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Effect of Papaya Leaves in Feed on the Immunity of Silver and Gold Arab Laying-Hens

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Abstract: The immunity of Arab Laying-hens is the key to success in the eggs production. The study aims to determine the effect of the level of papaya leaves (*Carica papaya* L.) in the feed to the immunity on Arab Laying-hens with different feather colors (Silver and Gold). The experimental design was completely randomized in a factorial arrangement (feather colors X level of papaya leaves). Immunity was determined by the number of CD4, CD8 and B220 after 60 days treatment. The results showed the feather colors and interaction of feather colors and the level of papaya leaves had no significant ($p>0.05$) effect toward CD4, CD8 and B220. However the number of B220 tended to increase by increasing the level of papaya leaves in feed in both of Silver and Gold Arab Laying-hens. In conclusion, the immunity expressed by CD4, CD8 and B220 was similar in both Silver and Gold Arab Laying-hens, as will go for the level of papaya leaves in feed.

Key words: Arab Laying-hens, feather colors, papaya leaves (*Carica papaya* L.), immunity, eggs production, poultry feed

INTRODUCTION

Arab Laying-hens are potential to be developed due to its ability to produce eggs which is higher than kampong Laying-hens. It has similar egg characteristics to kampong Laying-hens eggs. The marketing system of Arab Laying-hens egg is done similarly to kampong chickens, it is sold per egg which makes it expensive. Kampong Laying-hens is Indonesia Local Laying-hens and well known for many people in Indonesia. Its eggs are frequently consumed so that it increases the demand of eggs. Kampong Laying-hens eggs also offer many benefits for people. The eggs are suitable for traditional medicine. It taste savory without fishy smell.

Furthermore, antibiotics are often used to improve Laying-hens health and production. Nowadays, the use of antibiotics starts to cause problems, the long term use of antibiotic leads to give residues for consumers. The substitution of antibiotic for traditional medicine from medicinal plants is getting more common. Traditional medicine is safe and free from residue.

There are some problems in raising Arab laying-hens such as high death rate, side effect of antibiotic, expensive medicine and expensive feed cost. Besides, data and research of Arab laying-hens are still limited; it has not been published frequently. Papaya leaves can be used as an alternative for the problems. It reduces antibiotics usage, medicine costs and the feed cost. In addition, using papaya leaves for feed can result in producing healthy egg for consumers.

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Based on the feather colors, Arab laying-hens can be classified into two; Brakel Kriel-Silver and Brakel Kriel-Gold. The color of Silver Arab laying-hens from head to neck is silvery-white, while its body is white-black mottled or white-black barred. The color of Gold Arab laying-hens from head to neck is reddish-gold, while its body is red-black barred (Robert, 2008). The origin of White Arab laying-hens comes from the crossbreeding between original Arab rooster and local laying hens. While Red Arab (Gold) laying-hens have two possibilities of origins, it can be from the crossbreeding between original Arab rooster with Merawang hen or from the crossbreeding between original Arab rooster and laying hen (Pambudhi, 2003). The feather colors can be used to characterize certain types of chicken. Different feather colors may have different characteristics and ability to produce eggs.

Papaya is a medicinal plant often found in Indonesia; its leaves are abundant and very cheap. Papaya leaves contain feed substance and active substances which are beneficial to improve the egg production and health. It can be used as Arab laying-hens feed in the form of meal; meal form is more profitable compare to the juice form Muharlien and Nurgiartingsih (2015). Papaya

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leaves also contain crude protein, crude fiber, carbohydrate, Ca, Mg, Fe and K, high beta-carotene and Papain enzyme (Nwofia *et al*., 2012). In addition, it contains bio-active components that can improve the function of antioxidants in the blood and reduce the level of lipid peroxidation (Seigler *et al*., 2002). Part of the papaya plants such as roots, leaves, fruits and seeds contain *phytochemicals* in the form of polysaccharides, vitamins, minerals, proteins, Papain enzymes, alkaloids, glycosides, saponins and flavonoids; all of them can be used as nutrients and medicine (Krishna *et al*., 2008). *Saponins* are known as an immunostimulant and anticarcinogen agent (Francis *et al*., 2002). While *Flavonoids* are powerful antioxidants in preventing free radical (Miller, 1996). Papaya leaves contain alkaloids, carpaine, choline, carposide, vitamin C and vitamin E (Yogiraj *et al*., 2014; Aswani and Husain, 2012).

Immunity is the body's biological defenses against infections caused by micro-organisms such as bacteria, viruses, parasites and fungi (U.S. Department of Health and Human Services National Institutes of Health, 2003; Rifa'i, 2013). The immunity can be observed by identifying CD4, CD8 and B220. Identification of immune system can be done by measuring the amount of CD4, CD8 and B220 in the blood. CD4, CD8 and B220 are the types of white blood cells which have significant importance in the body's immunity. CD4 and CD8 profile can be used as the basis to infer an individual's health. CD4 molecule is a transmembrane protein which act as the activator in T helper cells. While CD8 molecule is a transmembrane protein that functions as co-receptors in killer T cells. Then, B220 or CD45R molecule is a transmembrane protein expressed by B cells (Rifa'I (2013). T cell (CD4) has an important role in the immune system, T cells and B cells (B220) jointly form antibody (Zhu and William, 2008). Based on the description above, there is an urgency to study in more detail about the effect of the usage level of papaya leaves containing in feed toward the Arab laying-hens immunity with different feather colors.

MATERIALS AND METHODS

The study was conducted in two steps. Step 1 was a field experiment, conducted at the Arab Laying-hens farm in Mulyoagung village, Dau, Malang. Step 2 was analysis by using Flow Cytrometry in Animal Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia. The research period was conducted in two months.

Research materials: The study was conducted in two steps, the materials in Step 1 were 60 Silver Arab Laying-hens and 60 Gold Arab Laying-hens, laying period with similar age and weight. The feed were a mixture of corn, rice bran and concentrate and it was added with papaya leaves 0, 6 and 12% according to treatment. Papaya leaves was given in the form of flour. The feed substances were similar for all treatments, 16% protein and ME 2650 Kcal/kg (SNI, 2006). Then, the materials in Step 2 were 12 Silver Arab Laying-hens and 12 Gold Arab Laying-hens taken from stage 1 random sampling.

Research method: The experimental design was completely randomized in a factorial arrangement (Steel and Torrie, 1991). The treatments consisted of the use of papaya leaves level (0, 6 and 12%) and feather colors (Silver and Gold). The variables observed were: CD4, CD8 and B220 which were analyzed by using Flow Citrometry (Rifa'i, 2013; Pradana *et al*., 2013) from whole blood collected at the end of the treatment. The data analysis by using GenStat soft ware program.

Blood sampling: The first step was collecting the blood sample by inserting the syringe on the backside of its wings. Before that, the targeted area had to be rubbed with alcohol swab to ensure its cleanness. Injection syringe was needed to take 3 cc of blood from each hen. After collecting the blood, the targeted are must be gently pressed with alcohol swab. Lastly, the blood sample was directly put into EDTA tubes and stored in a Styrofoam box to be taken to the Laboratory.

Blood isolation: The first step in blood isolation was taking all materials out from the refrigerator so that the materials could reach the room temperature. Then, 15 ml centrifuge tube was filled with Ficoll-Hipaque d = 1.077 g/ml (1: 1) and the blood sample. Blood sample in vacutainer EDTA to be tested were homogenized by tumbling it slowly and mixed it up with 1: 1 PBS. Next, the blood samples were taken by using micropipette and flowed slowly across the walls of a 15 ml centrifuge filled with Ficoll-Hipaque d = 1.077 g/ml. That procedure would generate two layers which cannot be mixed. Then, the blood sample was centrifuged at the room temperature (25°C) with the speed of 1600 rpm for 30 minutes. After centrifuging, it would form five separated layers; plasma, PBMCs cells, Ficoll-Hipaque, granulocytes and red blood cells. The PBMC ring was slowly taken by using a micropipette and placed in a new 15 ml centrifuge tube. PBMC solution was washed with 10 ml PBS and centrifuged at the temperature of 10°C, with the speed of 2.500 rpm for 5 min. However, supernatant was discarded in this step. The cell pellet formed was washed again with 1 ml PBS. After centrifuging process, it would form a pellet (red blood cells) on the basis of 15 ml centrifuge tube.

Staining: It was performed by using extracellular staining with antibody combinations. CD4 was stained by using FITC-conjugated rat anti-mouse; CD8 was stained by

using PE-conjugated rat anti-mouse; B220 was stained by using PE/Cy 5-conjugated rat anti-mouse. Extracellular staining was done by adding 50 µl of specific antibody to the cell (pellet) which was added 50 µl of PBS and 10% of FCS. Incubation with antibody was performed for 20 minutes at the temperature of 4°C. Then, it was added with 400 µl of PBS and transferred into a cuvette.

Flow cytrometry: It was performed by connecting the flow cytrometry tool with computer on Acquiring mode. Then, the software was set on BD CellQuest Pro™. Plot setting must be on acquiring mode and the flow cytometry must on Low Run condition. After flow cytometry was ready, the cuvette was hooked up to nozzle BD Biosciences FACs Calibur™ Flow Cytrometry. Thus, the result could be seen on computer.

RESULTS AND DISCUSSION

Effect of feather colors toward CD4, CD8 and B220 on Arab laying-hens: The immunity of Arab laying-hens in this research could be detected through CD4, CD8 and B220. The amount of CD4, CD8 and B220 in Silver and Gold Arab laying-hens were shown on Fig. 1.

The result of the analysis showed that feather colors had no significant effect ($p>0.05$) toward the amount of CD4, CD8 and B220. The amount of CD4, CD8 and B220 in Silver and Gold Arab laying-hens were almost the same because Silver and Gold Arab laying-hens were genetically close. Both of them still belonged to the types of Arab laying-hens. Besides, Silver and Gold Arab laying-hens came from the same original Arab rooster. Results of research on Silver Arab laying hens contained 3.46% of CD4, 3.23% of CD8 and 18.99% of B220. While Gold Arab laying-hens contained 5.51% of CD4, 4.08% of CD8 and 19.84% of B220. Those percentages signified fruitful results. In accordance with the research result from Pradana *et al*. (2013) finisher broiler, which was measured through the spleen, contains approximately 9.48% of CD4 and 15.22% of CD8. Besides, the results of the study from Shanmugasundaram and Selvaraj (2011) showed that the percentage of CD4 cells in the thymus and the blood was ranging from 3-7%; in the spleen was 10% and in the tonsils cecal, lung and bone marrow was 15%.

Effect of papaya leaves level toward CD4, CD8 and B220 on Arab laying-hens: The average amount of CD4, CD8 and B220 based on the level of the use of papaya leaves feed during the research can be seen on Fig. 2.

The average amount of CD4, CD8 and B220 (Fig. 2) and the results of the analysis showed that the usage level of papaya leaves in feed was not significant ($p>0.05$) toward the amount of CD4, CD8 and B220. However the number of B220 tended to increase by increasing the level of papaya leaves in feed in both of Silver and Gold

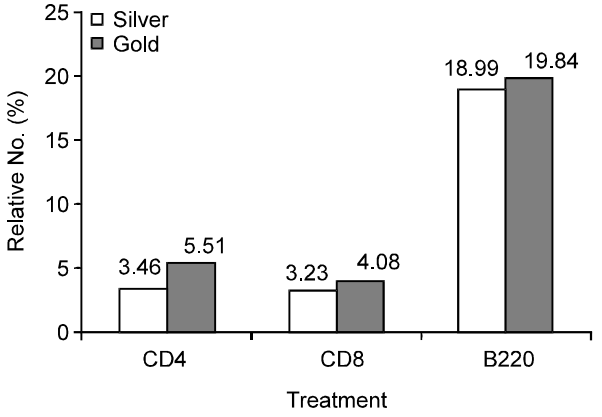

Fig. 1: Average amount of CD4, CD8 and B 220 on Silver and Gold Arab Laying-hens

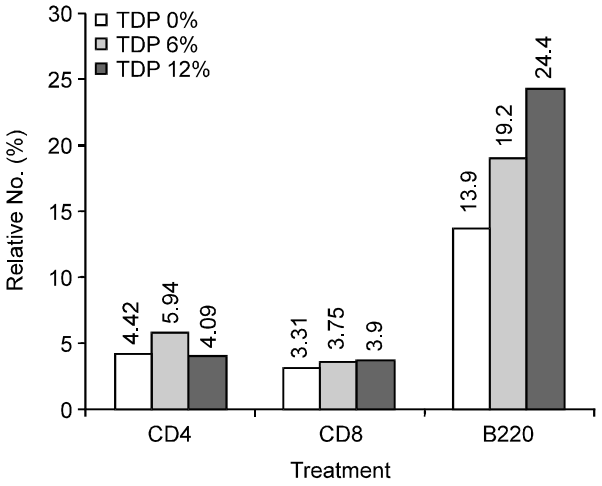

Fig. 2: Average amount of CD4, CD8 and B220 based on the level of the use of papaya leaves

Arab Laying-hens. It meant that papaya leaves could act as an immunostimulant to increase the immunity through B220. That could happen because of the element of flavonoids in papaya leaves. According to Sashidhara *et al*. (2007) flavonoids independently contribute to increase the production of *cytokines* such as IL-2. The IL-2 cytokines play a role in mediating a wide range of biological processes, including cell growth and proliferation of lymphocytes. According to Saxena *et al*. (2013) the alkaloid can act as an anti-bacterial, anti-fungal and anti-oxidants, while Flavonoids can act as anti-oxidants and detoxification (Lyu and Park, 2005). Flavonoids are the kind of flavonols which can be immunostimulant that increase IL-2. According to Hefni *et al*. (2013) the increase of B220 cells indicates that there is imunostimulator activity.

Number of CD4, CD8 and B220 based on the interaction of feather colors and papaya leaves level on Arab laying-hens: The average amount of CD4, CD8 and

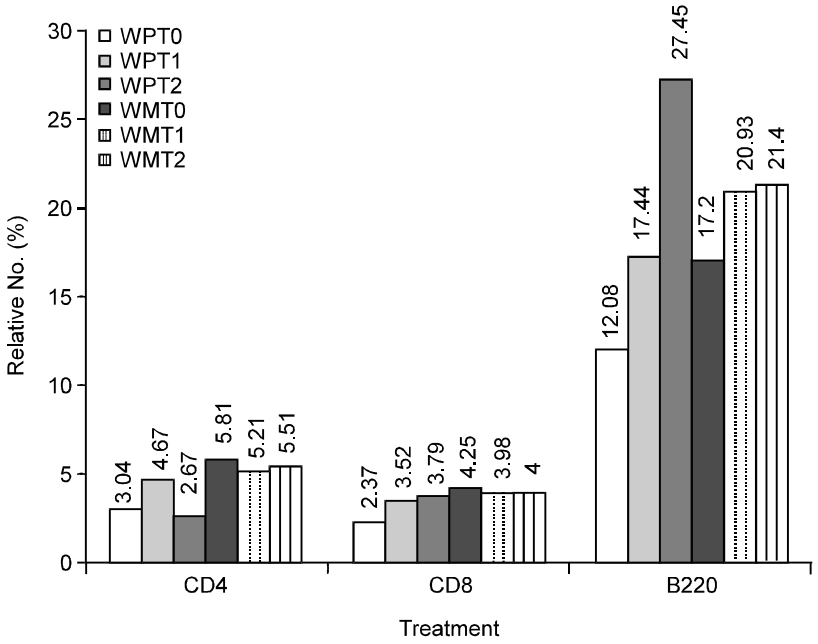

Fig. 3: Average amount of CD4, CD8 and B220 based on the interaction of feather colors and papaya leaves level

B220 based on the interaction of feather colors and papaya leaves level can be seen on Fig. 3. The amount of CD4, CD8 and B220 (Fig. 3) and the results of the analysis showed that the interaction of feather colors and the usage level of papaya leaves had no significant effect ($p>0.05$). It indicated that Silver and Gold Arab Laying-hens had the same response to the use of papaya leaves. It was supported by the results of this study which showed that the feather colors did not affect the amount of CD4, CD8 and B220 and the level of papaya leaves also did not affect CD4, CD8 and B220. However the number of B220 tended to increase by increasing the level of papaya leaves in feed, so it could be interpreted that the use of papaya leaves can be used as an immunostimulatory for Silver and Gold Arab Laying-hens.

Conclusion: Based on the feather colors, Arab Laying-hens could be classified into two groups, Silver and Gold. Silver and Gold Arab Laying-hens had equal immunity. In addition, Papaya leaves could be used to increase the immunity through B220. Silver and Gold Arab Laying-hens had the same response in the use of papaya leaves for increasing the immunity.

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