Effects of zeolite in aflatoxin B1 contaminated diet on aflatoxin residues and liver histopathology of laying duck

To cite this article: I Sumantri et al 2018 IOP Conf. Ser.: Earth Environ. Sci. 207 012017

View the article online for updates and enhancements.
Effects of zeolite in aflatoxin B1 contaminated diet on aflatoxin residues and liver histopathology of laying duck

I Sumantri¹, H Herliani¹, M Yuliani² and N Nuryono³

¹Department of Animal Science, Faculty of Agriculture, University of Lambung Mangkurat, Banjarbaru-70714, Indonesia
²Banjarbaru Veterinary Office, Ambulung No. 24, Banjarbaru-70712, Indonesia
³Faculty of Mathematics and Natural Science, Universitas Gadjah Mada, Yogyakarta-55281, Indonesia

E-mail: isumantri@ulm.ac.id

Abstract. This research was conducted to study the effects of zeolite in reducing aflatoxin residues and liver histopathology of laying duck. Sixty-four Indonesian local laying duck (Anas platyrinchos Borneo) were randomly allocated to 2 levels of aflatoxin B1 (AFB1) (low: 30 ppb; and high: 70 ppb) and 2 levels of zeolite inclusions (0 and 2%). The trial was conducted for 28 days and at the end of treatment, the ducks were sacrificed. Meat, liver, and egg samples were collected for AFB1 and aflatoxin M1 (AFM1) determinations. AFB1 and AFM1 concentrations were determined using ELISA analysis. Data were analyzed by analysis of variance using the general linear model of SPSS software. Liver samples were also analyzed for the histopathological study. Results showed that levels of AFB1 significantly (P<0.05) increase AFB1 concentration in the liver and egg. Zeolite inclusion did not significantly (P>0.05) reduce AFB1 and AFM1 concentrations in meat, liver, and egg. Examination of liver samples indicated moderate and severe liver pathology in the diet without zeolite. Therefore, it was concluded that zeolite inclusion in the high AFB1 contaminated diet does not reduce aflatoxins residue in the tissues but could prevent liver alteration of laying duck.

1. Introduction
Aflatoxin B1 (AFB1) is a toxic and carcinogenic substance produced by mainly toxigenic strains of Aspergillus flavus and A. parasiticus [1]. Factors of climate, the composition of the commodity, agronomic practices, harvesting, handling, and storage contribute on fungi to grow and produce mycotoxin [2]. Previous studies indicated a high occurrence of AFB1 in feed and feedstuffs collected in Indonesia [3-5].

Ingestion of AFB1-contaminated diet by animals will not only impact on their health and production but will also result in the excretion of AFB1 residues in the tissues, milk, and egg [6]. Duck is one of the sensitive animals to aflatoxin exposure that related to its liver biotransformation capacity [7]. Therefore, consumption of AFB1 contaminated diet will not only lead to a decrease in duck performance but potentially present aflatoxin residues in duck’s tissues and egg.

Nowadays, the use of adsorbents, such as zeolite, in the feed is widely recommended to inhibit aflatoxin absorption in the gastrointestinal tract. This approach is more effective and applicable due to
some reasons, such as relatively inexpensive, generally recognized as safe, and can be easily formulated in ration [8]. This research was objected to studying the efficacy of zeolite inclusion in AFB1 contaminated diet in reducing the adverse effects of AFB1 on laying duck.

2. Material and Method

2.1. AFB1 Contaminated Diet Production
AFB1-contaminated feed (CF) was produced by inoculation of Aspergillus flavus FNCC in a ground maize [9]. CF was estimated containing AFB1 at the level of 500 ppb. Then, CF was mixed into commercial feed to result in 2 levels of AFB1 in diet, namely low (30 ppb) and high (70 ppb).

2.2. Experimental Method
A total of 64 Alabio laying ducks (Anas platyrinchos Borneo) were used in the experiment. Ducks were weighed and randomly allocated to experimental units that consisted of 4 dietary treatments with 4 replications and 4 birds in each replication. The diets treatments were: AFB1 30 ppb+0% zeolite; AFB1 70 ppb+0% zeolite; AFB1 30 ppb +2% zeolite; and AFB1 70 ppb +2% zeolite. Experimental diet was provided restricted (150 g/d/bird) to ensure the amount AFB1 intake, whereas drinking water was provided ad libitum. The experiment was started when the ducks were aged 7 months with hen day average more than 60%. The experimental diet was conducted for 28 days. At the end of the experiment, ducks were sacrificed. Liver, thigh meat, and egg were collected for AFB1 and AFM1 content analyses. Liver samples were also examined for liver histopathology observation.

2.3. Histopathology Study
Representative liver samples were fixed in 10% buffered neutral formalin for histopathological study. Sections were cut at 5-micron thickness and stained by the hematoxylin and eosin method of Harris according to Manual Standard of Patologi Diagnose of Veterinary Laboratory.

2.4. Aflatoxin and Data Analysis
Aflatoxins contents of samples were determined using ELISA methods. ELISA kits used in the analysis were ELISA kit AgraQuant® Aflatoxin B1 (Romer Labs. Singapore) for AFB1 analysis and ELISA kit AgraQuant® Aflatoxin M1 Sensitive 25/50 (Romer Labs. Singapore) for AFM1 analysis. Data were analyzed using analysis of variance according to a completely randomized design. All statistical analysis was performed using software package SPSS version 18.0.

3. Results and Discussion

3.1. Aflatoxin B1 Residue
Results showed levels of AFB1 or zeolite have no significant effects on AFB1 residue in the liver and egg. However, zeolite 2% in the diet significantly reduced AFB1 levels in meat (Table 1.).

Table 1. The concentration of AFB1 residue in the liver, meat, and egg of laying duck (ppb).

| Treatment                  | Liver | Meat | Egg |
|---------------------------|-------|------|-----|
| AFB1 30 ppb+0% Zeolite    | 1.48  | 0.57a| 1.37|
| AFB1 70 ppb+0% Zeolite    | 2.37  | 1.29b| 1.91|
| AFB1 30 ppb+2% Zeolite    | 1.72  | 0.69a| 1.49|
| AFB1 70 ppb+2% Zeolite    | 2.18  | 0.45a| 1.35|

NS: Not Significant ($P > 0.05$)

a, b Means in each column with different superscripts are significantly different ($P < 0.05$)
AFB1 residues in meat, liver, and egg were detected in very low levels or almost in not detected levels for ELISA analysis. This study offered a low level of AFB1 contamination in experimental diet compare to many aflatoxin studies in poultry, such as 660-3000 ppb [10] or 1000-2000 ppb in broiler [11]. AFB1 residue in the animal product is a dose-dependent, thus low aflatoxin intake from feed will result in low AFB1 residue in the tissue, milk or egg [6].

Several studies showed the carry-over ratio of AFB1-feed into aflatoxin residues in the egg is very low [6]. In young laying hens, it was calculated to be 0.02% or almost not detected. However, AFB1 carry-over ratio is very high in the liver of poultry [12]. Study of [13] showed that after three weeks of trial, AFB1 levels in the liver of broiler fed different levels of AFB1 are similar. There is no difference of AFB1 levels between treatment in this study supports findings that hepatic metabolism of aflatoxin B1 become more efficient after three weeks of exposure and residue no longer accumulated in the liver.

Many studies have been conducted to study the use of adsorbents in the feed that will bind the aflatoxins and prevent its absorption in the gastrointestinal tract [14]. Several adsorbents, such as zeolite, bentonite, and synthetic aluminosilicates have the capability to bind aflatoxin by chemisorption mechanism that reduces aflatoxin bioavailability in the gastrointestinal tract. However, the efficacy of aflatoxin adsorbent has not always been demonstrated in an in vivo experiment.

3.2. Aflatoxin M1 Residue

Studies on AFM1 residue in duck’s tissues and egg are very limited. AFM1 was not detected (< 0.01 ppb) in the egg of laying hens fed with a diet containing 2,500 ppb AFB1 for four weeks [15]. Similarly, negative detection of AFM1 in the liver also resulted in that experiment, confirmed that only small quantities of aflatoxins are likely to be stored in the hen tissues. This study showed that AFB1 and zeolite levels have no significant effects on the levels of AFM1 in the liver, meat, and egg of laying duck. Our result confirmed that the highest level of AFM1 was found in the liver and the lowest was in the egg. And it was interesting, that AFM1 levels found in the liver of this study reach more than 100 ppt. The liver is considered the target organ for AFB1 because it is the organ where most aflatoxins are bioactivated to the reactive 8,9-epoxide form, which is known to bind DNA and proteins, damaging the liver structures and increasing liver weight [14].

| Treatment                | Liver | Meat | Egg |
|--------------------------|-------|------|-----|
| AFB1 30 ppb+0% Zeolite   | 125.20| 88.07| 51.33|
| AFB1 70 ppb+0% Zeolite   | 129.15| 90.77| 47.81|
| AFB1 30 ppb+2% Zeolite   | 101.86| 78.21| 67.49|
| AFB1 70 ppb+2% Zeolite   | 140.85| 95.79| 41.06|

AFM1 is a hydroxylated metabolite of AFB1 in the liver or tissue cells which can be excreted by the animal through urine, faeces, milk and egg [6, 16]. This study indicated zeolite inclusion in the diet has no significant effect in reducing AFM1 levels in laying duck tissues and egg. However, this study confirmed that AFM1 levels will be found at the highest level in the liver and the lowest level is in the egg.

3.3. Liver Histopathology

Observation of liver histopathology indicated mild acute degeneration of vacuoles in the liver of ducks received low-level of AFB1 but this degeneration was severe in high-level of AFB1. In zeolite groups, mild vacuoles degeneration was found in low-level AFB1 and medium degeneration was in high-level of AFB1 (Figure 1). Hepatic lesions correlated with aflatoxicosis is described as a vacuolation of hepatic cells due to fatty metamorphosis. This metamorphosis is classified as degenerative changes of the liver [17].
Adsorbent inclusion in the diet has a protective effect against aflatoxin exposure. This experiment showed zeolite inclusion seems to reduce the adverse effects of AFB1 exposure as indicated in the result of liver histopathology study of the zeolite group.

Study of [18] found that in low levels of AFB1 (50 to 100 ppb), all livers samples showed histopathological alterations, with an accumulation of fat vacuoles, except the normal appearance of livers from broiler received bentonite in the diet. Study of [19] suggested that ducks are a very sensitive species for aflatoxin injury and it would appear that they are also prone to develop hepatic tumours. The time taken for the tumour induction was about 90 days after oral exposure of AFB1 and histopathologically they were categorized as hepatocellular carcinoma, cholangiocellular carcinoma, and chronic hepatitis.

![Figure 1. Acute degenerative hepatocyte in liver samples: a. Mild (AFB1 30 ppb+0% zeolite); b. Severe (AFB1 70 ppb+0% zeolite); c. Mild (AFB1 30 ppb+2% zeolite); d. Medium (AFB1 70 ppb+2% zeolite).](image)

4. Conclusion
This study concluded that zeolite inclusion in AFB1 contaminated could not reduce aflatoxin residues in the tissues and egg but could prevent liver alteration of laying duck.

5. Acknowledgement
Authors thank the Indonesian Ministry of Research and Higher Education who supports this research through PTUPT Research Grant Project No. 119/UN8.2/PL/2017.
6. References

[1] International Agency for Research on Cancer (IARC) 2002 Monographs on the evaluation of carcinogenic risks to humans Number 82 *Some traditional herbal medicines, some mycotoxins, naphthalene and styrene* (Lyon: IARC) pp 171–300

[2] Bryden W L 2012 Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security *Animal Feed Science and Technology* **173** 134 – 158

[3] Sumantri I, Agus A, Irawan B, Habibah, Faizah N and Wulandari K J 2017 Aflatoxins contamination in feed and products of alabio duck (*Anas platyrinchos borneo*) collected from South Kalimantan, Indonesia *Bulletin of Animal Science* **41(2)** 163-168

[4] Agus A, Sumantri I, Murti T W and Boehm J 2013 Survey on the Occurrence of Aflatoxin B1 Contamination in Dairy Ration and Its Carry Over into the Milk in Yogyakarta and Central Java Provinces of Indonesia *Book of Abstract ISM-MycoRed International Conference* (Apulia: International Society of Mycotoxin)

[5] Rodrigues I and Nachrer K 2012 A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed *Toxins* **4** 663-675

[6] Voelkel I, Schroer-Merker E and Czerny C P 2011 The carry-over of mycotoxins in products of animal origin with special regards to its implications for the European Food Safety Legislation *Food and Nutrition Science* **2** 852-867

[7] Diaz G J and Murcia H W 2011 Biotransformation of aflatoxin B1and its relationship with the differential toxicological response to aflatoxin in commercial poultry species *Aflatoxin – Biochemistry and Molecular*, ed R G Guevara-Gonzalez (Rijeka: Intech Pub) p 3

[8] Kutz R E, Sampson J D, Pompeu L D, Ledoux D R, Spain J N, Vazquez-Anon M and Rottinghaus G E 2009 Efficacy of Solis, NovasilPlus, and MTB-100 to reduce aflatoxin M1 levels in milk of early to mid lactation cows fed aflatoxin B1 *Journal of Dairy Science* **92** 3959 – 3963

[9] Agus A, Maryudhani Y B, Yunianta, Wedhashri S and Nuryono 2010 Production of crude Aflatoxin B1 using different isolates and substrates *Book of Abstracts ISM International Conference* (Lyngby: International Society of Mycotoxin) p 130

[10] Galarza-Seeber R, Latorre J D, Bielke L R, Kuttappan V A, Wolfenden A D, Hernandez-Velasco X, Merino-Guzman R, Vicente J L, Donoghue A, Cross D, Hargis B M and Tellez G 2016 Leaky gut in mycotoxins: Aflatoxin B1 does not increase gut permeability in broiler chickens *Frontiers in Veterinary Science* **3** 1-10

[11] Diaz G J and Sugahara M 1995 Individual and combined effects of aflatoxin and gizzardosine in broiler chickens *British Poultry Science* **36 (5)** 729-736

[12] Bintvihok A, Thiengnin S, Doi K and Kumagi S 2002 Residues of aflatoxins in the liver, muscle and eggs of domestic fowls *Journal of Veterinary Medicine Science* **64 (11)** 1037-1039

[13] Fowler J, Li W and Bailey C 2015 Effects of a calcium bentonite clay in diets containing aflatoxin when measuring liver residues of aflatoxin B1 in starter broiler chicks *Toxins* **7** 3455-3464

[14] Denli M, Blandon J C, Guynot M E, Salado S and Perez J F 2009 Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1 *Poultry Science* **88** 1444–1451

[15] Zaghini A, Martelli G, Roncada P, Simioli M and Rizzi L 2005 Mannanoligosaccharides and aflatoxin B1 in feed for laying hens: effects on egg quality, aflatoxins B1 and M1 residues in eggs, and aflatoxin B1levels in liver *Poultry Science* **84** 825-832

[16] Iqbal S Z, Jinap S, Pirouz A A and Faizal A R A 2015 Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review *Trends in Food Science and Technology* **46** 110-119

[17] Espada Y, Domingo M, Gomez J and Calvo M A 1992 Pathological lesions following an experimental intoxication with aflatoxin B1 in broiler chickens *Research in Veterinary Science* **53** 275-279
[18] Magnoli A P, Monge M P, Miazzo R D, Cavaglieri L R, Magnoli C E, Merkis C I, Cristofolini A L, Dalcero A M and Chiacchiera S M 2011 Effect of low levels of aflatoxin B1 on performance, biochemical parameters, and aflatoxin B1 in broiler liver tissues in the presence of monensin and sodium bentonite Poultry Science 90 48–58

[19] Leenadive T, Valsala K V and Rajan A 1995 Aflatoxin induced hepatocarcinogenesis in ducks Mycotoxin Research 11 2-8