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HAEMATOLOGICAL PROFILE OF BLOOD IN LAYING HENS GROWTH PHASE CONSUMING AFLATOXIN CONTAMINATED RANSUM

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ABSTRACT

The purpose of this study was to determine the effect of aflatoxin-contaminated ratio on the haematological profile of the growth phase of laying hens which includes the number of erythrocytes, hemoglobin levels, Packed Cell Volume (PCV), and the number of leukocytes. Day Old Chick (DOC) of 36 animals used in this study were then divided into two groups (P0 and P1). P0 (control group) was given commercial CP 524 100% and P1 (treatment group) was given 80% CP-524 basal feed and 20% aflatoxin contaminated feed was added. Chicken was adapted for 20 days then the treatment of giving aflatoxin was carried out for 40 days with a sampling period every 20 days, namely days 20, 40, and 60. The results of this study showed that the changes that occur in the blood profile did not have a significant difference ($p > 0.05$). Overall the number of erythrocytes, hemoglobin levels, and Packed Cell Vollume (PCV) has increased due to the absence of erithropoesis disorders and an increase in leukocytes due to infection but within the norm. The increase in the hematological profile is influenced by several factors, namely age, sex, environmental temperature, and climate factors. The conclusion of this study was that 20% of aflatoxin-contaminated feed did not have an effect on the haematological profile of the growth phase layer chicken.

KEY WORDS: Aflatoxin, Haematological, Layer chicken

INTRODUCTION

Laying hens are poultry that have the potential to produce eggs commercially. High demand for animal origin food products has encouraged poultry sector farmers to increase their business. Based on data from the Ministry of Agriculture (2017) (Ministry of Agriculture Republic of Indonesia., 2018), the laying hens population reached 176.9 million and the consumption of eggs was 106,418 kg / capita / year. The increasing population causes the demand and need for egg consumption in Indonesia to increase.

Laying hens are able to produce until the age of two years, starting at 20 weeks old, the eggs produced reach 250 to 280 eggs per year (Zulfikar, 2013). Day Old Chicken for laying hens needs to be prepared until it reaches the beginning of production as a maintenance stage that cannot be ignored. One factor that needs to be considered is the problem of feed quality which plays an important role in increasing growth and nutritional requirements needed for chicken reproduction. A good feed is determined by the ingredients of quality ration, has a balanced nutritional value, and does not contain contaminants.

One of contamination in the ransum is aflatoxin. Aflatoxins include natural contaminants produced by several species of *Aspergillus fungi* that are found in hot and humid climates, especially at temperatures of 27 - 40 °C and 85% relative humidity (Widiastuti, 2006). Aflatoxin is a secondary metabolite produced by a toxigenic strain of *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin commonly found in animal feed is aflatoxin B1, B2, G1, G2, among all aflatoxin types,

Aflatoxin B1 is the most dangerous aflatoxin type (Iswari, 2006).

In general, body health can be detected through the condition of the blood feature. Blood has a very complex role for the occurrence of physiological processes that run well, so that livestock productivity can run optimally (Ismoyowati *et al*., 2006) .Invalid blood profile can be influenced from within the body for example caused by health problems, stress, nutritional status and body temperature, then it can also be influenced by external factors such as contamination of aflatoxin in feed. Aflatoxins have the potential to cause carcinogenic, mutagenic, teratogenic, and are immunosuppressive (Samuel, 2009).

In the study of Valchev *et al.*, (2014) explained that the influence of aflatoxin on kidney function is that it can cause degeneration and necrotic in renal peritubular. The formation of erythrocytes (erythropoesis) occurs in the medulla of the bone marrow. The main factor that plays a role in the formation of red blood cells is the erythropoetin hormone (Guyton and Hall, 2006). This hormone is 90% produced by renal peritubular intestinal cells, so it can be assumed that if chickens experience malfunctioning in the kidneys will cause impaired erythrocyte formation and can affect the hemoglobin.

Hematological examination in animals serves as a screening test to assess general health, the body's ability to fight infection, to evaluate the physiological status of animals and to help establish the diagnosis of disease (Jain, 1993). Based on the above background, this study is very important to know how the effect of aflatoxin exposure on growth phase laying hens on the blood haematological response includes erythrocyte count, hemoglobin level, Packed Cell Volume (PCV), number of leukocytes in poultry.

MATERIALS AND METHODS

Experimental animal and sample preparation

The experimental animals used in this study were 36 laying hens. Isa Brown's Commercial DOC Layer. 36 chickens were divided into two treatments (P0 and P1). Each treatment consisted of 18 chickens. Furthermore, each treatment is divided into three subgroups according to the sampling interval, namely when chickens are 20, 40, and 60 days or with the understanding that when animals receive feed treatment for 0, 20, and 40 days, so that each subgroup consists of 6 chickens .

The feeds used in this study were basal feed and aflatoxin contaminated feed. Aflatoxin Contamination Feed is obtained from the independent processed products of Blitar Regency farmers that have been stored for a long time in a warehouse. Contaminated feed was tested for its combined mycotoxin content (aflatoxin B1, B2, G1, G2) in laboratory tests and had a concentration of 9.58 ppb. The aflatoxin-contaminated feed was analyzed for its nutrient content in the animal feed laboratory of the Faculty of Veterinary Medicine, Airlangga University.

The research cages used brooder cages for DOC maintenance for 20 days. Then 36 battery cages each measuring 30 cm x 40 cm were prepared for maintenance during the treatment for 36 treatment chickens. The cage is equipped with a place to feed and drink. This research used tools such as food and drink containers, syringes, gloves, masks, laboratory coats, ballpoint pens, cameras. The equipment used in the inspection is the EDTA tube, syringe and needle and hematology analyzer.

Procedure of treatment

The first stage 36 DOC tails were adapted to the brooder cage for 20 days then put into the battery cage by randomization method using a lottery. At the time of adaptation of chickens P0 and P1 groups were given initial feed growth of CP-521 laying eggs with a protein content of 19-21% and were given an ad libitum drink. The ND-IB vaccine was given at 3 days, then at 14 days the ND vaccination was given, then at the age of 18 days the IBD-Intermediate vaccine was applied through mouth drops.

The second stage, after the chicken is 21 days old, feeding and treatment is carried out in accordance with the experiments to be observed. Group P0 gets commercial ration for the growth phase of CP-524. Group P1 gets 80% commercial feed ration CP-524 growe feed with 20% ration Aflatoxin B1, B2, G1, G2 with a concentration of 9.58 ppb. This feed is mixed until homogeneous before it is given. At the age of 36 days ND CLONE vaccination is given by mouth drops.

Blood sampling

Blood sampling is carried out in three periods namely at the end of the adaptation period, namely the 0th treatment day, then after the animals get treatment for up to 20 days and treatment for up to

40 days. 0, 20, and 40 days treatment is coinciding with the age of the 20th, 40th, and 60th day of the chicken. Each collection period is set to 6 chickens to collect blood from groups P0 and P1. Blood collection through the brachial vein and accommodated in a sterile vacuum tube containing 3ml EDTA anticoagulant and labeled each tube, then examined using a Hematology Analyzer.

Data analysis

Results data obtained from blood hematological tests were tabulated and then analyzed using ANOVA by split plot test. If there are differences the results are then evaluated by the distance test at the significance level of 0.05. The entire analysis process was carried out with the SPSS program.

RESULTS AND DISCUSSION

Overall, 20% aflatoxin-contaminated ration with a concentration of 9.58 ppb did not have a negative effect on changes in blood picture including erythrocyte count, hemoglobin level, Packed Cell Vollume (PCV), and leukocyte count. The results of this study the effect of aflatoxin on the length of time treatment on the hematological profile of blood has increased, but the effect of the treatment of the control group with the treatment group did not have a significant difference (p> 0.05)

Erythrocytes

The absence of influence on the number of erythrocytes is suspected because there is no damage to the kidneys. This is comparable to previous studies that giving aflatoxin of 0.5 mg/kg of feed for 4 weeks did not have a significantly different effect on kidney function. (Ayu, 2018) reported the results of research that administration of aflatoxin <50 ppb did not have a significant negative effect on the number of erythrocytes.

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Aflatoxin is a poisonous substance, an increase in the number of erythrocytes can be due to an increase in young erythrocytes (immaturus), it occurs due to the response of the spinal cord to anemia caused by poisoning so that the spinal cord releases imaturus red blood cells into the circulation (Hamdani and Dwinna, 2010).

Hemoglobin levels

Hemoglobin in red blood cells comes from the synthesis of acetic acid and glycin which produce porphyrin. Porphyrin combined with Fe will produce heme molecules, then proceed with the addition of globin which consists of amino acids to form hemoglobin (Rosmalawati, 2008). Hemoglobin levels and the number of erythrocytes is directly proportional. There were no significant changes in hemoglobin levels because the amount of erythrocytes during the study was at normal levels.

Table 1. mount of blood erythrocytes in laying hens given growth rations aflatoxin contaminated.

Group	Amount of erythrocytes (mm^3)		
	20 (Hari)	40(day)	60 (hari)
P0	2.60 ± 0.10^a	2.75 ± 0.22^b	2.91 ± 0.14^b
P1	2.60 ± 0.10^a	2.90 ± 0.35^b	3.03 ± 0.26^b

Note: Different superscripts in the same row or column show significant differences (p <0.05)

Table 2. Blood growth hemoglobin levels in laying hens fed aflatoxin ration.

Group	Hemoglobin levels g%		
	20 (Day)	40 (Day)	60 (Day)
P0	9.40 ± 0.63^a	10.01 ± 1.19^a	10.48 ± 0.67^b
P1	9.40 ± 0.63^a	10.18 ± 1.16^a	11.4 ± 0.98^b

Note: Different superscripts in the same row or column show significant differences (p <0.05).

Ayu's research report (2018) (Ayu, 2018) with the result that the number of erythrocytes increased from 2.27 million/mm^3 to 2.39 million/mm^3 in the treatment of aflatoxin <50 ppb, followed by hemoglobin values also increased with the same treatment from 10.39 g /% to 11.14 g /%. High and low levels of hemoblobin in the blood are influenced by the health of the age of the animal, species, environment, blood handling when examined, feed, and the presence or absence of damage to erythrocytes (Stockham and Scott, 2008).

Packed Cell Vollume (PCV)

Packed Cell Volume (PCV)is a comparison between the volume of blood erythrocytes with other blood components. The volume of erythrocytes in the blood is directly proportional to the amount of erythrocytes and hemoglobin levels. PCV is an erythrocyte that has been separated from other blood components such as leukocytes, platelets and plasma so that if there is a decrease in erythrocytes there will be a decrease in PCV because PCV functions to determine the number of erythrocytes per unit volume of blood (Clark, 2009). Factors that influence it are age, molting, reproduction cycle and air temperature. Factors that influence erythrocytes also affect PCV.

Leukocyte count

Leukocytes are active units of the immune system by providing fast and strong defense against any infectious agent (Cahyaningsih, 2007). The results in this study did not have a noticeable change in the treatment of time and the treatment group with the control group. Provision of 20% aflatoxin contaminated ration with a concentration of 9.58 ppb is thought to have no negative effect on the number of leukocytes.

An increase in leukocyte values from the normal number indicates an infection while a decrease in leukocytes indicates a bone marrow depression, which is caused by a viral infection or a toxic reaction to a chemical agent (Rastogi, 2007). In this study the average number of leukocytes increased but not significantly in the control group of 1.32 million/L and in the treatment group of 1.54 million / L on day 40 to day 60. The normal number of leukocytes in chickens was 20.0 - 30.0 x 10^3 / L so that it can be concluded that the increase in leukocytes in this study is still within normal limits, it is presumably because it is influenced by the age of chickens which is increasing so that it causes the number of leukocytes to increase (Rosmalawati N., 2008).

CONCLUSION

Based on the research that has been done, it can be concluded that the administration of aflatoxin-contaminated ration has no effect on changes in blood profile in laying hens, the growth phase which includes the number of erythrocytes, hemoglobin levels, Packed Cell Volume (PCV), and the number of leukocytes.

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Table 3. The value of Packed Cell Volume (PCV) blood of laying hens in the growth phase that was fed aflatoxin ration.

Group	Packed Cell Volume (PCV) %		
	20 (day)	40 (day)	60 (day)
P0	24.5 ± 2.25^a	27.83 ± 2.04^b	28.5 ± 2.16^b
P1	24.5 ± 2.25^a	28.67 ± 2.06^b	29.67 ± 3.20^b

Note: Different superscripts in the same row or column show significant differences ($p < 0.05$).

Table 4. The number of leukocytes in the growth phase of laying hens fed by aflatoxin ration.

Group	Leukocyte count g/mm^3		
	20 (day)	40 (day)	60 (day)
P0	22.9 ± 1.05^a	23.21 ± 0.18^a	24.53 ± 2.20^b
P1	22.9 ± 1.05^a	23.21 ± 0.18^a	24.75 ± 1.31^b

Note: Different superscripts in the same line show significant differences ($p < 0.05$).

content was analyzed in the animal feed laboratory of the Faculty of Veterinary Medicine, Airlangga University. Brooder cages have facilities that are very helpful for maintaining DOC for 20 days, and are very grateful to them for giving me the opportunity and support in this research.

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