International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Effect of Sepiolite and Tomato Powder on Serum Biochemical Profile of Broiler Exposed to Aflatoxin

Nur Wulan Purnama[1*], Kemilau Bunga Nusantara[1], Eko Widodo[2], Osfar Sjofjan[2]
[1]Student, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia-65145
[2]Lecturer, Faculty of Animal Science, Brawijaya University, Malang, East Java, Indonesia-65145
*Corresponding author: eko.widodo@ub.ac.id

***Abstract*—** *The purpose of this research was to evaluate the effect of adding combination of sepiolite as aflatoxin binder and tomato powder (TP) as antioxidant source in aflatoxin contaminated feed on serum biochemical profile of broiler chicken. The method was in vivo experiment set in Completely Randomized Design. One hundred and twelve unsexed day old broiler chicks of Lohmann strain randomly distributed into 4 treatments and 4 replications. The treatments were consist of T0= positive control (corn contaminated 10 ppb aflatoxin), T1=negative control (corn contaminated 102.5 ppb aflatoxin), T2=T1+0.25% sepiolite, and T3=T1+0.25% sepiolite+1.5%tomato powder. The measured variables were: blood lipid profile (cholesterol and triglycerides), kidney physiology indicators (creatinine and blood urea nitrogen), and blood glucose. The data were analyzed with ANOVA and continued with Duncan's multiple range test. The result showed that addition of sepiolite and tomato powder significantly increase the triglycerides level (P<0.05), but ithad no influence on total serum cholesterol, creatinine, blood urea nitrogen, and glucose. Conclusion of this research was addition of sepiolite and tomato powder in feed with mild aflatoxin contamination could not improve the serum biochemical profiles, except triglyceride, in broiler chicken.*

***Keywords*—** *Sepiolite, tomato powder, blood serum, broiler, aflatoxin.*

I. INTRODUCTION

Mycotoxin contamination in poultry, especially in broiler feed is a persistent problem in several Asian countries which still need attention for effective and efficient problem solving. Mycotoxin is a secondary metabolites product of filamentous molds which grow on cereal crops, particularly corn, and could cause a reduction in feed intake and even kill the poultry. There are several important mycotoxin types includeaflatoxin, ochratoxin, T-2 toxin, fumonisin, zearalenone, and deoxinivalenol. Among those types, aflatoxin is the most often found to contaminate feed [1]. Clinical signs due to aflatoxin poisoning are such as decrease feed consumption and body weight, increase mortality, cause damages to liver, kidney, and gizzard [2].

Aflatoxin contamination in local corn in Indonesia was between 59 ppb and the highest was 236 ppb. These facts exceed the threshold set by Indonesian standard of maximum aflatoxin contamination in corn as 50 ppb. This is mainly due to the tropical climate is an ideal conditions for the growth of mold and fungi. Besides that, the conventional post harvest handling, inadequate storage and transportation have also been reported to increase aflatoxin contamination in corn as the main feed raw material for poultry [3].

A strategy to prevent and eliminate the negative impact of aflatoxin that could controlled by feed mills or farmers was by using aflatoxin binder. Aflatoxin binder is an ingredient added to feed which does not affect the nutritional value, but able to binding aflatoxin in gastrointestinal tract and then is excreted into the feces. Sepiolite is a magnesium silicate complex which has large surface area, so it has high capacity in binding aflatoxin. Addition 1.0% sepiolite improve growth performance and decrease blood cholesterol and triglyceride in broiler [4].

In early 2018 Indonesian government has banned the use of antibiotic growth promoter (AGP) in poultry. Combining aflatoxin binder with other feed additive such as phytobiotics rich in antioxidants might show a more beneficial effect. Tomato is a potential antioxidant source. Tomato powder contains lycopene as major phytochemical content which could protect cells and tissues from oxidative damage caused by reactive oxygen species. Lycopene also plays a role in prevention of cancer and heart disease, improve immune system, and metabolic pathways. Tomato is a source of various micro and macro minerals such as iron 3.99 mg, phosphor 173 mg, calcium 80 mg, magnesium 126 mg, zinc 2.71 mg, copper 0.876 mg, manganese 1.83 mg, sodium 121.60 mg and potassium 2805.8 mg per 100 g [5].

Studies of blood constituent play a vital role in assessing the physiological, pathological, and nutritional status of poultry. It provides the presence of several metabolites and other constituents in the body. Changes in the constituent compounds of blood when compared to normal values could serve as a reflector of the metabolic stage of an animal as well as status of nutrition[6]. Considering the benefits of tomato powder and sepiolite, as well as the negative effects of aflatoxin in feed, it is necessary to investigate the effects of adding combination of sepiolite and tomato powder to serum biochemical profile of broiler chicken exposed to aflatoxin containing feed.

II. MATERIALS AND METHODS

A. Birds and Dietary Treatments

One hundred and twelve unsexed day old chicken of Lohmann broiler strain with initial body weights 39.93±3.09 g were randomly allocated into 4 treatments, 7 birds per treatments, and each was replicated 4 times. Each experimental unit had an area of 1 $m^2$of having litter covered floor. The brooding period was carried out for 14 days by adjusting surrounding temperatures of 32-34^{0}Cby providing

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International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

gasolec heater. The lighting used 10 watt LED lamp provided between 17.00 p.m. -06.00 a.m, except during brooding period. Feed and water was given *ad libitum*.

The treatments which were used in this experiment included:

T0= positive control (basal feed with normal corn)
T1= negative control (basal feed with moldy corn)
T2= negative control + sepiolite 0.25%
T3= negative control + sepiolite 0.25% + tomato powder 1.5%

Normal corn and moldy corn had different nutritional qualities which described in the following table:

TABLE 1. Nutritional values of corn as raw material

Corn	Normal	Moldy
Moisture (%)	13,81	18,50
Crude protein (%)	8,92	8,11
Fat (%)	2,01	2,74
Crude Fiber (%)	2,43	1,80
Ash (%)	1,19	1,07
Aflatoxin (ppb)	10	102,5
ME (Kcal/kg)	3144,73	2876,01

Tomato powder was obtained from UPT Materia Medica, Batu, Indonesia had nutritional content of : moisture 8.14%, crude protein 6.85%, crude fat 4.27%, crude fiber 15.50%, ash 0.73%, carotenoid total 180.04 µg/g, and estimated lycopene 140 µg/g. Sepiolite was obtained from PT. Anpario Biotechnology Indonesia trade name *sorbasafe* contain 100% sepiolite, the recommended dose for chicken is 0.25%.

The formulation and nutrient contents of basal diet explained in the following table:

TABLE 2. Formulation and nutritional content of basal diets

Raw Materials (%)	Starter (1-14 days old)	Grower-finisher (15-42 days old)
Corn	55	57
Rice bran	8	9.5
Palm oil	2.5	3.5
SBM	27	24
MBM	6	4.5
Premix	1.4	1,4
DL-methionine	0.1	0.1
Nutritional content		
Moisture	12.34	12.20
Crude protein	22.39	19.38
Crude fat	5.94	7.37
Crude fiber	3.86	3.99
Ash	6.87	6.44
Calcium	1.16	0.96
Phosphor	0.74	0.68
Aflatoxin of T0 (ppb)*	5.5	5.7
Aflatoxin of T1-T3 (ppb)	56.37	58.42

*****maximum levels of aflatoxin contamination in broiler feed set by Indonesian standard: 40 ppb (starter phase), 50 ppb (grower finisher phase)

Analysis of feed nutrient contents used near infrared spectroscopy. Analysis aflatoxin used ultraviolet box aflatoxin detector. A 200 g ground corn sample placed in dark box 20x20x20 cm. Corn was highlighted by the use of an ultraviolet flashlight in a dark room. Part of corn which shows a purplish green glow counted. The present of 1 whole corn seed is equivalent to 15 ppb, 1 smaller seed with the size of a mungbean is equal to 5 ppb.

B. Measured Variables

Variables measured included cholesterol, triglycerides, blood urea nitrogen, creatinine, and glucose. At 38 days of age, 16 birds were randomly selected from each group (1 bird per replication). Blood samples were taken using 2 ml injection syringe through the brachial vein. Each sample placed in yellow vacutainer (serum separator tube) containing silica as activator to separate serum from blood plasma. After that, samples were centrifuged 5000 rpm for 10 minutes. The serumobtained were analyzed by using Pentra C200 automatic biochemical analyzer.

C. Statistical Analysis

The data obtained were tabulated in Microsoft Excel for one way analysis of variance. If there are significant differences between the treatments followed by Duncan's multiple range test (DMRT).

III. Results and Discussion

A. Blood Lipid Profile

Data describing the effect of adding tomato powder and sepiolite onblood lipid profile of broiler chicken presented in table 3.

TABLE 3. Blood lipid profile of broiler chicken with different treatments of sepiolite and tomato powder addition

Treatments	Cholesterol (mg/dL)	Triglycerides (mg/dL)
T1	175.00±13.54	92.00±24.43^{a}
T2	141.75±27.94	82.25±24.96^{a}
T3	162.75±50.28	137.75±18.39^{b}
T4	184.75±7.72	113.50±21.27^{ab}

With the exception of triglycerides, no significant different were observed for all the biochemical profiles measured in this study. The triglycerides levels obtained in this study were significantly different ($P<0.05$) among the treatments group. Addition of 0.25% sepiolite (T2) with or without combination with 1.5% tomato powder (T3) showed higher triglycerides level compared to positive and negative controls (T0 and T1). Lower level of triglycerides in T1 had a positive correlation with lower feed consumption caused by musty odor in moldy corn (data were not shown). So, nutritional deficiencies could occur and might decrease the triglycerides level in blood. Triglycerides are synthesized in the intestinal mucosa and in the liver from the digestion of dietary nutrients and the absorption of fatty acids. Triglycerides level influenced by sex, diet, and hormonal factors [7]. All triglycerides level among the treatments reported in this experiment is still in accordance with the normal range for healthy chicken (less than 150 mg/dL) [8]. However, previous study showed that a lower triglycerides levels when boiler chicken treated with essential oil and pepper (at approximately 29.37-39.81 mg/dL) [9].

Tomato powder (lycopene content) as an antioxidant source was expected could decrease the total cholesterol level

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of broiler chickens exposed to aflatoxin. The mechanisms of which antioxidant decreasing cholesterol were : 1) inhibit the activity of HMG-CoA reductase to decrease mevalonate syntesis as a basis of cholesterol formation; 2) Inhibit the Cholesterol Acil Transferase (ACAT) which will reduce the storage of ester cholesterol in the tissue; 3) increase the activity of LDL receptor to decrease LDL cholesterol in blood [10]. Nevertheless, the data of total cholesterol level showed no significant different among the treatments. Cholesterol levels among the treatments in this study (141.75-184.75 mg/dL) were higher compared to research result reported by [9] at 104.56-118.23 mg/dL, and [6]even reported the lower cholesterol serum levels of broiler chicken fed with graded levels of baobab (*Adansonia digitata* L.) seed meal were approximately 47.38-60.36 mg/dL.

B. Kidney Physiology

Data describing the effect of adding tomato powder and sepiolite onkidney physiology of broiler chicken presented in table 4.

TABLE 4. Kidney physiology of broiler chicken with different treatments of sepiolite and tomato powder addition

Treatments	Creatinine (mg/dL)	Blood urea nitrogen (mg/dL)
T1	0.42±0.05	5.10±0.44
T2	0.40±0.00	4.33±0.72
T3	0.40±0.00	3.73±0.90
T4	0.40±0.00	4.25±0.73

Creatinine is a byproduct of phosphocreatine breakdown in skeletal muscle. The kidney maintain the blood creatinine in a normal range by filter almost all of it from the blood and release it into the excreta. Higher level of creatinine could indicates kidneys damage. Aflatoxin could interfere the kidneys function by reducing natural antioxidant glutathione peroxidase and superoxidase dismutase that causing the formation of reactive oxygen species (ROS). ROSs that are not balanced with antioxidant could cause oxidation [1]. Tomato powder as lycopene source and sepiolite as aflatoxin binder were expected to decrease creatinine level in broiler chicken exposed to aflatoxin by improving kidneys function. Antioxidants inhibit ROS by producing electrons donor. Based on statistical analysis, addition of sepiolite and tomato powder in broiler chicken fed with mild aflatoxin contamination from corn did not significantly affect the creatinine level. Creatinine values obtained in this study was approximately 0.40-0.42 mg/dL. Quite similar with this study, creatinine level of broiler chicken fed with urea and cooper sulphate was 0.46-0.64 mg/dL [11]. Lower creatinine level was reported on broiler chicken fed with rosemary oil and vitamin E at 0.23-0.25 mg/dL [12]. Creatinine level was also influenced by age of broiler chicken. Under thermoneutral environment, creatinine level of broiler chickens approximately at 0.41-0.51 mg/dL [13].

Blood urea nitrogen (BUN) is another important indicator of kidneys function. Protein metabolism produces urea nitrogen which will be excreted by the kidneys. BUN level represents the balance of urea production and excretion function in the kidneys. Higher BUN levels indicate abnormal kidneys function. BUN values obtained in this study did not show significant different among the treatment groups. The BUN values obtained in this study was approximately 3.73-5.10 mg/dL. The BUN levels of broiler chicken fed with rosemary oil and vitamin E was approximately 4.00-4.10 mg/dL [12].BUN level in that study was approximately 7.38-8.28 mg/dL was also reported by [14] who studied broiler chicken exposed to mycotoxin and treated with hydrated sodium aluminium silicate, esterified glucomanan and their combination compare with control without addition of feed additive.

C. Blood Glucose

Datadescribing the effect of adding tomato powder and sepiolite onblood glucose levels of broiler chicken presented in table 5.

TABLE 5. Blood glucose levels of broiler chicken with different treatments of sepiolite and tomato powder addition

Treatments	Glucose (mg/dL)
T1	330.00±62.43
T2	386.75±83.18
T3	302.25±56.12
T4	358.50±62.42

Blood glucose level influenced by nutrition intake especially carbohydrates. Excessive or deficiency of blood glucose level could cause health problem both in short or long term. Broiler chicken had blood glucose level within the normal range 200-500 mg/dL. Blood glucose level was influenced by age. The highest glucose level occurred at 35 days of age [13]. Glucose level obtained in this study was approximately 302.25-386.75 mg/dL which means still within the normal range of healthy chicken. Lower blood glucose level was reported by [14] who studied broiler chicken exposed to mycotoxin and treated with hydrate sodium calcium aluminium silicate, esterified glucomannan and their combination which was in the range of 227.027-234.23 mg/dL.

IV. CONCLUSION

Addition of tomato powder and sepiolite to feed with mild aflatoxin contamination had no influence on total serum cholesterol, creatinine, urea, and glucose, but increase the triglycerides level of broiler chicken.

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