughtered, then carcass and giblet were collected and weighed. The observed variables were body weight changes, egg production (egg weight and DDA), the percentages of carcass, liver, and intestine weights, and histopathology of the liver.

Liver histopathology was diagnosed as follow: representative liver samples were fixed in 10% buffered neutral formalin. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris (Bancroft and Gamble, 2008).

Data Analysis

Feed samples were analysed for AFB1 concentrations by ELISA method using ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of body weight, egg weight, DDA percentage, carcass percentage, liver percentage, and intestine weight were analysed by the general linear model procedure using the IBM SPSS 21.0 statistical program. Significant differences between treatment means were separated using Duncan's multiple range test with a 5% probability.

RESULTS AND DISCUSSIONS

Body Weight

Treatments had a significant effect on live weight changes ($P<0.05$). Table 2 showed that AFB1 exposure at 70 ppb caused a decrease in duck weight, as seen in AFC treatment, which experienced an average weight loss of -0.87% after four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be reduced by zeolite inclusion in the diet. This was indicated by the average of live weight of ducks in AFC + 2% zeolite diet that increased by 3.20%. Zeolite also significantly improved duck performance, as seen in control diet +2% zeolite, that had the highest body weight gain (4.86%). This gain was higher than the control ducks (P1) which only increased by 2.57%.

The adverse effects of AFB1 on growth performance was_related with a decrease in the efficiency of protein and energy utilization due to the deterioration of the digestive system of the birds (Denly *et al*., 2009). Recent studies in broilers suggested that absorptive surface of small intestine would deteriorate during chronic exposure to low levels of AFB1, thus declines absorption of nutrient in the intestine (Galarza-Seeber *et al*., 2016). Figure 1 clearly shows that the presence of AFB1 in the diet (P2) decreased the growth performance of the duck.

Study on the effects of AFB1 on the performance of laying ducks is still limited. In 1st-day-old ducklings which received a feed containing AFB1 at levels up to 100 ppb for 21 days showed that an increase in AFB1 level caused a decrease in weight gain (Wan *et al*., 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al*., 2001). Yunus *et al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss, decreased feed consumption, and increased feed conversion. The percentage of weight loss reported varies depending on the dose and duration of exposure, such as 5% weight loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15% weight loss at a dose of

Table 2. Effects of Treatment Diets on Live Weight Change of Laying Ducks

Treatment Diets	Initial Weight (g)	Final Weight (g)	Body weight Change	
			Gram	%
Control diet (P1)*	1,382±139	1,411±110	29.06±102^{ab}	2.57±7.8^{ab}
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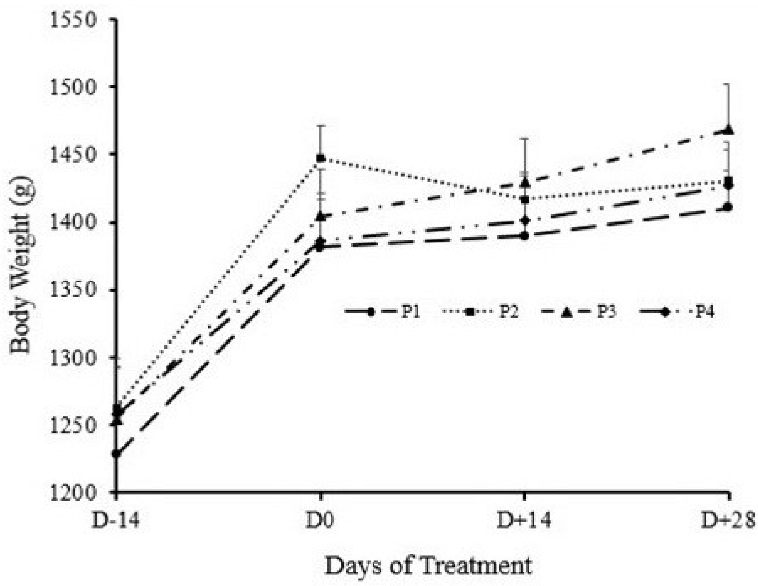

Figure 1. Live Weight Curves of Laying Ducks Treated with Control Diet (P1); AFB1-contaminated Diet 70 ppb (P2); P1+2% zeolite (P3); and P2+2% zeolite (P4)

1,000 ppb for 21 days (Yunus *et al*., 2011).

This study indicated the positive effect of zeolite inclusion in the diet for laying duck. The addition of 2% zeolite in the control diet (P3) resulted in the highest final live weight. By zeolite inclusion, the growth performance of duck receiving AFB1 contaminated diet (P4) was still higher than those of the control ducks. This finding suggests that the use of zeolite can reduce the impact of exposure to AFB1 on body weight. Chemically, zeolite is a clay group of aluminosilicate minerals which has a three-dimensional structure consisting of skeletons of SiO_4 and AlO_4 which form interconnected channels wherein the channel cavity there are weak bonds of H_2O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO_4 (Mallek *et al*., 2012).

In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of AFB1 exposure (Wan *et al*., 2013). In broiler, the dietary use of natural or synthetic zeolites has been reported to improve feed efficiency, thus resulting in better growth performance of broilers (Mallek *et al*., 2012). Zeolites (montmorillonites) are a class of the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals, bacteria, and toxic antinutritive agents (Fowler *et al*., 2015; Sulzberger *et al*., 2017). The binding between aflatoxin and the adsorbent forms an inert and stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract (Huwig *et al*., 2001).

Egg Production

In this study, the treatments had no effect on egg production and egg weight (P>0.05). However, as shown in Table 3, AFB1 contamination in the diet tended_to reduce egg weight, and the addition of 2% zeolite tended to increase egg weight despite the presence of AFB1 contamination in the feed ($p = 0.433$).

Aflatoxin-contaminated feed causes a decrease in egg production, as shown in the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40% in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and Awer (2009) showed that the production and egg weight of white leghorn laying hens were not affected by feed contaminated with aflatoxin at the levels of 100 ppb for 60 days, although feeding with aflatoxin contamination caused a decrease in feed consumption. Research of Zaghini *et al*. (2005) showed a decrease in egg weight of laying hens receiving AFB1 2,500 ppb for 4 weeks, this was due to a decrease in the percentage of eggshell weight and thinner eggshells due to the AFB1 exposure through contaminated feed consumption. Evidences suggest that AFB1 causes induction or inhibition of liver mixed-function-oxygenase activities that affect the metabolism of exogenous and endogenous substrates in the liver.

Weights of Carcass, Liver, and Intestine

This experiment showed that the treatments have a significant effect (P<0.05) on final body weight. Nevertheless, the treatments did not have significant effects on carcass percentage, the relative weight of giblet, and relative weight of the liver (Table 4 and Table 5).

Duck carcass meat is mostly located in breast and thigh, therefore the weights of breast and thigh muscles are the main factor of carcass yield in_duck. Study of Chang *et al.* (2016)

Table 3. Effects of Treatment Diets on Egg Production of Laying Ducks

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Means in the same column indicate are not significantly different
Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb*

Table 4. Effects of Treatment Diets on Final Body Weight, Carcass Weight, Giblet Weight, Liver Weight, and Small Intestinum Weight of Laying Ducks

Treatment Diets	Final Body Weight (g)	Carcass Weight (g)	Giblet Weight (g)	Liver Weight (g)
Control diet (P1)*	1,460±110^{ab}	833± 29	352±123	43.8± 8.4
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Table 5. Effects of Treatment Diets on the Percentages of Carcass, Giblet, and Liver of Laying Ducks

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P2+2% zeolite (P4)	60±1.5	34±3.2	15±1.9

*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

showed that_diet containing aflatoxin at 62-65 ppb significantly reduced_live weight, breast muscle weight, and thigh muscle weight of meat male ducks at various age.

Although the statistical test did not show any difference in the relative weight of all variables, it was seen that in AFB1 contaminated diet groups (P2 and P4) was found an enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier than the control (13.54%). These results were also found in the relative weights of the intestines for P2 and P4 groups, namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

This study applied a relatively low of AFB1 contamination level in the diet, but this is a common level of AFB1 contamination that found in feed and feedstuffs for a duck in Indonesia according to Sumantri *et al*. (2017). At a low dose of aflatoxin exposure, the performance of birds were relatively similar, but the_changes in liver size and pathology can be detected (Magnoli *et al*., 2011). Denli *et al*. (2009) found broiler liver enlargement after receiving feed containing AFB1 at 1,000 ppb.

Liver is the target organ of AFB1 because most of the AFB1 absorbed will undergo bioactivation to form a compound 8.9-epoxide which then binds to protein and DNA (Pasha *et al*., 2007). Our study indicated mild acute degeneration of vacuoles in the liver of ducks received control diet, but this degeneration was severe in P2 diet. In zeolite groups, mild vacuoles degeneration was found in P3 ducks and medium degeneration was in P4 ducks (Figure 2).

Hepatic lesions correlated with aflatoxicosis is described as vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver (Espada *et al*., 1992). Study of Leenadevi *et al*. (1995) revealed that ducks are a very sensitive species for aflatoxin injury and it would appear

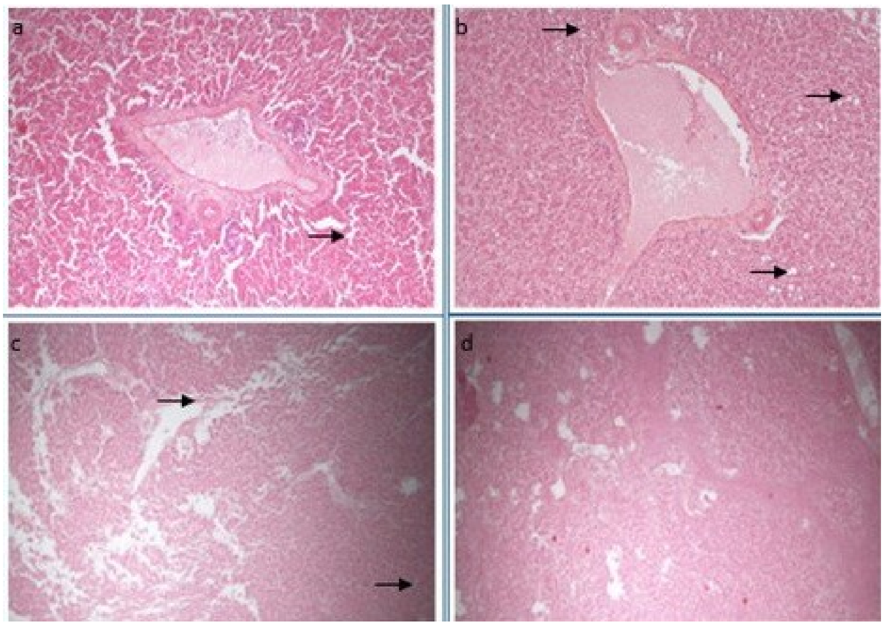

Figure 2. Acute Degenerative Hepatocyte in Liver Samples: a. Mild (P1: control diet); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically, they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed that zeolite inclusion seems to reduce the adverse effects of AFB1 exposure as indicated in the result of liver histopathology study of P3 and P4 groups. Similarly, Magnolli *et al*. (2011) found that in low levels of AFB1 (50 to 100 ppb), all livers samples of broilers showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet.

CONCLUSION

Zeolite inclusions in AFB1-contaminated diet for laying ducks could reduce the adverse effects of AFB1 exposure, especially on body weight and liver histopathology, however it did not appear to increase the egg production.

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9. Pengembaliaan proof sheet check naskah tanggal 12 September 2019

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Dr. Ika Sumantri, M.Sc.
Department of Animal Science
University of Lambung Mangkurat

2 attachments

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1.	2	2	37	Seven	Eight
2.	2	2	46	(P1); Control + 2% zeolite (P2); and AFC + 2%	(P2); Control + 2% zeolite (P3); and AFC + 2%
3.	2	2	47	zeolite (P3)	zeolite (P4)

10. Revisi naskah terakhir sebelum published tanggal 13 September 2019

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Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of laying duck

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ABSTRAK

Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2% zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil memperlihatkan paparan AFB1 70 pbb menyebabkan penurunan bobot badan sekitar 1,12% (P<0,05). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak berpengaruh terhadap produksi telur dan bobot telur (p>0,05). Paparan AFB1 menghasilkan bobot potong yang lebih rendah (P<0,05), namun dengan imbuhan zeolit, itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4% dengan imbuhan zeolit. Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan bobot badan.

Kata kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit

ABSTRACT

The research was objected to study the effect of zeolite inclusion in aflatoxin B1 (AFB1) contaminated diet on the performance of laying duck. A completely randomized design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1) commercial feed (Control); (2) AFB1-contaminated feed 70 ppb (AFC); (3) Control + 2% zeolite; and (4) AFC + 2% zeolite. Each treatment had 4 replications with 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data were analyzed according to the general linear model of SPSS 21.0 statistical software. Results indicated that AFB1 exposure significantly ($P<0.05$) decreased the body weight of laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that increased by 2.95% in AFC+2% zeolite. Treatments had no significant effect on egg production and egg weight ($P>0.05$). Zeolite inclusion resulted in the highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight ($P<0.05$). Relative liver weight of duck fed_AFC diet was 16.62% and to be 15.4% by zeolite addition in the diet. In conclusion, 2% of zeolite inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying duck.

Keywords:Aflatoxin B1, laying duck, performance, zeolite

INTRODUCTION

Aflatoxin B1 (AFB1) is highly carcinogenic and genotoxic compounds produced by fungi, especially toxigenic species of *Aspergillus flavus* and *A. parasiticus*. The consumption of feed containing AFB1 by the animal can result in excretion of a hydroxylated metabolite of aflatoxin, namely aflatoxin M1 (AFM1), in the animal products, such as milk, meat, and eggs (Voelkel *et al*., 2011; van der Fels-Klerx and Camenzuli, 2016). In order to avoid AFB1 exposure on livestock and ingestion of its residues by consumers, the Indonesian government has established the maximum limit of AFB1 contamination in feed for the industry. However, tropical climate causes high occurrences and levels of AFB1 contamination in feed for ruminant and poultry in Indonesia (Agus *et al*., 2013; Sumantri *et al*., 2017).

Several strategies have been developed to minimize the toxic effects of aflatoxins on animal and the transfer of its residues into animal products, such as physical, chemical, and biological methods. However, in recent years the use of aflatoxin adsorbent is the most frequently studied method because it is considered as an effective, safe, economical and applicable method (Kutz *et al*., 2009). One of the aflatoxin adsorbents is zeolite, a tectosilicates mineral that has the ability to bind aflatoxin so that it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al* ., 2010). Zeolites have a microporous structure that forming a large internal surface. This is associated with their high cation exchange capacity (Ca2+) that makes zeolites efficiently adsorbing polar molecules such as AFB1 (Di Gregorio *et al*., 2014).

Many studies have been conducted to determine the efficacy of processed clays, including zeolite, in response to aflatoxin challenge to dairy cow, broiler and meat duck (Sulzberger *et al*., 2017; Fowler *et al*., 2015; Mallek *et al*., 2012; Chang *et al*., 2016). However, little information on the use of natural zeolite dealing with laying duck fed AFB1-contaminated diet has been found. Duck is highly sensitive to aflatoxin exposure because of differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz and Murcia, 2011). Consumption of AFB1 contaminated diet will not only adversely affect on duck's performance but may result in residues in the products (Zhang *et al*., 2016). Therefore, this research aimed to investigate the ameliorate effects of natural zeolite inclusion in AFB1 contaminated diet on the performance and health of laying duck.

MATERIALS AND METHODS

Experimental Diet

AFB1-contaminated diet (AFC) was produced as follow: commercial feed for laying duck (IP333, PT. Wonokoyo) was used as a medium. The medium was added with aquadest to reach 30% of moisture content. The medium was inoculated with *A. flavus* FNCC 612 then it was incubated in temperature 35^{o}C for 10 days. The concentration of AFB1 in the medium was analyzed by ELISA test to calculate the dilution factor of the medium in the experimental diet. Indonesian National Standard of Industry (SNI) has established the threshold level of AFB1 in complete commercial feed that was 20 ppb (Kementan RI, 2009). Previous studies showed the detrimental effects of AFB1 on the performance of duck was observed in the level of 50 ppb or more (Ostrowski-Meisnerr, 1983; Sumantri *et al*., 2017). Therefore, this experiment was applied the level of contamination at 70 ppb. The medium then were mixed with commercial feed based on the dilution factor to obtain AFB1 levels of AFC at 70 ppb.

The zeolite used in the experiment was a natural zeolite which is mined and purchased in Central Java (PT. Brataco Chemika). Zeolite was ground using a mortar and sieved through 100 mesh. Experimental diet composition is presented in Table 1.

In Vivo Experiment

Eight months, sixty-four female Alabio ducks (*Anas platyrinchos* Borneo) were used in the experiment. Ducks were weighed and randomly assigned to 4 dietary treatments with 4 replicates of 4 ducks in each experimental unit. The mean of the duck's body weight when randomized into dietary treatment was 1,247±145 g. The treatments were: commercial feed as a control diet (P1); AFB1-contaminated diet 70 ppb (P2); Control + 2% zeolite (P3); and AFC + 2% zeolite (P4).

Dietary treatment was given_when the egg production, Duck Day Average (DDA), reached 70%. The experiment was carried out for 4 weeks. The experimental diet was provided twice a day and restricted, about 150 g/head/day, to ensure the level of AFB1 exposure on the animal was

Table 1. Composition of Experimental Diets

Ingredients	Control diet (P1)	AFC** (P2)	P1+2% zeolite (P3)	P2+2% zeolite (P4)
Commercial complete feed (%)*	100	90	100	90
AFB1-production medium (%)	0	10	0	10
Natural zeolite (%)	0	0	2	2
Analysis				
Dry matter (%)	88	88	88	88
Crude protein (%)	18	18	18	18
Crude fat (%)	7	7	7	7
Crude fiber (%)	6	6	6	6
Ash (%)	14	14	14	14
Calcium (%)	3.3	3.3	3.3	3.3
Phosphorous	0.8	0.8	0.8	0.8
Metabolizable energy (kkal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.
**AFC= Aflatoxin B1 contaminated diet

controlled. Water was provided *ad libitum*.
Egg production was recorded and weighed daily, starting at 15th day until the 28th day of treatment. Body weight of duck was measured individually at two weeks before treatment (D-14), the beginning of treatment (D0), the second week of treatment (D14), and the fourth week of treatment (D28). At the end of the experiment (D28), ducks were slaughtered, then carcass and giblet were collected and weighed. The observed variables were body weight changes, egg production (egg weight and DDA), the percentages of carcass, liver, and intestine weights, and histopathology of the liver.

Liver histopathology was diagnosed as follow: representative liver samples were fixed in 10% buffered neutral formalin. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris (Bancroft and Gamble, 2008).

Data Analysis

Feed samples were analysed for AFB1 concentrations by ELISA method using ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of body weight, egg weight, DDA percentage, carcass percentage, liver percentage, and intestine weight were analysed by the general linear model procedure using the IBM SPSS 21.0 statistical program. Significant differences between treatment means were separated using Duncan's multiple range test with a 5% probability.

RESULTS AND DISCUSSIONS

Body Weight

Treatments had a significant effect on live weight changes ($P<0.05$). Table 2 showed that AFB1 exposure at 70 ppb caused a decrease in duck weight, as seen in AFC treatment, which experienced an average weight loss of -0.87% after four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be reduced by zeolite inclusion in the diet. This was indicated by the average of live weight of ducks in AFC + 2% zeolite diet that increased by 3.20%. Zeolite also significantly improved duck performance, as seen in control diet +2% zeolite, that had the highest body weight gain (4.86%). This gain was higher than the control ducks (P1) which only increased by 2.57%.

The adverse effects of AFB1 on growth performance was_related with a decrease in the efficiency of protein and energy utilization due to the deterioration of the digestive system of the birds (Denly *et al*., 2009). Recent studies in broilers suggested that absorptive surface of small intestine would deteriorate during chronic exposure to low levels of AFB1, thus declines absorption of nutrient in the intestine (Galarza-Seeber *et al*., 2016). Figure 1 clearly shows that the presence of AFB1 in the diet (P2) decreased the growth performance of the duck.

Study on the effects of AFB1 on the performance of laying ducks is still limited. In 1st-day-old ducklings which received a feed containing AFB1 at levels up to 100 ppb for 21 days showed that an increase in AFB1 level caused a decrease in weight gain (Wan *et al*., 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al*., 2001). Yunus *et al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss, decreased feed consumption, and increased feed conversion. The percentage of weight loss reported varies depending on the dose and duration of exposure, such as 5% weight loss at a dose of 500 ppb; 10% weight loss at a dose of 800

Table 2. Effects of Treatment Diets on Live Weight Change of Laying Ducks

Treatment Diets	Initial Weight (g)	Final Weight (g)	Body weight Change	
			Gram	%
Control diet (P1)*	1,382±139	1,411±110	29.06±102^{ab}	2.57±7.8^{ab}
AFC (P2)**	1,447± 94	1,431± 92	-16.25±101^{a}	-0.87±7.4^{a}
P1+2% zeolite (P3)	1,405±139	1,469±134	64.06± 99^{b}	4.86±7.3^{b}
P2+2% zeolite (P4)	1,386±142	1,427±130	40.94± 66^{ab}	3.20±5.5^{ab}

*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb
$^{a, b}$ means in the same column with different superscripts differ significantly (P<0.05)

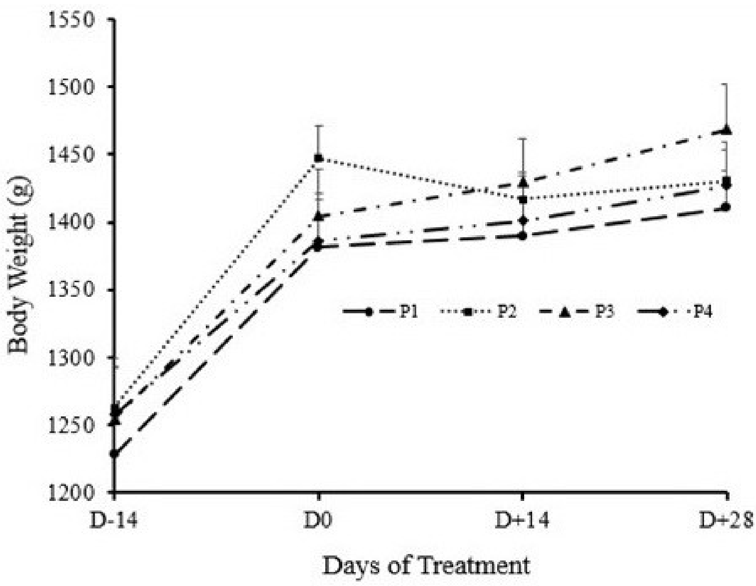

Figure 1. Live Weight Curves of Laying Ducks Treated with Control Diet (P1); AFB1-contaminated Diet 70 ppb (P2); P1+2% zeolite (P3); and P2+2% zeolite (P4)

ppb for 28 days; and 15% weight loss at a dose of 1,000 ppb for 21 days (Yunus *et al*., 2011).

This study indicated the positive effect of zeolite inclusion in the diet for laying duck. The addition of 2% zeolite in the control diet (P3) resulted in the highest final live weight. By zeolite inclusion, the growth performance of duck receiving AFB1 contaminated diet (P4) was still higher than those of the control ducks. This finding suggests that the use of zeolite can reduce the impact of exposure to AFB1 on body weight. Chemically, zeolite is a clay group of aluminosilicate minerals which has a three-dimensional structure consisting of skeletons of SiO_4 and AlO_4 which form interconnected channels wherein the channel cavity there are weak bonds of H_2O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO_4 (Mallek *et al*., 2012).

In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of AFB1 exposure (Wan *et al*., 2013). In broiler, the dietary use of natural or synthetic zeolites has been reported to improve feed efficiency, thus resulting in better growth performance of broilers (Mallek *et al*., 2012). Zeolites (montmorillonites) are a class of the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals, bacteria, and toxic antinutritive agents (Fowler *et al*., 2015; Sulzberger *et al*., 2017). The binding between aflatoxin and the adsorbent forms an inert and stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract (Huwig *et al*., 2001).

Egg Production

In this study, the treatments had no effect on egg production and egg weight (P>0.05). However, as shown in Table 3, AFB1 contamination in the diet tended_to reduce egg weight, and the addition of 2% zeolite tended to increase egg weight despite the presence of AFB1 contamination in the feed (p = 0.433).

Aflatoxin-contaminated feed causes a decrease in egg production, as shown in the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40% in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and Awer (2009) showed that the production and egg weight of white leghorn laying hens were not affected by feed contaminated with aflatoxin at the levels of 100 ppb for 60 days, although feeding with aflatoxin contamination caused a decrease in feed consumption. Research of Zaghini *et al*. (2005) showed a decrease in egg weight of laying hens receiving AFB1 2,500 ppb for 4 weeks, this was due to a decrease in the percentage of eggshell weight and thinner eggshells due to the AFB1 exposure through contaminated feed consumption. Evidences suggest that AFB1 causes induction or inhibition of liver mixed-function-oxygenase activities that affect the metabolism of exogenous and endogenous substrates in the liver.

Weights of Carcass, Liver, and Intestine

This experiment showed that the treatments have a significant effect (P<0.05) on final body weight. Nevertheless, the treatments did not have significant effects on carcass percentage, the relative weight of giblet, and relative weight of the liver (Table 4 and Table 5).

Duck carcass meat is mostly located in breast and thigh, therefore the weights of breast and thigh muscles are the main factor of carcass

Table 3. Effects of Treatment Diets on Egg Production of Laying Ducks

Treatment Diets	DDA (%)	Egg Production (g)	Egg Weight (g)
Control diet (P1)*	54.40±3.7	4,147±217	70.70±3.7
AFC (P2)**	64.58±9.2	4,842±736	69.23±3.4
P1+2% zeolite (P3)	56.71±8.4	4,417±590	72.10±1.5
P2+2% zeolite (P4)	58.56±12.6	4,488±886	70.81±1.1

Means in the same column indicate are not significantly different
Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb*

Table 4. Effects of Treatment Diets on Final Body Weight, Carcass Weight, Giblet Weight, Liver Weight, and Small Intestinum Weight of Laying Ducks

Treatment Diets	Final Body Weight (g)	Carcass Weight (g)	Giblet Weight (g)	Liver Weight (g)
Control diet (P1)*	1,460±110[ab]	833± 29	352±123	43.8± 8.4
AFC (P2)**	1,426±55[a]	847±128	289±105	47.5±15.9
P1+2% zeolite (P3)	1,406±90[a]	835±121	300± 80	43.0± 9.6
P2+2% zeolite (P4)	1,576±104[b]	951± 76	324± 33	49.5± 5.1

[a, b] Means in the same column with different superscripts differ significantly (P<0.05)
* Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

Table 5. Effects of Treatment Diets on the Percentages of Carcass, Giblet, and Liver of Laying Ducks

Treatment Diets	Carcass (%)	Giblet (%)	Liver (%)
Control diet (P1)*	57±5.0	42±15.6	14±4.8
AFC (P2)**	59±7.3	36±15.9	17±1.3
P1+2% zeolite (P3)	59±5.5	37±13.2	15±2.2
P2+2% zeolite (P4)	60±1.5	34±3.2	15±1.9

*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)
**AFC is a diet containing AFB1 at the level of 70 ppb

yield in_duck. Study of Chang *et al.* (2016) showed that_diet containing aflatoxin at 62-65 ppb significantly reduced_live weight, breast muscle weight, and thigh muscle weight of meat male ducks at various age.

Although the statistical test did not show any difference in the relative weight of all variables, it was seen that in AFB1 contaminated diet groups (P2 and P4) was found an enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier than the control (13.54%). These results were also found in the relative weights of the intestines for P2 and P4 groups, namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

This study applied a relatively low of AFB1 contamination level in the diet, but this is a common level of AFB1 contamination that found in feed and feedstuffs for a duck in Indonesia according to Sumantri *et al*. (2017). At a low dose of aflatoxin exposure, the performance of birds were relatively similar, but the_changes in liver size and pathology can be detected (Magnoli *et al*., 2011). Denli *et al*. (2009) found broiler liver enlargement after receiving feed containing AFB1 at 1,000 ppb.

Liver is the target organ of AFB1 because most of the AFB1 absorbed will undergo bioactivation to form a compound 8.9-epoxide which then binds to protein and DNA (Pasha *et al*., 2007). Our study indicated mild acute degeneration of vacuoles in the liver of ducks received control diet, but this degeneration was severe in P2 diet. In zeolite groups, mild vacuoles degeneration was found in P3 ducks and medium degeneration was in P4 ducks (Figure 2).

Hepatic lesions correlated with aflatoxicosis is described as vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver (Espada *et al*., 1992). Study of Leenadevi *et al*. (1995) revealed that ducks are a very sensitive

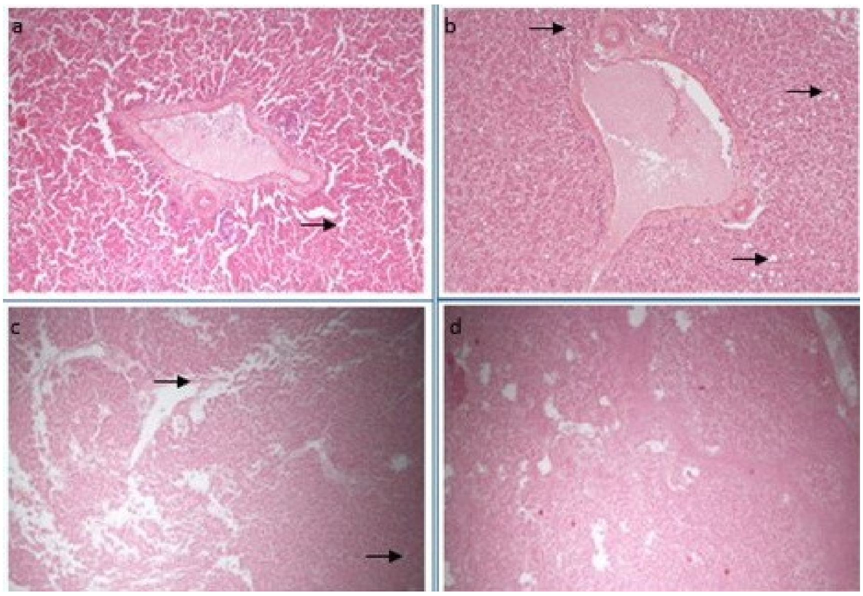

Figure 2. Acute Degenerative Hepatocyte in Liver Samples: a. Mild (P1: control diet); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

species for aflatoxin injury and it would appear that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically, they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed that zeolite inclusion seems to reduce the adverse effects of AFB1 exposure as indicated in the result of liver histopathology study of P3 and P4 groups. Similarly, Magnolli *et al*. (2011) found that in low levels of AFB1 (50 to 100 ppb), all livers samples of broilers showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet.

CONCLUSION

Zeolite inclusions in AFB1-contaminated diet for laying ducks could reduce the adverse effects of AFB1 exposure, especially on body weight and liver histopathology, however it did not appear to increase the egg production.

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