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

### Kronologi Publikasi

#### 1. Submission 24 Maret 2019



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Ika Sumantri <isumantri@ulm.ac.id>

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**[JITAA] Submission Acknowledgement**  
1 message

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**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:

Manuscript URL:  
<https://ejournal.undip.ac.id/index.php/jitaa/author/submission/22495>  
Username: ika\_sumantri

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Edy Kurnianto  
Journal of the Indonesian Tropical Animal Agriculture

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Journal of the Indonesian Tropical Animal Agriculture  
<http://ejournal.undip.ac.id/index.php/jitaa>

#### 2. Telaah oleh reviewer tanggal 12 Juni 2019



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Ika Sumantri <isumantri@ulm.ac.id>

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**Hasil Telaah Manuskrip #22495**  
3 messages

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**JITAA JPPT** <jppt.fpundip@gmail.com> Wed, Jun 12, 2019 at 11:40 PM  
To: isumantri@ulm.ac.id

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,

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**2 attachments**

 **22495-61421-SS-reviewer IIS.docx**  
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1 **Effect of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of**  
2 **laying duck**  
3

4  
5  
6 **ABSTRAK**  
7

8 Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben  
9 aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik  
10 petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) P1:  
11 pakan komersial; (2) P2: pakan terkontaminasi AFB1 70 ppb; (3) P3: P1 + 2% zeolit; (4)  
12 P4: P2 + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap  
13 ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina berumur  
14 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi  
15 menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil  
16 memperlihatkan paparan AFB1 70 ppb menyebabkan penurunan bobot badan sekitar  
17 1,12% ( $P<0,05$ ). Imbuan zeolit dapat menghindari dampak paparan AFB1 terlihat  
18 dengan naiknya bobot badan sekitar 2,95% pada P4. Perlakuan tidak berpengaruh  
19 terhadap produksi telur ( $P>0,05$ ). Paparan AFB1 cenderung menurunkan bobot telur dan  
20 imbuan zeolit cenderung mengurangi penurunan bobot telur. Paparan AFB1  
21 menghasilkan bobot potong yang lebih rendah ( $P<0,05$ ), namun dengan imbuan zeolit,  
22 itik pada P4 menghasilkan bobot potong yang paling tinggi. Paparan AFB1 cenderung  
23 menghasilkan bobot relatif hati yang lebih besar (16,62%), namun dampak ini menurun  
24 dengan imbuan zeolit (15,4%). Disimpulkan bahwa imbuan zeolit sebesar 2% dapat  
25 mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan  
26 bobot badan.  
27

28 *Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit*  
29

30  
31 **ABSTRACT**  
32

33 The research was objected to studying the effect of zeolite inclusion in aflatoxin  
34 B1 (AFB1) contaminated diet on the performance of laying duck. A completely  
35 randomized design was adopted in the in vivo experiment that consisted of 4 treatments,  
36 namely: (1) P1: commercial feed; (2) P2: AFB1-contaminated feed 70 ppb; (3) P1 + 2%  
37 zeolite; and (4) P4: P2 + 2% zeolite. Each treatment had 4 replications with 4 ducks in  
38 each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos*  
39 *Borneo*) was used in 28 days of the feeding experiment. Data was analyzed according to  
40 the general linear model of SPSS 21.0 statistical software. Results indicated AFB1  
41 exposure significantly ( $P<0.05$ ) decreases body weight of laying duck by 1.12%. Zeolite  
42 inclusion could prevent the adverse effect of AFB1 on body weight that increased by  
43 2.95% in P4. Treatments had no significant effect on egg production ( $P>0.05$ ). The lowest  
44 egg weight was found in P2 and zeolite inclusion seemed to increase egg weight, but this  
45 was not statistically significant ( $P>0.05$ ). Zeolite inclusion resulted in the highest final  
46 body weight whilst AFB1 diet without zeolite resulted in the lowest final body weight  
47 ( $P<0.05$ ). Ducks received AFB1 contaminated had heavier liver weight (16.62%), and

48 liver weight was decreased by zeolite inclusion (15.4%). In conclusion, 2% zeolite  
49 inclusion could reduce the adverse effects of AFB1 exposure on the performance of laying  
50 duck.

51  
52 *Key words: Aflatoxin B1, laying duck, performans, zeolite*

53  
54

## 55 INTRODUCTION

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

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

73 Compared to chickens, duck is highly sensitive to aflatoxin exposure because of  
74 differences in hepatic and extra-hepatic enzymes responsible for AFB1 metabolism (Diaz

75 and Murcia, 2011). Therefore, consumption of AFB1 contaminated diet will adversely  
76 affect duck's performance. [This] research aims to investigate the effects of zeolite  
77 inclusion in AFB1 contaminated diet on the performance of laying duck.

**Commented [T1]:** What is the novelty of the present manuscript?? 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

78

## 79 MATERIALS AND METHODS

### 80 Experimental Diet

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

**Commented [T2]:** Moisture of feed.....??

**Commented [T3]:** How???

**Commented [T4]:** You have to provide the reference in determining this doses

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

90

### 91 In Vivo Experiment

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

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

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

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

113

#### 114 **Analysis**

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

121

## RESULTS AND DISCUSSIONS

122

### 123 Live Weight Changes

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

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

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

141 Study on the effects of AFB1 on the performance of laying ducks is still very  
142 limited. In 1-day-old ducklings which received a feed containing AFB1 at levels up to  
143 100 ppb for 21 days, an increase in AFB1 level caused a decrease in weight gain (Wan *et*  
144 *al.*, 2013). Research on chickens showed a decrease in broiler body weight fed 200 ppb  
145 AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al.*, 2001). Yunus *et al.* (2011)

Commented [T5]: Please elaborate more!

Commented [T6]: How zeolite exert such positive effect?

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

151 This study shows the use of zeolite can increase weight gain and reduce the  
152 impact of exposure to AFB1. Chemically, zeolite is a clay group of aluminosilicate  
153 minerals which has a three-dimensional structure consisting of skeletons of  $\text{SiO}_4$  and  $\text{AlO}_4$   
154 which form interconnected channels where in the channel cavity there are weak bonds of  
155  $\text{H}_2\text{O}$  molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge  
156 negative from  $\text{AlO}_4$  (Mallek *et al.*, 2012).

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

165

### 166 **Egg Production**

167 In this study, the treatments had no effect on egg production and weight ( $p >$   
168 0.05). However, as shown in Table 2., the presence of AFB1 contamination tends to

Commented [T7]: Please provide the p-value

169 reduce egg weight (P2), and the addition of adsorbent tends to increase egg weight (P3)  
170 despite the presence of AFB1 contamination in the feed (P4) ( $P > 0.05$ ).

Commented [T8]:

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

Commented [T9]: How AFB1 influence the egg production and weight?

181

### 182 **Weights of Carcass, Liver, and Intestine**

183 This experiment showed the treatments have a significant effect ( $P < 0.05$ )  
184 on final body weight. Nevertheless the treatments did not have significant effects ( $P >$   
185  $0.05$ ) on carcass percentage, relative weight of giblet, and relative weight of liver (Table  
186 3. and Table 4.).

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

191 Although the statistical test did not show any difference in the relative weight of all  
192 variables, it was seen that in AFB1 contaminated diet group (P2 and P4) there was an



193 enlargement of the liver, with a relative weight of 16.62% and 15.40%, which was heavier  
194 than the control (13.54%). This results were also found in the relative weights of the  
195 intestines for P2 and P4, namely 16.93% and 18.2% respectively, which were heavier  
196 than the controls (14.19%).

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

204 Liver is the target organ of AFB1 because most of the AFB1 absorbed will  
205 undergo bioactivation to form a compound 8,9-epoxide which then binds to protein and  
206 DNA ( Pasha *et al.*, 2007 ). Our study indicated mild acute degeneration of vacuoles in  
207 the liver of ducks received P1 diet, but this degeneration was severe in P2 diet. In zeolite  
208 groups, mild vacuoles degeneration was found in P3 and medium degeneration was in P4  
209 (Figure 2).

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



233

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237

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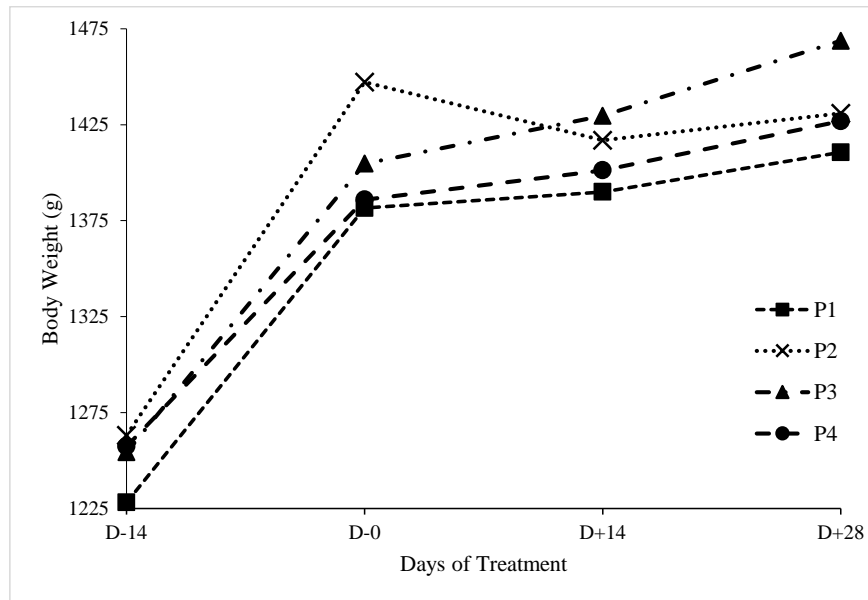
312

313

314 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 <sup>ab</sup>	2.10 <sup>ab</sup>
P1 + AFB1 70 ppb (P2)	1447	1431	-16.25 <sup>a</sup>	-1.12 <sup>a</sup>
P1 + 2% zeolite (P3)	1405	1469	64.06 <sup>b</sup>	4.56 <sup>b</sup>
P2 + 2% zeolite (P4)	1386	1427	40.94 <sup>ab</sup>	2.95 <sup>ab</sup>

315 <sup>a, b</sup> means in same column with different superscripts differ significantly ( $P < 0.05$ )  
 316



317  
 318 Figure 1. Live weight curves of laying ducks treated with control diet (P1); P1 containing  
 319 AFB1 70 ppb (P2); P1 + 2% zeolite (P3); and P2 + 2% zeolite (P4)  
 320

321

322  
323 Tabel 2. Effects of Treatment Diet on Egg Production of Laying Duck

Treatment Diet	DDA (%) <sup>ns</sup>	Egg Production (g) <sup>ns</sup>	Egg Weight (g) <sup>ns</sup>
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

324 <sup>ns</sup> means in the same column are not significantly different ( $P > 0.05$ )

325  
326 Tabel 3. Effects of Treatment Diet on Final Body Weight, Carcass Weight, Giblet  
327 Weight, Liver Weight, and Small Intestinum Weight of Laying Duck

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

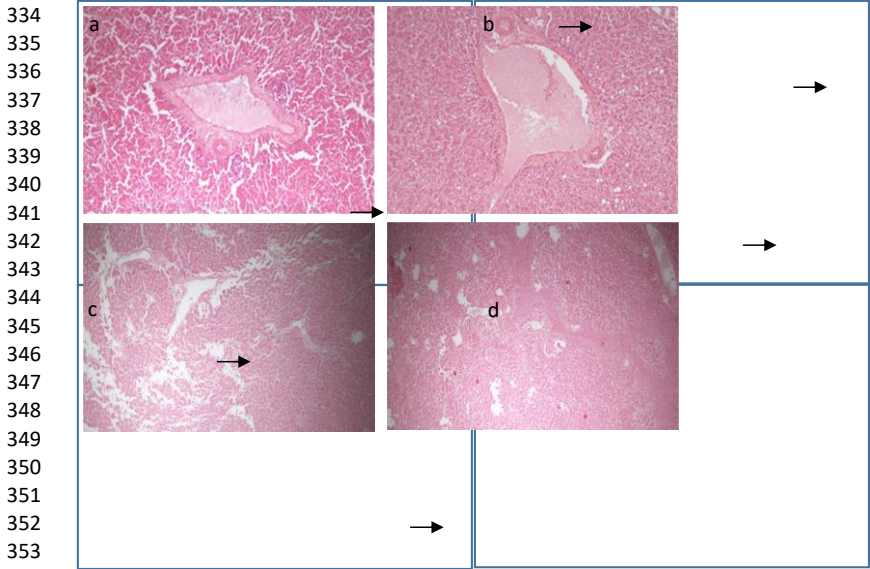
328 <sup>a, b</sup> means in same column with different superscripts differ significantly ( $P < 0.05$ )

329 <sup>ns</sup> means in the same column are not significantly different ( $P > 0.05$ )

330  
331 Tabel 4. Effects of Treatment Diet on the Percentages of Carcass, Giblet, and Liver of  
332 Laying Duck

Treatment Diet	Percentages		
	Carcass (%) <sup>ns</sup>	Giblet (%) <sup>ns</sup>	Liver (%) <sup>ns</sup>
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

333 <sup>ns</sup> means in the same column are not significantly different ( $P > 0.05$ )



355 Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial feed);  
356 b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium  
357 (P4: P2 + 2% zeolite).  
358

1  
2 **Effect of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of**  
3 **laying duck**  
4

5  
6  
7 **ABSTRAK**  
8

9 Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben  
10 aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik  
11 petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) P1:  
12 pakan komersial; (2) P2: pakan terkontaminasi AFB1 70 ppb; (3) P3: P1 + 2% zeolit;  
13 (4) P4: P2 + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik setiap  
14 ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina  
15 berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi  
16 menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil  
17 memperlihatkan paparan AFB1 70 ppb menyebabkan penurunan bobot badan sekitar  
18 1,12% ( $P<0,05$ ). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat  
19 dengan naiknya bobot badan sekitar 2,95% pada P4. Perlakuan tidak berpengaruh  
20 terhadap produksi telur ( $P>0,05$ ). Paparan AFB1 cenderung menurunkan bobot telur  
21 dan imbuhan zeolit cenderung mengurangi penurunan bobot telur. Paparan AFB1  
22 menghasilkan bobot potong yang lebih rendah ( $P<0,05$ ), namun dengan imbuhan zeolit,  
23 itik pada P4 menghasilkan bobot potong yang paling tinggi. Paparan AFB1 cenderung  
24 menghasilkan bobot relatif hati yang lebih besar (16,62%), namun dampak ini menurun  
25 dengan imbuhan zeolit (15,4%). Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat  
26 mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan  
27 bobot badan.

28  
29 *Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit*  
30

31  
32 **ABSTRACT**  
33

34 The research was objected to studying the effect of zeolite inclusion in aflatoxin  
35 B1 (AFB1) contaminated diet on the performance of laying duck. A completely  
36 randomized design was adopted in the in vivo experiment that consisted of 4 treatments,  
37 namely: (1) P1: commercial feed; (2) P2: AFB1-contaminated feed 70 ppb; (3) P1 + 2%  
38 zeolite; and (4) P4: P2 + 2% zeolite. Each treatment had 4 replications with 4 ducks in  
39 each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos*  
40 *Borneo*) was used in 28 days of the feeding experiment. Data was analyzed according to  
41 the general linear model of SPSS 21.0 statistical software. Results indicated AFB1  
42 exposure significantly ( $P<0.05$ ) decreases body weight of laying duck by 1.12%. Zeolite  
43 inclusion could prevent the adverse effect of AFB1 on body weight that increased by  
44 2.95% in P4. Treatments had no significant effect on egg production ( $P>0.05$ ). The  
45 lowest egg weight was found in P2 and zeolite inclusion seemed to increase egg weight,  
46 but this was not statistically significant ( $P>0.05$ ). Zeolite inclusion resulted in the

**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%.

**Commented [SST2]:** Angka ini imbuhan zeolitnya atau level penurunan? Saran: letakkan angka dalam kurung setelah kata menurun.

**Commented [SST3]:** Konten disesuaikan dengan masukan yg di versi Indonesia.



47 highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final  
48 body weight ( $P < 0.05$ ). Ducks received AFB1 contaminated had heavier liver weight  
49 (16.62%), and liver weight was decreased by zeolite inclusion (15.4%). In conclusion,  
50 2% zeolite inclusion could reduce the adverse effects of AFB1 exposure on the  
51 performance of laying duck.

52

53 *Key words: Aflatoxin B1, laying duck, performans, zeolite*

54

55

56

## INTRODUCTION

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

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

**Commented [SST4]:** Why zeolite? How about others toxin binder? Facts about zeolite as a treatment agent in this investigation is so poor stated in introduction section. Please describe more detail about zeolites and previous studied related to its utilization as toxin binder in the feed of poultry.

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

79

## 80 MATERIALS AND METHODS

### 81 **Experimental Diet**

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

88 The zeolite that was used in the experiment was a natural zeolite which is mined  
89 and purchased in Central Java. Zeolite was ground using a mortar and sieved through  
90 100 mesh.

91

### 92 **In Vivo Experiment**

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

**Commented [SST5]:** It was suggested you show the table of feed formulation/content.

**Commented [SST6]:** Branded name or product specification?

**Commented [SST7]:** How did average weight of the ducks?

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

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

103 Egg production was recorded and weighed daily, starting at 15th day until the  
104 28th day of treatment. Body weight of duck was measured individually at two weeks  
105 before treatment (D-14), the beginning of treatment (D0), the second week of treatment  
106 (D14), and the fourth week of treatment (D28). At the end of the experiment (D28),  
107 ducks were sacrificed, then carcass and giblet were collected and weighed. The  
108 observed variables were changes in body weight, egg production (egg weight and  
109 DDA), the weight percentages of carcass, liver, and intestine, and histopathology of  
110 liver.

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

115

#### 116 **Analysis**

117 Feed samples were analysed for AFB1 concentrations by ELISA method using  
118 ELISA kit AgraQuant® ELISA Aflatoxin B1 (Romer Labs, Singapore). Data of live  
119 weight changes, egg weight, DDA percentage, carcass percentage, liver percentage, and  
120 intestine weight were analysed by the general linear model procedure using

121 the IBM SPSS 21.0 statistical program. Significant differences between treatment  
122 means were separated using the Duncan's multiple range test with a 5% probability.

123

124

## RESULTS AND DISCUSSIONS

### 125 Live Weight Changes

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

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

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

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

145 100 ppb for 21 days, an increase in AFB1 level caused a decrease in weight gain  
146 (Wan *et al.*, 2013). Research on chickens showed a decrease in broiler body weight fed  
147 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al.*, 2001). Yunus *et*  
148 *al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss,  
149 decreased feed consumption, and increased feed conversion. The percentage of weight  
150 loss reported varies depending on the dose and duration of exposure, such as 5% weight  
151 loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15%  
152 weight loss at a dose of 1,000 ppb for 21 days.

153 This study shows the use of zeolite can increase weight gain and reduce the  
154 impact of exposure to AFB1. Chemically, zeolite is a clay group of aluminosilicate  
155 minerals which has a three-dimensional structure consisting of skeletons of SiO<sub>4</sub> and  
156 AlO<sub>4</sub> which form interconnected channels where in the channel cavity there are weak  
157 bonds of H<sub>2</sub>O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the  
158 charge negative from AlO<sub>4</sub> (Mallek *et al.*, 2012).

159 In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of  
160 AFB1 exposure (Wan *et al.*, 2013). In broiler, the dietary use of natural or synthetic  
161 zeolites has been reported to improve feed efficiency, thus resulting to a better growth  
162 performance of broilers (Mallek *et al.*, 2012). Zeolites (montmorillonites) are a class of  
163 the smectite clay group, which has 3-layer structures that allow to adsorb heavy metals,  
164 bacteria, and toxic antinutritive agents (Fowler *et al.*, 2015; Sulzberger *et al.*,  
165 2017). The binding between aflatoxin and the adsorbent forms an inert and  
166 stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract  
167 (Huwig *et al.*, 2001).

168

169 **Egg Production**

170 In this study, the treatments had no effect on egg production and weight ( $p >$   
171 0.05). However, as shown in Table 2., the presence of AFB1 contamination tends to  
172 reduce egg weight (P2), and the addition of adsorbent tends to increase egg weight (P3)  
173 despite the presence of AFB1 contamination in the feed (P4) ( $P > 0.05$ ).

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

184

185 **Weights of Carcass, Liver, and Intestine**

186 This experiment showed the treatments have a significant effect ( $P < 0.05$ )  
187 on final body weight. Nevertheless the treatments did not have significant effects ( $P >$   
188 0.05) on carcass percentage, relative weight of giblet, and relative weight of  
189 liver (Table 3. and Table 4.).

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

193 weight, breast muscle weight, and thigh muscle weight of meat male ducks at various  
194 age.

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

201 This study applied a relatively low of AFB1 contamination level in the diet, but  
202 this is a common level of AFB1 contamination that found in feed and feedstuffs for  
203 duck in Indonesia according to previous study of Sumantri *et al.* (2017). At low dose of  
204 aflatoxin exposure, the performance of birds are relatively unchanged, but changes in  
205 liver size and pathology can be detected (Magnoli *et al.*, 2011). Study of Denli *et*  
206 *al.* (2009) found broiler liver enlargement after receiving feed containing AFB1 at  
207 1,000 ppb.

208 Liver is the target organ of AFB1 because most of the AFB1 absorbed will  
209 undergo bioactivation to form a compound 8,9-epoxide which then binds to protein and  
210 DNA (Pasha *et al.*, 2007). Our study indicated mild acute degeneration of vacuoles in  
211 the liver of ducks received P1 diet, but this degeneration was severe in P2 diet. In  
212 zeolite groups, mild vacuoles degeneration was found in P3 and medium degeneration  
213 was in P4 (Figure 2).

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





237

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241

242

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319

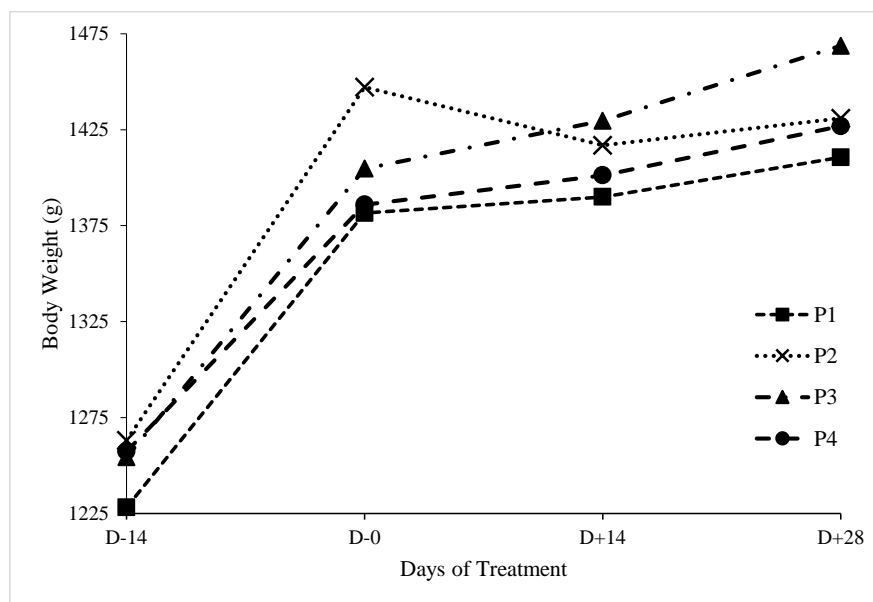
320 Table 1. Effects of Treatment Diet on Live Weight Change of Laying Duck

Commented [SST9]: Please show the STDEV (Standard deviation) beside the means!

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

321 <sup>a, b</sup> means in same column with different superscripts differ significantly ( $P < 0.05$ )

322



323

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

Commented [SST10]: Please show the error bars of those data!

326

327

328  
329

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

Commented [SST11]: STDEV???

Treatment Diet	DDA (%) <sup>ns</sup>	Egg Production (g) <sup>ns</sup>	Egg Weight (g) <sup>ns</sup>
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

330 <sup>ns</sup> means in the same column are not significantly different ( $P > 0.05$ )

331

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

Commented [SST12]: STDEV???

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

334 <sup>a, b</sup> means in same column with different superscripts differ significantly ( $P < 0.05$ )

335 <sup>ns</sup> means in the same column are not significantly different ( $P > 0.05$ )

336

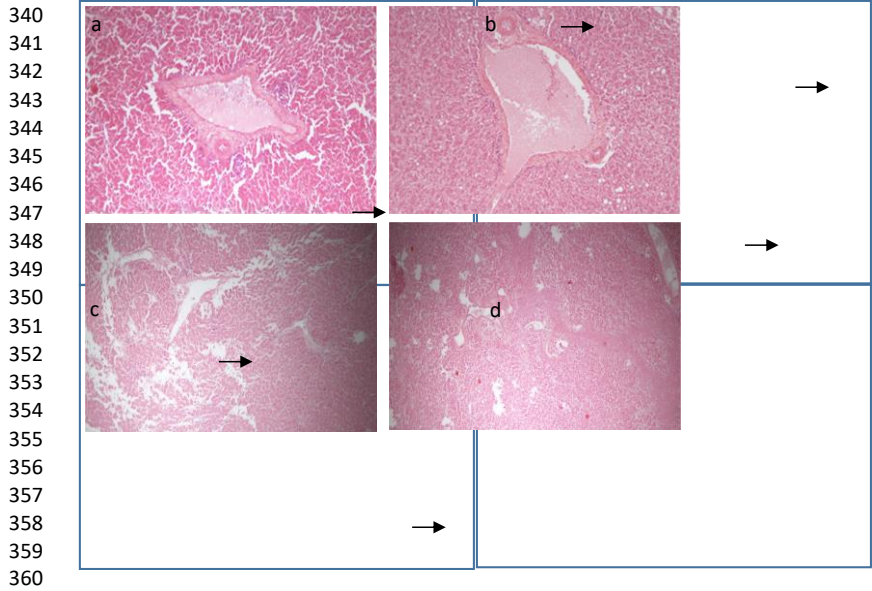
337 Tabel 4. Effects of Treatment Diet on the Percentages of Carcass, Giblet, and Liver of  
338 Laying Duck

Commented [SST13]: STDEV???

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 (%) <sup>ns</sup>	Giblet (%) <sup>ns</sup>	Liver (%) <sup>ns</sup>
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

339 <sup>ns</sup> means in the same column are not significantly different ( $P > 0.05$ )



361 Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial  
 362 feed); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d.  
 363 Medium (P4: P2 + 2% zeolite).  
 364

### 3. Submission revisi artikel tanggal 26 Juni 2019

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To: JITAA JPPT <jppt.fpundip@gmail.com>

Wed, Jun 26, 2019 at 3:37 PM

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

[Quoted text hidden]

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1  
2 **Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of**  
3 **laying duck**  
4

5  
6  
7 **ABSTRAK**  
8

9 Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben  
10 aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik  
11 petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan  
12 komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2%  
13 zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik  
14 setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina  
15 berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi  
16 menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil  
17 memperlihatkan paparan AFB1 70 ppb menyebabkan penurunan bobot badan sekitar  
18 1,12% ( $P<0,05$ ). Imbuan zeolit dapat menghindari dampak paparan AFB1 terlihat  
19 dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak  
20 berpengaruh terhadap produksi telur dan bobot telur ( $p>0,05$ ). Paparan AFB1  
21 menghasilkan bobot potong yang lebih rendah ( $P<0,05$ ), namun dengan imbuan zeolit,  
22 itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang  
23 paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4%  
24 dengan imbuan zeolit. Disimpulkan bahwa imbuan zeolit sebesar 2% dapat  
25 mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan  
26 bobot badan.  
27

28 *Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit*  
29  
30

31 **ABSTRACT**  
32

33 The research was objected to studying the effect of zeolite inclusion in aflatoxin  
34 B1 (AFB1) contaminated diet on the performance of laying duck. A completely  
35 randomized design was adopted in the in vivo experiment that consisted of 4 treatments,  
36 namely: (1) commercial feed (Control); (2) AFB1-contaminated feed 70 ppb (AFC); (3)  
37 Control + 2% zeolite; and (4) AFC + 2% zeolite. Each treatment had 4 replications with  
38 4 ducks in each replication. A total of 64 eight months-female Alabio duck (*Anas*  
39 *platyrinchos Borneo*) was used in 28 days of the feeding experiment. Data were  
40 analyzed according to the general linear model of SPSS 21.0 statistical software.  
41 Results indicated AFB1 exposure significantly ( $p<0.05$ ) decreases the body weight of  
42 laying duck by 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on  
43 body weight that increased by 2.95% in AFC+2% zeolite. Treatments had no significant  
44 effect on egg production and egg weight ( $p>0.05$ ). Zeolite inclusion resulted in the  
45 highest final body weight whilst AFB1 diet without zeolite resulted in the lowest final  
46 body weight ( $p<0.05$ ). Relative liver weight of AFC diet was 16.62% and to be 15.4%

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

49  
50  
51  
52

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

53

## INTRODUCTION

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

64 Several strategies have been developed to minimize the toxic effects of  
65 aflatoxins on animal and the transfer of its residues into animal products, such as  
66 physical, chemical, and biological methods. However, in recent years the use of  
67 aflatoxin adsorbent is the most studied method because it is considered as an effective,  
68 safe, economical and applicable method (Kutz *et al.*, 2009). One of the aflatoxin  
69 adsorbents is zeolite, a tectosilicates mineral that has the ability to bind aflatoxin so that  
70 it can prevent the absorption of AFB1 in the digestive tract of livestock (Li *et al.* , 2010  
71 ). **Zeolites have a microporous structure that forming a large internal surface. This is  
72 associated with their high cation exchange capacity (Ca<sup>2+</sup>) that making zeolites  
73 efficiently adsorbing polar molecules such as AFB1 (Di Gregorio *et al.*, 2014)**



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

85

## 86 MATERIALS AND METHODS

### 87 Experimental Diet

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

98 Sumantri *et al.*, 2017). Therefore, this experiment applied the level of contamination at  
99 70 ppb. Production medium then mixed with commercial feed based on the dilution  
100 factor to obtain AFB1 levels of AFC at 70 ppb.

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

105

### 106 **In Vivo Experiment**

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

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

117 Egg production was recorded and weighed daily, starting at 15th day until the  
118 28th day of treatment. Body weight of duck was measured individually at two weeks  
119 before treatment (D-14), the beginning of treatment (D0), the second week of treatment  
120 (D14), and the fourth week of treatment (D28). At the end of the experiment (D28),  
121 ducks were sacrificed, then carcass and giblet were collected and weighed. The

122 observed variables were changes in body weight, egg production (egg weight and  
123 DDA), the weight percentages of carcass, liver, and intestine, and histopathology of the  
124 liver.

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

129

### 130 **Analysis**

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

137

## 138 **RESULTS AND DISCUSSIONS**

### 139 **Live Weight Changes**

140 Treatments had a significant effect on live weight changes ( $p < 0.05$ ). Table  
141 2. showed that AFB1 exposure at 70 ppb would cause a decrease in duck weight, as  
142 seen in AFC treatment, which experienced an average weight loss of -0.87% after four  
143 weeks of treatment. This adverse effect of AFB1 exposure on body weight can be  
144 reduced by zeolite inclusion in the diet. This was indicated by the average of live weight  
145 of ducks in AFC + 2% zeolite diet that increased by 3.20%. Zeolite also significantly

146 improved duck performance, as seen in control diet +2% zeolite, that had the highest  
147 body weight gain, namely 4.86%. This gain was higher than the control feed which only  
148 increased by 2.57%.

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

156 Study on the effects of AFB1 on the performance of laying ducks is still very  
157 limited. In 1-day-old ducklings which received a feed containing AFB1 at levels up to  
158 100 ppb for 21 days, an increase in AFB1 level caused a decrease in weight gain  
159 (Wan *et al.*, 2013). Research on chickens showed a decrease in broiler body weight fed  
160 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al.*, 2001). Yunus *et*  
161 *al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss,  
162 decreased feed consumption, and increased feed conversion. The percentage of weight  
163 loss reported varies depending on the dose and duration of exposure, such as 5% weight  
164 loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15%  
165 weight loss at a dose of 1,000 ppb for 21 days.

166 This study indicated the positive effect of zeolite inclusion in the diet for laying  
167 duck. Addition of 2% zeolite in the control diet resulted in the highest final live weight  
168 (Control + 2% zeolite). By zeolite inclusion, the growth performance of duck receiving  
169 AFB1 contaminated diet (AFC + 2% zeolite) was still higher than the control diet. This

170 finding suggests that the use of zeolite can reduce the impact of exposure to AFB1 on  
171 body weight. Chemically, zeolite is a clay group of aluminosilicate minerals which has  
172 a three-dimensional structure consisting of skeletons of SiO<sub>4</sub> and AlO<sub>4</sub> which form  
173 interconnected channels wherein the channel cavity there are weak bonds of H<sub>2</sub>O  
174 molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge  
175 negative from AlO<sub>4</sub> (Mallek *et al.*, 2012).

176 In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of  
177 AFB1 exposure (Wan *et al.*., 2013). In broiler, the dietary use of natural or synthetic  
178 zeolites has been reported to improve feed efficiency, thus resulting in better growth  
179 performance of broilers (Mallek *et al.*, 2012). Zeolites (montmorillonites) are a class of  
180 the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals,  
181 bacteria, and toxic antinutritive agents (Fowler *et al.*., 2015; Sulzberger *et al.*.,  
182 2017). The binding between aflatoxin and the adsorbent forms an inert and  
183 stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract  
184 (Huwig *et al.*., 2001).

185

## 186 **Egg Production**

187 In this study, the treatments had no effect on egg production and weight ( $p >$   
188 0.05). However, as shown in Table 3., AFB1 contamination in the diet tends to reduce  
189 egg weight, and the addition of 2% zeolite tends to increase egg weight despite the  
190 presence of AFB1 contamination in the feed ( $p = 0.433$ ).

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

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

203

#### 204 **Weights of Carcass, Liver, and Intestine**

205 This experiment showed the treatments have a significant effect ( $p < 0.05$ )  
206 on final body weight. Nevertheless, the treatments did not have significant effects ( $p >$   
207  $0.05$ ) on carcass percentage, the relative weight of giblet, and relative weight of the  
208 liver (Table 4. and Table 5.).

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

214 Although the statistical test did not show any difference in the relative weight of all  
215 variables, it was seen that in AFB1 contaminated diet groups (AFC and AFC+2%  
216 zeolite) there was an enlargement of the liver, with a relative weight of 16.62% and  
217 15.40%, which was heavier than the control (13.54%). These results were also found in

218 the relative weights of the intestines for AFC and AFC+2% zeolite groups, namely  
219 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

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

227 Liver is the target organ of AFB1 because most of the AFB1 absorbed will  
228 undergo bioactivation to form a compound 8,9-epoxide which then binds to protein and  
229 DNA ( Pasha *et al.*, 2007 ). Our study indicated mild acute degeneration of vacuoles in  
230 the liver of ducks received control diet, but this degeneration was severe in AFC diet. In  
231 zeolite groups, mild vacuoles degeneration was found in Control + 2% zeolite and  
232 medium degeneration was in AFC + 2% zeolite (Figure 2).

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

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

248

## 249 **CONCLUSION**

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

253

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258

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353

354

355 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 (kcal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

356 \*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.

357 \*\*AFC= Aflatoxin B1 contaminated diet

358

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

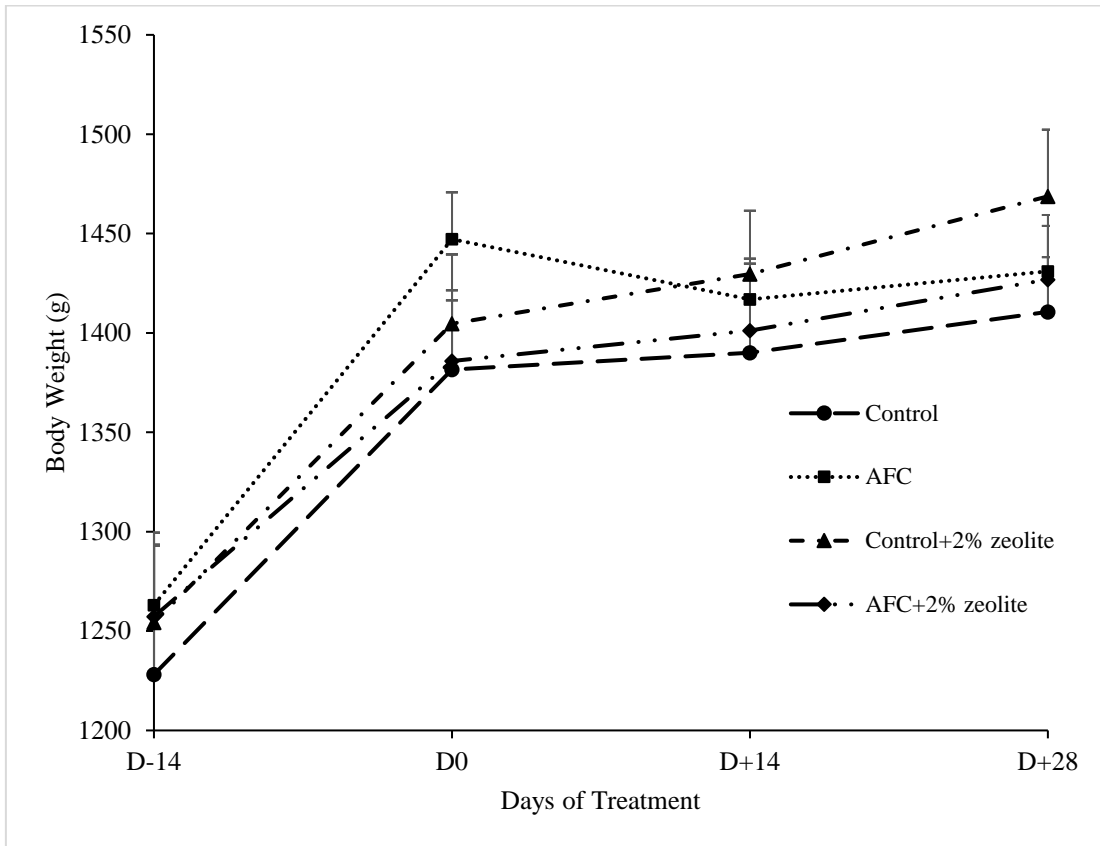
Treatment Diet	Initial (g)	Final (g)	Live weight Change	
			gram	%
Control diet*	1,382±139	1,411±110	29.06±102 <sup>ab</sup>	2.57±7.8 <sup>ab</sup>
AFC**	1,447±94	1,431±92	-16.25±101 <sup>a</sup>	-0.87±7.4 <sup>a</sup>
Control + 2% zeolite	1,405±139	1,469±134	64.06±99 <sup>b</sup>	4.86±7.3 <sup>b</sup>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

360 \*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)

361 \*\*AFC is a diet containing AFB1 at the level of 70 ppb

362 <sup>a, b</sup> means in the same column with different superscripts differ significantly ( $P < 0.05$ )

363



365

366 Figure 1. Live weight curves of laying ducks treated with control diet; AFB1-  
367 contaminated diet (AFC) 70 ppb (P2); Control+2% zeolite; and AFC+2% zeolite  
368

369

370

371 Tabel 3. Effects of Treatment Diet on Egg Production of Laying Duck<sup>ns</sup>

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

372 <sup>ns</sup> means in the same column are not significantly different ( $p > 0.05$ )

373 \*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)

374 \*\*AFC is a diet containing AFB1 at the level of 70 ppb

375

376

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

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

379 <sup>a, b</sup> means in the same column with different superscripts differ significantly ( $p < 0.05$ )380 <sup>ns</sup> means in the same column are not significantly different ( $p > 0.05$ )

381 \*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)

382 \*\*AFC is a diet containing AFB1 at the level of 70 ppb

383

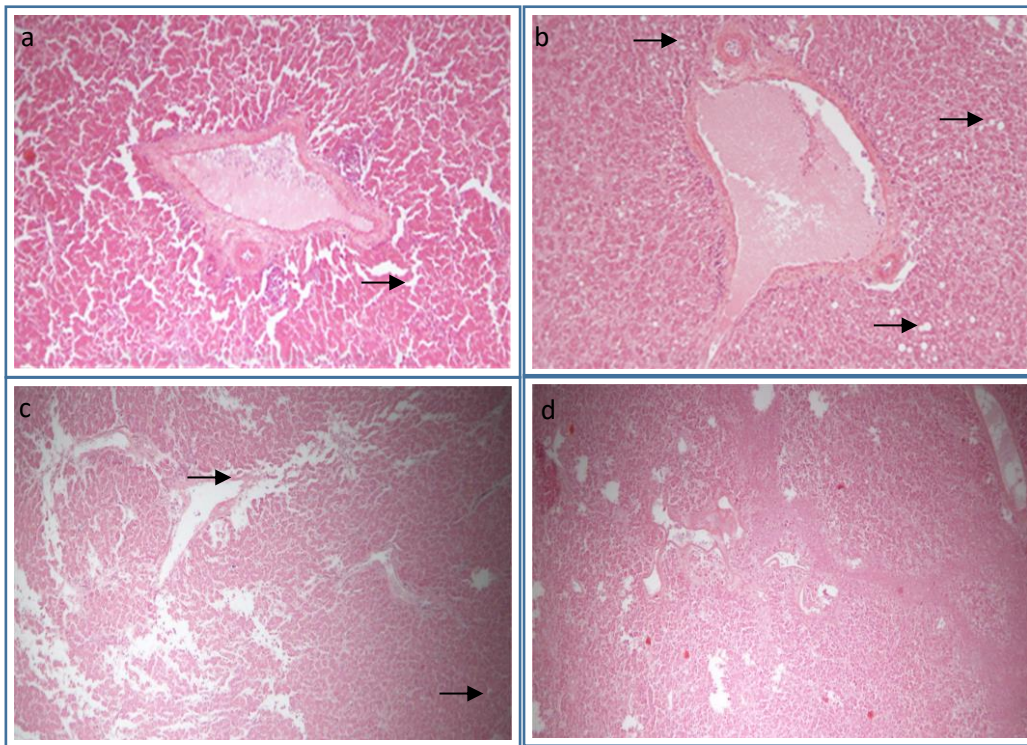
384

385

386 Tabel 5. Effects of Treatment Diet on the Percentages of Carcass, Giblet, and Liver of  
 387 Laying Duck<sup>ns</sup>

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

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



413 Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial  
 414 feed); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d.  
 415 Medium (P4: P2 + 2% zeolite).  
 416

#### 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

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



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Ika Sumantri <isumantri@ulm.ac.id>

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### Manuskrip-cek bhs Inggris

6 messages

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**JITAA JPPT** <jppt.fpundip@gmail.com>  
To: Ika Sumantri <isumantri@ulm.ac.id>


Thu, Aug 1, 2019 at 11:46 AM

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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1  
2 **Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of**  
3 **laying duck**  
4

5  
6  
7 **ABSTRAK**  
8

9 Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben  
10 aflatoksin dalam pakan terkontaminasi aflatoksin B1 (AFB1) terhadap performans itik  
11 petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan  
12 komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2%  
13 zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik  
14 setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina  
15 berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi  
16 menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil  
17 memperlihatkan paparan AFB1 70 ppb menyebabkan penurunan bobot badan sekitar  
18 1,12% ( $P<0,05$ ). Imbuhan zeolit dapat menghindari dampak paparan AFB1 terlihat  
19 dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak  
20 berpengaruh terhadap produksi telur dan bobot telur ( $p>0,05$ ). Paparan AFB1  
21 menghasilkan bobot potong yang lebih rendah ( $P<0,05$ ), namun dengan imbuhan zeolit,  
22 itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang  
23 paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4%  
24 dengan imbuhan zeolit. Disimpulkan bahwa imbuhan zeolit sebesar 2% dapat  
25 mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan  
26 bobot badan.

27  
28 *Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit*  
29

30  
31 **ABSTRACT**  
32

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

47 16.62% and to be 15.4% by zeolite addition in the diet. In conclusion, 2% of zeolite  
48 inclusion could reduce the adverse effects of AFB1 exposure on the performance of  
49 laying duck.

50  
51 *Key words: Aflatoxin B1, laying duck, performance, zeolite*

52  
53

## 54 INTRODUCTION

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

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

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

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

87

## 88 MATERIALS AND METHODS

### 89 Experimental Diet

90 AFB1-contaminated diet (AFC) was produced as follow: ~~commercial feed for~~  
91 ~~laying duck (IP333, PT. Wonokoyo) was used as a production medium. The medium~~  
92 ~~was added with aquadest to reach 30% of moisture content of production medium be~~  
93 ~~30%. The medium was inoculated with *A. flavus* FNCC 612 then ~~it~~ incubated in  
94 temperature 35°C for 10 days. The concentration of AFB1 in the medium was analyzed  
95 by ELISA test to calculate the dilution factor of the medium in the experimental diet.  
96 Indonesian National Standard of Industry (SNI) has established the threshold level of  
97 AFB1 in complete commercial feed, ~~namely~~ that was 20 ppb (Kementan RI, 2009).~~

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

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

108

#### 109 **In Vivo Experiment**

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

116 Dietary treatment was ~~started~~ given when the egg production, Duck Day  
117 Average (DDA), ~~reaches~~ reached 70%. The experiment was carried out for 4  
118 weeks. The experimental diet was provided twice a day and restricted, ~~namely~~ about  
119 150 g/head/day, to ensure the level of AFB1 exposure on the animal ~~is~~ was  
120 controlled. Water was provided *ad libitum*.

121 Egg production was recorded and weighed daily, starting at 15th day until the  
122 28th day of treatment. Body weight of duck was measured individually at two weeks  
123 before treatment (D-14), the beginning of treatment (D0), the second week of treatment  
124 (D14), and the fourth week of treatment (D28). At the end of the experiment (D28),  
125 ducks were ~~sacrificed~~ [slaughtered](#), then carcass and giblet were collected and weighed.  
126 The observed variables were **changes in body weight**, egg production (egg weight and  
127 DDA), **the weight percentages of carcass**, liver, and intestine, and histopathology of the  
128 liver.

**Commented [a1]:** the average daily gain ??

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

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

133

#### 134 **Analysis**

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

**Commented [a3]:** body weight??

141

## 142 **RESULTS AND DISCUSSIONS**

### 143 **Live Weight Changes → [Body weight](#)**

144 Treatments had a significant effect on live weight changes ( $p < 0.05$ ). Table  
145 2, showed that AFB1 exposure at 70 ppb ~~would cause~~ **caused** a decrease in duck weight,  
146 as seen in AFC treatment, which experienced an average weight loss of -0.87% after  
147 four weeks of treatment. This adverse effect of AFB1 exposure on body weight can be  
148 reduced by zeolite inclusion in the diet. This was indicated by the average of live weight  
149 of ducks in AFC + 2% zeolite diet that increased by 3.20%. Zeolite also significantly  
150 improved duck performance, as seen in control diet +2% zeolite, that had the highest  
151 body weight gain, ~~namely~~ **(4.86%)**. This gain was higher than the control ~~feed~~ **ducks**  
152 which only increased by 2.57%.

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

160 Study on the effects of AFB1 on the performance of laying ducks is still ~~very~~  
161 limited. In **1st**-day-old ducklings which received a feed containing AFB1 at levels up to  
162 100 ppb for 21 days, **showed that** an increase in AFB1 level caused a decrease in weight  
163 gain (Wan *et al.*, 2013). Research on chickens showed a decrease in broiler body weight  
164 fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al.*, 2001). Yunus *et*  
165 *al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss,  
166 decreased feed consumption, and increased feed conversion. The percentage of weight  
167 loss reported varies depending on the dose and duration of exposure, such as 5% weight

168 loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15%  
169 weight loss at a dose of 1,000 ppb for 21 days (reference).

170 This study indicated the positive effect of zeolite inclusion in the diet for laying  
171 duck. The addition of 2% zeolite in the control diet resulted in the highest final live  
172 weight (Control + 2% zeolite). By zeolite inclusion, the growth performance of duck  
173 receiving AFB1 contaminated diet (AFC + 2% zeolite) was still higher than those of the  
174 control diet ducks. This finding suggests that the use of zeolite can reduce the impact of  
175 exposure to AFB1 on body weight. Chemically, zeolite is a clay group of  
176 aluminosilicate minerals which has a three-dimensional structure consisting of skeletons  
177 of SiO<sub>4</sub> and AlO<sub>4</sub> which form interconnected channels wherein the channel cavity there  
178 are weak bonds of H<sub>2</sub>O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which  
179 offset the charge negative from AlO<sub>4</sub> (Mallek *et al.*, 2012).

180 In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of  
181 AFB1 exposure (Wan *et al.*, 2013). In broiler, the dietary use of natural or synthetic  
182 zeolites has been reported to improve feed efficiency, thus resulting in better growth  
183 performance of broilers (Mallek *et al.*, 2012). Zeolites (montmorillonites) are a class of  
184 the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals,  
185 bacteria, and toxic antinutritive agents (Fowler *et al.*, 2015; Sulzberger *et al.*,  
186 2017). The binding between aflatoxin and the adsorbent forms an inert and  
187 stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract  
188 (Huwig *et al.*, 2001).

189

190 **Egg Production**

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

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

207

### 208 **Weights of Carcass, Liver, and Intestine**

209 This experiment showed that the treatments have a significant effect ( $p < 0.05$ )  
210 on final body weight. Nevertheless, the treatments did not have significant effects ( $p >$   
211  $0.05$ ) on carcass percentage, the relative weight of giblet, and relative weight of the  
212 liver (Table 4 and Table 5).

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

Commented [a4]: mostly located



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

218 Although the statistical test did not show any difference in the relative weight of all  
219 variables, it was seen that in AFB1 contaminated diet groups (AFC and AFC+2%  
220 zeolite) ~~there was~~ was found an enlargement of the liver, with a relative weight of  
221 16.62% and 15.40%, which was heavier than the control (13.54%). These results were  
222 also found in the relative weights of the intestines for AFC and AFC+2% zeolite groups,  
223 namely 16.93% and 18.2% respectively, which were heavier than the controls (14.19%).

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

231 Liver is the target organ of AFB1 because most of the AFB1 absorbed will  
232 undergo bioactivation to form a compound 8,9-epoxide which then binds to protein and  
233 DNA ( Pasha *et al.*, 2007 ). Our study indicated mild acute degeneration of vacuoles in  
234 the liver of ducks received control diet, but this degeneration was severe in AFC diet. In  
235 zeolite groups, mild vacuoles degeneration was found in Control + 2% zeolite and  
236 medium degeneration was in AFC + 2% zeolite (Figure 2).

237 Hepatic lesions correlated with aflatoxicosis is described as vacuolation of  
238 hepatic cells due to fatty metamorphosis. This metamorphosis is classified as

Commented [a5]: the same  
or  
similar



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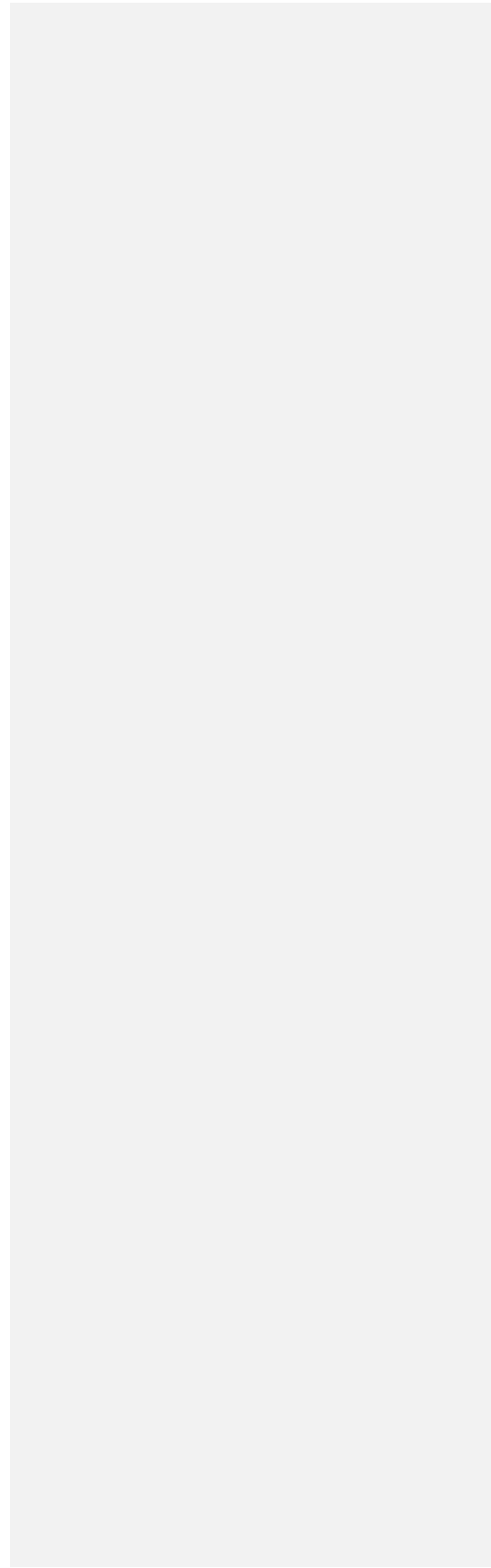
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357



359 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 (kcal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

360 \*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.

361 \*\*AFC= Aflatoxin B1 contaminated diet

362

363 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 <sup>ab</sup>	2.57±7.8 <sup>ab</sup>
AFC**	1,447±94	1,431±92	-16.25±101 <sup>a</sup>	-0.87±7.4 <sup>a</sup>
Control + 2% zeolite	1,405±139	1,469±134	64.06±99 <sup>b</sup>	4.86±7.3 <sup>b</sup>
AFC + 2% zeolite	1,386±142	1,427±130	40.94±66 <sup>ab</sup>	3.20±5.5 <sup>ab</sup>

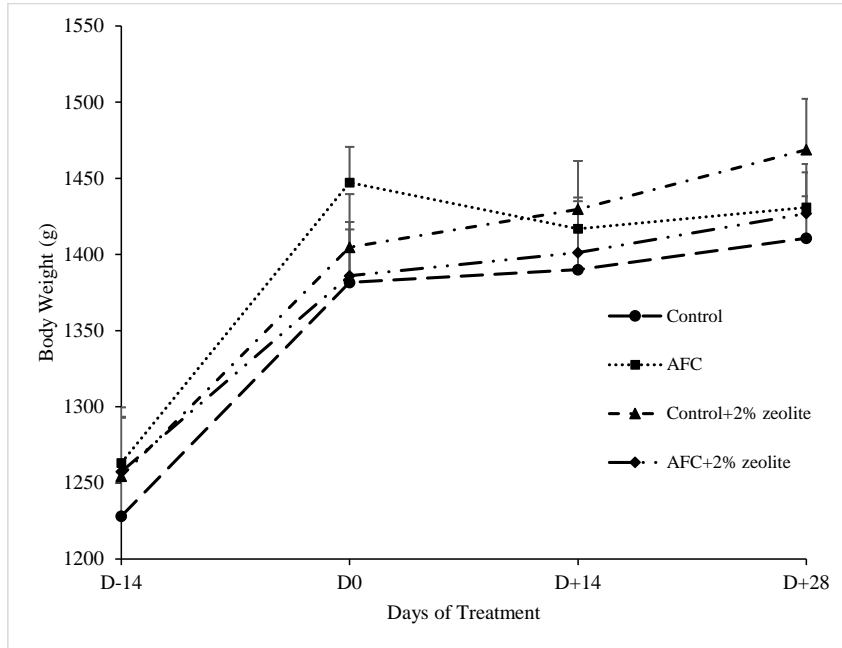
364 \*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)

365 \*\*AFC is a diet containing AFB1 at the level of 70 ppb

366 <sup>a, b</sup> means in the same column with different superscripts differ significantly ( $P < 0.05$ )

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370 Figure 1. Live weight curves of laying ducks treated with control diet; AFB1-  
371 contaminated diet (AFC) 70 ppb (P2); Control+2% zeolite; and AFC+2% zeolite  
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374  
375 Tabel 3. Effects of Treatment Diets on Egg Production of Laying Ducks<sup>ns</sup>

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

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

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

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

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

389



390 Tabel 5. Effects of Treatment Diets on the Percentages of Carcass, Giblet, and Liver of  
 391 Laying Ducks<sup>ns</sup>

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

392 <sup>ns</sup> means in the same column are not significantly different ( $p > 0.05$ )

393 \*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)

394 \*\*AFC is a diet containing AFB1 at the level of 70 ppb

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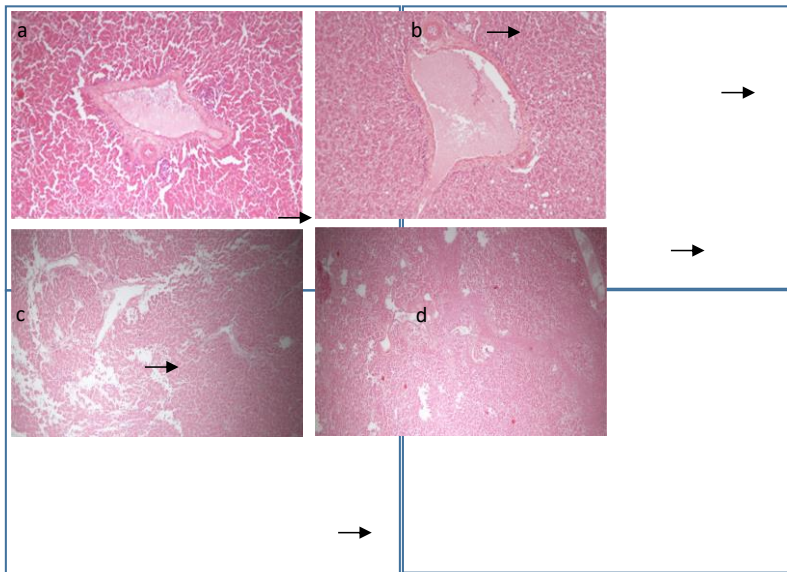
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417 Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: commercial  
 418 feed); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d.  
 419 Medium (P4: P2 + 2% zeolite).

420

## 7. Submission revisi Bahasa Inggris tanggal 3 Agustus 2019

**Ika Sumantri** <isumantri@ulm.ac.id>  
To: JITAA JPPT <jppt.fpundip@gmail.com>

Sat, Aug 3, 2019 at 4:00 PM

Dear JITAA Editors,  
Terlampir file yang sudah diperbaiki bahasa Inggris-nya sesuai saran editor.

Salam,

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



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1  
2 **Effects of zeolite inclusion in aflatoxin B1-contaminated diet on the performance of**  
3 **laying duck**  
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7 **ABSTRAK**  
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9 Penelitian bertujuan mengetahui efektivitas penggunaan zeolit sebagai adsorben  
10 aflatoxin dalam pakan terkontaminasi aflatoxin B1 (AFB1) terhadap performans itik  
11 petelur. Penelitian menggunakan rancangan acak lengkap dengan perlakuan: (1) pakan  
12 komersial (Control); (2) pakan terkontaminasi AFB1 70 ppb (AFC); (3) Control + 2%  
13 zeolit; (4) AFC + 2% zeolit. Setiap perlakuan memiliki 4 ulangan dengan 4 ekor itik  
14 setiap ulangan. Penelitian menggunakan itik Alabio (*Anas platyrinchos* Borneo) betina  
15 berumur 8 bulan. Perlakuan pakan berlangsung selama 28 hari. Data dianalisis variansi  
16 menggunakan prosedur *General Linear Model* software SPSS 21.0. Hasil  
17 memperlihatkan paparan AFB1 70 ppb menyebabkan penurunan bobot badan sekitar  
18 1,12% ( $P<0,05$ ). Imbuan zeolit dapat menghindari dampak paparan AFB1 terlihat  
19 dengan naiknya bobot badan sekitar 2,95% pada pakan AFC+2% zeolit. Perlakuan tidak  
20 berpengaruh terhadap produksi telur dan bobot telur ( $p>0,05$ ). Paparan AFB1  
21 menghasilkan bobot potong yang lebih rendah ( $P<0,05$ ), namun dengan imbuan zeolit,  
22 itik yang menerima pakan terkontaminasi AFB1 menghasilkan bobot potong yang  
23 paling tinggi. Bobot relatif hati itik pada AFC sebesar 16,62% turun menjadi 15,4%  
24 dengan imbuan zeolit. Disimpulkan bahwa imbuan zeolit sebesar 2% dapat  
25 mengurangi dampak paparan AFB1 terhadap kinerja itik petelur, khususnya penurunan  
26 bobot badan.

27  
28 *Kata Kunci: Aflatoksin B1, itik petelur, kinerja produksi, zeolit*  
29

30  
31 **ABSTRACT**  
32

33 The research was objected to study the effect of zeolite inclusion in aflatoxin B1  
34 (AFB1) contaminated diet on the performance of laying duck. A completely randomized  
35 design was adopted in the in vivo experiment that consisted of 4 treatments, namely: (1)  
36 commercial feed (Control); (2) AFB1-contaminated feed 70 ppb (AFC); (3) Control +  
37 2% zeolite; and (4) AFC + 2% zeolite. Each treatment had 4 replications with 4 ducks in  
38 each replication. A total of 64 eight months-female Alabio duck (*Anas platyrinchos*  
39 *Borneo*) was used in 28 days of the feeding experiment. Data were analyzed according  
40 to the general linear model of SPSS 21.0 statistical software. Results indicated that  
41 AFB1 exposure significantly ( $p<0.05$ ) decreased the body weight of laying duck by  
42 1.12%. Zeolite inclusion could prevent the adverse effect of AFB1 on body weight that  
43 increased by 2.95% in AFC+2% zeolite. Treatments had no significant effect on egg  
44 production and egg weight ( $p>0.05$ ). Zeolite inclusion resulted in the highest final body  
45 weight whilst AFB1 diet without zeolite resulted in the lowest final body weight  
46 ( $p<0.05$ ). Relative liver weight of duck fed AFC diet was 16.62% and to be 15.4% by

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

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

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## 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. 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).

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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 (Ca<sup>2+</sup>) that makes zeolites efficiently adsorbing polar molecules such as AFB1 (Di Gregorio *et al.*, 2014).

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

85

## 86 MATERIALS AND METHODS

### 87 **Experimental Diet**

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

98 applied the level of contamination at 70 ppb. The medium then were mixed with  
99 commercial feed based on the dilution factor to obtain AFB1 levels of AFC at 70 ppb.

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

103

#### 104 **In Vivo Experiment**

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

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

115 Egg production was recorded and weighed daily, starting at 15th day until the  
116 28th day of treatment. Body weight of duck was measured individually at two weeks  
117 before treatment (D-14), the beginning of treatment (D0), the second week of treatment  
118 (D14), and the fourth week of treatment (D28). At the end of the experiment (D28),  
119 ducks were slaughtered, then carcass and giblet were collected and weighed. The  
120 observed variables were body weight changes, egg production (egg weight and

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

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

126

### 127 **Analysis**

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

134

## 135 **RESULTS AND DISCUSSIONS**

### 136 **Body Weight**

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

144 gain (4.86%). This gain was higher than the control ducks (P1) which only increased by  
145 2.57%.

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

153 Study on the effects of AFB1 on the performance of laying ducks is still very  
154 limited. In 1st-day-old ducklings which received a feed containing AFB1 at levels up to  
155 100 ppb for 21 days showed that an increase in AFB1 level caused a decrease in weight  
156 gain (Wan *et al.*, 2013). Research on chickens showed a decrease in broiler body weight  
157 fed 200 ppb AFB1 for 8 weeks, from 1,999 g to 1,853 g (Mani *et al.*, 2001). Yunus *et*  
158 *al.* (2011) concluded that in chickens, consumption of aflatoxin caused weight loss,  
159 decreased feed consumption, and increased feed conversion. The percentage of weight  
160 loss reported varies depending on the dose and duration of exposure, such as 5% weight  
161 loss at a dose of 500 ppb; 10% weight loss at a dose of 800 ppb for 28 days; and 15%  
162 weight loss at a dose of 1,000 ppb for 21 days (Yunus *et al.*, 2011).

163 This study indicated the positive effect of zeolite inclusion in the diet for laying  
164 duck. The addition of 2% zeolite in the control diet (P3) resulted in the highest final live  
165 weight. By zeolite inclusion, the growth performance of duck receiving AFB1  
166 contaminated diet (P4) was still higher than those of the control ducks. This finding  
167 suggests that the use of zeolite can reduce the impact of exposure to AFB1 on body



168 weight. Chemically, zeolite is a clay group of aluminosilicate minerals which has a  
169 three-dimensional structure consisting of skeletons of SiO<sub>4</sub> and AlO<sub>4</sub> which form  
170 interconnected channels wherein the channel cavity there are weak bonds of H<sub>2</sub>O  
171 molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge  
172 negative from AlO<sub>4</sub> (Mallek *et al.*, 2012).

173 In ducklings, the use of 0.1% clay adsorbent can reduce the negative impact of  
174 AFB1 exposure (Wan *et al.*, 2013). In broiler, the dietary use of natural or synthetic  
175 zeolites has been reported to improve feed efficiency, thus resulting in better growth  
176 performance of broilers (Mallek *et al.*, 2012). Zeolites (montmorillonites) are a class of  
177 the smectite clay group, which has 3-layer structures that allow adsorbing heavy metals,  
178 bacteria, and toxic antinutritive agents (Fowler *et al.*, 2015; Sulzberger *et al.*,  
179 2017). The binding between aflatoxin and the adsorbent forms an inert and  
180 stable complex, so it will prevent the absorption of aflatoxin in the gastrointestinal tract  
181 (Huwig *et al.*, 2001).

182

### 183 **Egg Production**

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

188 Aflatoxin-contaminated feed causes a decrease in egg production, as shown in  
189 the study of Exarhos and Gentry (1982), namely egg production fell from 85% to 40%  
190 in laying eggs given AFB1 1,000 ppb for 6 weeks. At lower doses, the study of Aly and  
191 Awer (2009) showed that the production and egg weight of white leghorn laying hens

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

200

### 201 **Weights of Carcass, Liver, and Intestine**

202 This experiment showed that the treatments have a significant effect ( $p < 0.05$ )  
203 on final body weight. Nevertheless, the treatments did not have significant effects ( $p >$   
204  $0.05$ ) on carcass percentage, the relative weight of giblet, and relative weight of the  
205 liver (Table 4 and Table 5).

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

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

215 of the intestines for P2 and P4 groups, namely 16.93% and 18.2% respectively, which  
216 were heavier than the controls (14.19%).

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

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

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

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

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

244

245

## CONCLUSION

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

249

250

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254

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351

352

353 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 (kcal/kg)	2800	2800	2800	2800
Aflatoxin B1 (ppb)	0	70	0	70

354 \*The commercial complete feed for laying duck is IP333 produced by PT. Wonokoyo Tbk.

355 \*\*AFC= Aflatoxin B1 contaminated diet

356

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

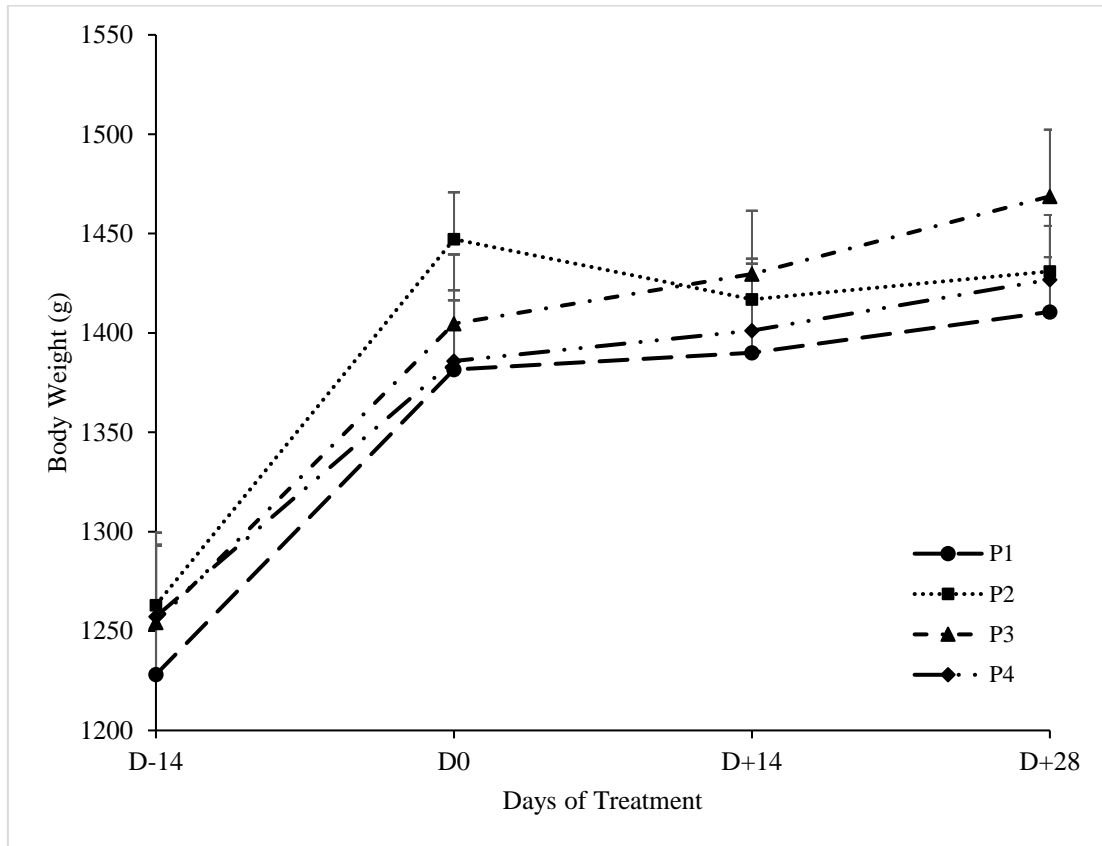
Treatment Diets	Initial weight (g)	Final weight (g)	Body weight Change	
			gram	%
Control diet (P1)*	1,382±139	1,411±110	29.06±102 <sup>ab</sup>	2.57±7.8 <sup>ab</sup>
AFC (P2)**	1,447±94	1,431±92	-16.25±101 <sup>a</sup>	-0.87±7.4 <sup>a</sup>
P1+2% zeolite (P3)	1,405±139	1,469±134	64.06±99 <sup>b</sup>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

358 \*Control diet is a commercial complete feed for laying duck (IP333 PT. Wonokoyo Tbk.)

359 \*\*AFC is a diet containing AFB1 at the level of 70 ppb

360 <sup>a, b</sup> means in the same column with different superscripts differ significantly ( $p < 0.05$ )

361



363

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

366

367



Tabel 3. Effects of Treatment Diets on Egg Production of Laying Ducks<sup>ns</sup>

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

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

373  
374

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

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

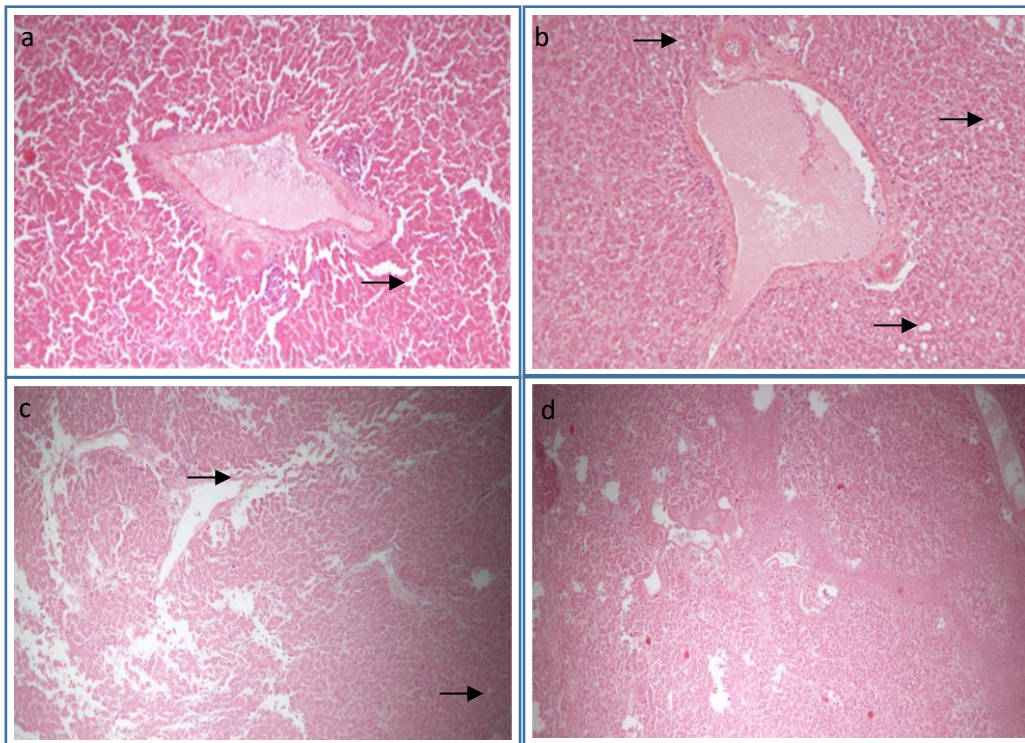
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384 Tabel 5. Effects of Treatment Diets on the Percentages of Carcass, Giblet, and Liver of  
 385 Laying Ducks<sup>ns</sup>

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

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



411 Figure 2. Acute degenerative hepatocyte in liver samples: a. Mild (P1: control diet); b.  
 412 Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium  
 413 (P4: P2 + 2% zeolite).  
 414

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



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Ika Sumantri <isumantri@ulm.ac.id>

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### Proof sheet dan biaya publikasi

5 messages

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**JITAA JPPT** <jppt.fpundip@gmail.com>  
To: Ika Sumantri <isumantri@ulm.ac.id>

Mon, Sep 9, 2019 at 11:21 PM

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.

Bila dalam waktu 5 hari tersebut tidak ada respon Sdr, maka penerbitan artikel kami tunda, atau bahkan kami batalkan.

Salam,  
Pengelola JITAA

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#### 2 attachments

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3537K

## 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 ppb 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 (Ca<sup>2+</sup>) 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°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-Meisner, 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 gilet 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 <sup>ab</sup>	2.57±7.8 <sup>ab</sup>
AFC (P2)**	1,447± 94	1,431± 92	-16.25±101 <sup>a</sup>	-0.87±7.4 <sup>a</sup>
P1+2% zeolite (P3)	1,405±139	1,469±134	64.06± 99 <sup>b</sup>	4.86±7.3 <sup>b</sup>
P2+2% zeolite (P4)	1,386±142	1,427±130	40.94± 66 <sup>ab</sup>	3.20±5.5 <sup>ab</sup>

\*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

<sup>a, b</sup> means in the same column with different superscripts differ significantly (P<0.05)

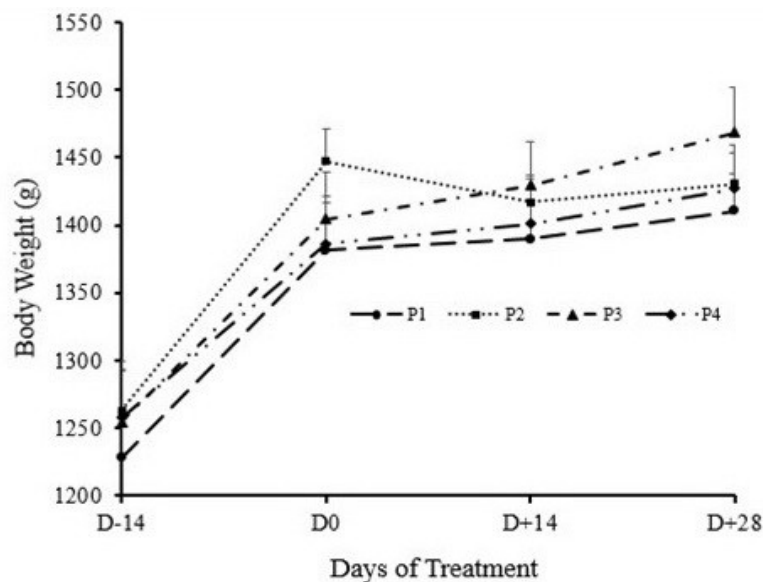


Figure 1. Live Weight Curves of Laying Ducks Treated with Control Diet (P1); AFB1-contaminated Diet 70 ppb (P2); P1+2% zeolite (P3); and P2+2% zeolite (P4)

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<sub>4</sub> and AlO<sub>4</sub> which form interconnected channels wherein the channel cavity there are weak bonds of H<sub>2</sub>O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO<sub>4</sub> (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

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 <sup>ab</sup>	833± 29	352±123	43.8± 8.4
AFC (P2)**	1,426±55 <sup>a</sup>	847±128	289±105	47.5±15.9
P1+2% zeolite (P3)	1,406±90 <sup>a</sup>	835±121	300± 80	43.0± 9.6
P2+2% zeolite (P4)	1,576±104 <sup>b</sup>	951± 76	324± 33	49.5± 5.1

<sup>a, b</sup> 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

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

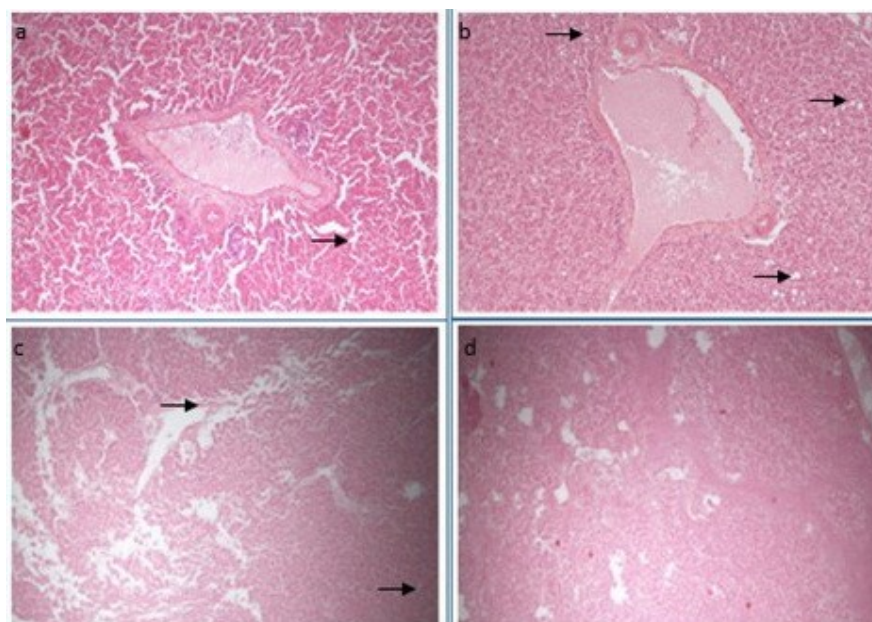


Figure 2. Acute Degenerative Hepatocyte in Liver Samples: a. Mild (P1: control diet); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

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. Pengembalian proof sheet check naskah tanggal 12 September 2019

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**2 attachments**

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No.	Halaman	Kolom	Baris	Tertulis	Seharusnya
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 ppb 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 (Ca<sup>2+</sup>) 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°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-Meisner, 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 (kcal/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 <sup>ab</sup>	2.57±7.8 <sup>ab</sup>
AFC (P2)**	1,447± 94	1,431± 92	-16.25±101 <sup>a</sup>	-0.87±7.4 <sup>a</sup>
P1+2% zeolite (P3)	1,405±139	1,469±134	64.06± 99 <sup>b</sup>	4.86±7.3 <sup>b</sup>
P2+2% zeolite (P4)	1,386±142	1,427±130	40.94± 66 <sup>ab</sup>	3.20±5.5 <sup>ab</sup>

\*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

<sup>a, b</sup> means in the same column with different superscripts differ significantly (P<0.05)

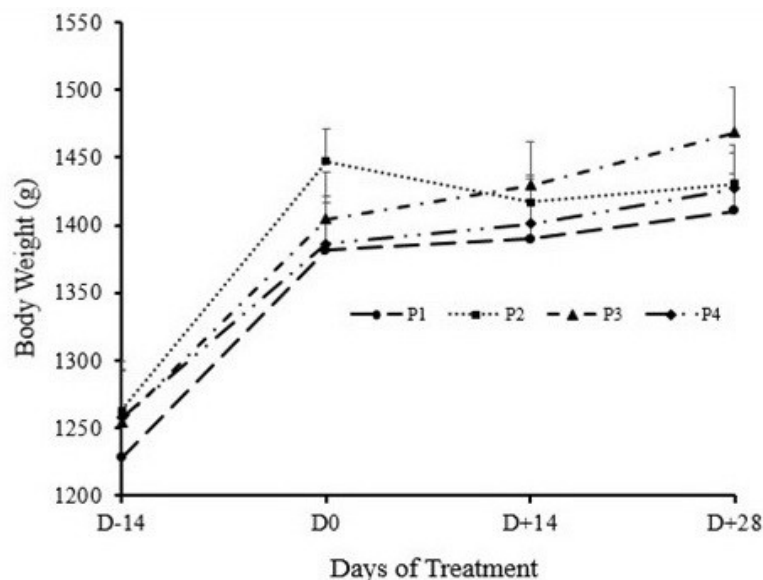


Figure 1. Live Weight Curves of Laying Ducks Treated with Control Diet (P1); AFB1-contaminated Diet 70 ppb (P2); P1+2% zeolite (P3); and P2+2% zeolite (P4)

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<sub>4</sub> and AlO<sub>4</sub> which form interconnected channels wherein the channel cavity there are weak bonds of H<sub>2</sub>O molecules and alkali cations (Na, K, Li, Ca, Mg, Ba, Sr) which offset the charge negative from AlO<sub>4</sub> (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 <sup>ab</sup>	833± 29	352±123	43.8± 8.4
AFC (P2)**	1,426±55 <sup>a</sup>	847±128	289±105	47.5±15.9
P1+2% zeolite (P3)	1,406±90 <sup>a</sup>	835±121	300± 80	43.0± 9.6
P2+2% zeolite (P4)	1,576±104 <sup>b</sup>	951± 76	324± 33	49.5± 5.1

<sup>a, b</sup> 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



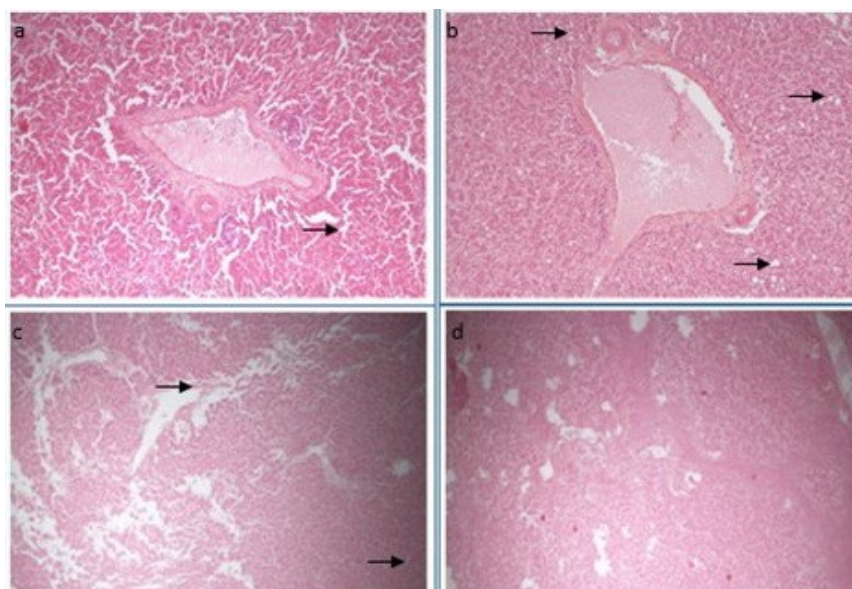


Figure 2. Acute Degenerative Hepatocyte in Liver Samples: a. Mild (P1: control diet); b. Severe (P2: P1 containing AFB1 70 ppb); c. Mild (P3: P1 + 2% zeolite); d. Medium (P4: P2 + 2% zeolite).

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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11. Pemberitahuan naskah telah diterbitkan pada 29 September 2019



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