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

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

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Abstract. This research was conducted to study the effects of zeolite in reducing aflatoxin residues and liver histopathology of laying duck. Sixty-four of 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^{NS}	Meat	Egg^{NS}
AFB1 30 ppb+0% Zeolite	1.48	0.57^{a}	1.37
AFB1 70 ppb+0% Zeolite	2.37	1.29^{b}	1.91
AFB1 30 ppb+ 2% Zeolite	1.72	0.69^{a}	1.49
AFB1 70 ppb+ 2% Zeolite	2.18	0.45^{a}	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 AFB_1 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].

Table 2. The concentration of AFM1 residue in the liver, meat, and egg of laying duck (ppt).

Treatment	Liver^{NS}	Meat^{NS}	Egg^{NS}
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

NS: Not Significant ($P > 0.05$)

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.

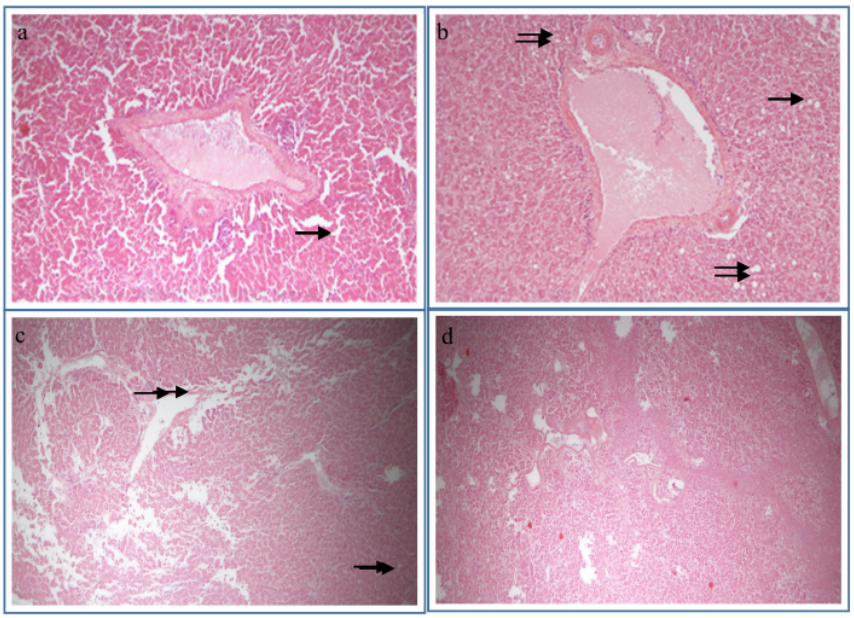

Figure 1. Acute degenerative hepatocyte in liver samples: a. Mild (AFB1 30 ppb+0% zeolite); b. Severe (AFB1 70 ppb+0% zeolite); c. Mild (AFB1 30 ppb+2% zeolite); d. Medium (AFB1 70 ppb+2% zeolite).

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

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