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Blood Profile of Quails (*Coturnix coturnix japonica*) Fed Ration Containing Silkworm Pupae (*Bombyx mori*) Powder Extract

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ABSTRACT

The aim of this research was to assess the use of silkworm pupae (*Bombyx mori*) powder extract on the blood cells and hematological variables of quails (*Coturnix coturnix japonica*). The treatments consisted of R0= isoenergy and isoprotein ration without pupae powder extract, R1= isoenergy and isoprotein ration + 1% of pupae powder extract, R2= isoenergy and isoprotein ration + 10% of pupae powder extract, R3= isoenergy and isoprotein ration + 1% of residue of pupae powder extract, R4= isoenergy and isoprotein ration + 10% of residue of pupae powder extract, R5= isoenergy and isoprotein ration + 1% of pupae powder, and R6= isoenergy and isoprotein ration + 10% of pupae powder. The variables measured were blood cell and hematological profiles including the number of leukocytes, erythrocytes, and leukocytes differentiation. Immune response was observed at 12, 24, and 48 h by counting the white blood cells type. The results showed that feed consumption ranged 79.19-154.70 g/quail/wk and the body weight was 71.45-149 g/quail. The addition of pupae extract at a dose of 10% in the diet significantly (P<0.05) increased the number of quail white blood cells. The addition of pupae extract, pupae powder residue, and pupae powder in the ration did not significantly affect the number of quail erythrocytes, hemoglobin, and PCV (packed cell volume). Mean number of white blood cells increased during 24 h after treatment of 10% pupae powder extract (R2) and decreased at the 48th h. It is concluded that the value of leukocytes, lymphocytes, monocytes, and heterophile increased in quails supplemented with 10% pupae powder extract (R2).

Key words: quail, silk worm pupae powder, the immune system, white blood cells

ABSTRAK

Tujuan penelitian ini ialah mengkaji pengaruh penggunaan ekstrak tepung pupa ulat sutera (*Bombyx mori*) pada sel darah dan peubah hematologi puyuh (*Coturnix coturnix japonica*). Perlakuan terdiri atas R0= ransum isoenergi dan isoprotein tanpa ekstrak tepung pupa, R1= ransum isoenergi dan isoprotein + 1% ekstrak tepung pupa, R2= ransum isoenergi dan isoprotein + 10% ekstrak tepung pupa, R3= ransum isoenergi dan isoprotein + 1% residu ekstrak tepung pupa, R4= ransum isoenergi dan isoprotein + 10% residu ekstrak tepung pupa, R5= ransum isoenergi dan isoprotein + 1% tepung pupa, dan R6= ransum isoenergi dan isoprotein + 10% tepung pupa. Peubah yang diamati adalah profil hematologi meliputi: jumlah leukosit, eritrosit, dan diferensiasi leukosit. Respons kekebalan tubuh diamati pada 12, 24, dan 48 jam setelah pemberian pakan dengan menghitung jenis sel darah putih. Hasil penelitian menunjukkan bahwa konsumsi pakan berkisar 79,19-154,70 g/puyuh/minggu dan bobot badan puyuh memiliki rataan 71,45-149 g. Penambahan ekstrak pupa dosis 10% dalam ransum nyata (P<0,05) meningkatkan jumlah sel darah putih puyuh. Penambahan ekstrak tepung pupa, residu ekstrak tepung pupa, dan tepung pupa dalam ransum tidak berpengaruh pada jumlah eritrosit puyuh, hemoglobin, dan PCV (*packed cell volume*). Jumlah rata-rata sel darah putih meningkat selama 24 jam setelah penambahan 10% ekstrak tepung pupa (R2) dan menurun pada jam ke-48. Disimpulkan bahwa ada peningkatan nilai leukosit, limfosit, monosit, dan heterofil pada puyuh dengan menambahkan 10% ekstrak tepung pupa (R2).

Kata kunci: puyuh, sel darah putih, sistem kekebalan tubuh, tepung pupa ulat sutera

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INTRODUCTION

Insects have great potential as sources of food and feed as well as a source of nutrients that is comparable to meat and fish and also can be a source of animal protein as the main stay protein in the future for the purposes of poultry farming and fish farming (Usub *et al*., 2008; Oh *et al*., 2012; Rumpold & Schluter, 2013). The utilization of some insects such as silkworms and bees for large-scale production with the use of technology was done to anticipate the problem of global food and feed supply (Vantomme *et al*., 2010; Huis *et al*., 2013).

Another potential owned by insects is the discovery of a various bioactive substances with beneficial characteristics (Hirose *et al*., 2013). These substances included compounds with diverse pharmacological properties, including anti-cancer, anti-tumor, anti-viral, and antimicrobial as well as the capacity to boost the immune system against various diseases in human, livestock, poultry, and fish (Chernysh *et al*., 2002). Insects are not only useful as a source of animal protein in food production as well as feed, but they provide benefits in the pharmacological and medical areas which are able to protect animals and livestock against various risks of many diseases.

In the life cycle of insects, the eggs will hatch into larvae, pupae, and adult metamorphosis. In the stage of insect metamorphosis, pupae under fasting conditions to grow in the cocoon (protective hard). Silkworm cocoon thread is used for spinning into yarn silk, while the pupae are used as animal feed cultivation. Cocoon production for silk in Indonesia since the 2005-2009 period reached 166 tons, which produced about 23 tons of yarn. The wastes produced from the process were around 143 tons.

In some countries such as Korea, Japan, and China pupae are used to produce snacks, canned food, alternative foods for diabetics, candles and beauty products such as soap and hair tonic (Yi *et al*., 2010). Pupae silkworms can be made into flour pupae that can be used as an ingredient in the manufacture of complementary cookies (crackers and flakes), sponge cake, and nuggets (Astuti & Kusharto, 2009). In addition, the pupae silkworm can be utilized as a bioreactor in the development of antiviral agents and as an immunostimulant in vertebrates (Yao *et al*., 2006; Swevers *et al*., 2013; Yeo *et al*., 2013).

The addition of insect pupae powder on red sea bream feed could boost immune activity against pathogenic bacteria (Ido *et al*., 2015). Powder extract of *Bactrocera cucurbitae* pupae was known to activate the innate immunity on rat macrophage cells (Ohta *et al*., 2014). This immunostimulatory effect was identified and it was derived from the active ingredient in the form of polysaccharides contained in the body of the pupae (Ohta *et al*., 2014). Silkworm pupae contained polysaccharides (27.9%) (Long *et al*., 2007) and high protein (55.6%) with a balanced amino acid (Zhou & Han, 2006; Tomotake *et al*., 2010).

Immunostimulatory effect of silkworm pupae was identified from the polysaccharide contents. High molecular weight polysaccharide can activate the innate immune system. The polysaccharide is the site identifier (marker). Dipterose polysaccharide is synthesized by the Diptera insect pupae such as melons fly and has a molecular weight of $1.01x10^6$ mol%. Similarly, it occurs also in insect Lepidoptera such as silkworm, polysaccharides molecular components, namely silk rose, that is synthesized in the pupae and has a molecular weight of $3.15x10^5$ mol%. The composition of silkrose and dipterose consist of nine monosaccharides i.e., D-glucose, D-mannose, D-galactose, D-ribose, L-rhamnose, L-fucose, D-glucoronic acid, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine (Ohta *et al*., 2014). Silkrose is ethanol-soluble polysaccharide group, so that silkrose bioactive substances can be obtained from the extraction of silkworm pupae powder with ethanol solvent.

L-rhamnose is one component of dipterose with the highest molar ratio as compared to the other monosaccharides. At silkrose, the highest monosaccharide component is D-Galactose with molar ratio reached 48.9%. L-Rhamnose can be found in natural organic components such as flavonoids, terpenoids and saponins (Ohta *et al*., 2014). Saponins in a particular dosage are secondary compounds that can play a role as immunostimulant. Bomford (1980) reported that saponins can function resemble a membrane antigen of polysaccharide cell at the outside of the cell membrane and it is used as the site identifier (marker) in the immune system of vertebrates.

Dipterose and Silkrose were obtained from *Musca domestica*, melon fly, *Hermetia illucens*, *Antheraea yamamai* and *Bombyx mori* and were able to activate the innate immune response against pathogenic microorganisms and viral infections (Ohta *et al*., 2014; Ido *et al*., 2015). Conversely the waste of silkworm pupae in unrefined pupae powder will make some rat macrophage cells die (Ido *et al*., 2015). The ability of bioactive substances owned by the insects in activating the immune response against pathogens and viral infections in fish and mammals, become the reason to conduct a research to assess the use of silkworm pupa powder extract (*B. mori*) on the blood profile of quail (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

A total of 70 laying quails (*Coturnix coturnix japonica*) with the age of 4 weeks and average body weight of 76.36 g were used in this study and half of them (35 laying quails) were used for blood profile analysis. Raw materials used to formulate the experimental ration were corn, rice bran, soybean meal, fish meal, palm oil, DCP, $CaCO_3$, NaCl, Premix, DL-Methionine, and silkworm pupae powder. Additional feed was given in the form of silkworm pupae powder, residue of silkworm pupae powder extraction and pupae powder extraction on the prescribed dose. Pupae powder extraction refers to the maceration extraction method with 95% ethanol as a solvent (Liu & Zhang, 2006). Feed rations were prepared with isoenergy and isoprotein i.e. the energy content of 2950 kcal/kg and protein content of 18% (Table 1) (Leeson & Summers, 2005). The experimental quails were maintained for 8 weeks, with details of treatment

were first week for environmental adaptation, second week for control ration adaptation (R0), third week for additional treatment rations, and the last five weeks without ration treatments (R0). Rations and water were given *ad libitum*.

This study used a completely randomized design with 7 treatments and 5 replications. The treatments were R0= isoenergy and isoprotein ration without pupae powder extract (control), R1= isoenergy and isoprotein ration + 1% pupae powder extract, R2= isoenergy and isoprotein ration + 10% pupae powder extract, R3= isoenergy and isoprotein ration + 1% residue of pupae powder extraction, R4= isoenergy and isoprotein ration + 10% residue of pupae powder extraction, R5= isoenergy and isoprotein ration + 1% pupae powder, and R6= isoenergy and isoprotein ration + 10% pupae powder. Pupae powder was used as a feed supplement with doses of 0% (control), 1% and 10% (Ohta *et al*., 2014). Parameters measured were blood profile of the experimental quails consisted of number of erythrocytes, hemoglobin concentration, hematocrit percentage, leukocyte count and leukocyte differentiation (Jalees *et al*., 2011). Blood samples of quail were taken on the 15th day at the time of observation 12, 24, and 48 h. It was based on the estimation that time of circulating white blood cells in the blood may be brief (Guyton & Hall, 1997). Blood sampling at different times was conducted in order to look at the treatment that had significantly impacted on the number of white blood cells. One milliliters of blood sample was taken and put into EDTA tubes. Preparations of the blood smear with Giemsa staining were made to calculate the number of leukocyte. Total white blood cells and total red blood cells were calculated by using a Neubauer hemocytometer, with a modified diluent solution of Rees & Ecker. Data were tested by using Analysis of Variance (ANOVA) and the differences among treatments' means were examined by Duncan Multiple Range Test (Mattjik & Sumertajaya, 2002). Data were processed by using SPSS 16.0 statistical software.

Table 1. Composition and nutrient contents of the basal ration in laying period

Feed ingredient	(%)
Yellow corn	50.13
Soybean meal	23.00
Fish meal	8.00
Rice bran	6.87
Palm oil	4.50
$CaCO_3$	6.60
Premix	0.50
DL=Methionine	0.20
NaCl	0.20
Total	100.00
Nutrient content*)	
Metabolizable energy (kkal/kg)	2950.08
Crude protein (%)	18.82
Crude fat (%)	5.36
Crude fiber (%)	2.41
Methionine (%)	0.58
Lysine (%)	1.06
Cystine (%)	0.30
Linoleic acid (%)	1.50
Ca (%)	3.19
P (%)	0.48
Na (%)	0.15
Cl (%)	0.17

Note: *)Based on calculation using the formula of Leeson & Summers (2005).

RESULTS AND DISCUSSION

Quail Feed Consumption

Feed consumption during the research ranged from 79.19 g/quail/wk to 154.70 g/quail/wk (Table 2). The results showed that the supplementation of pupae extract, residue of pupae extraction and pupae powder in R1, R2, R3, R4, R5, and R6 did not affect the average feed consumption. In poultry, there is a limiting factor of feed intake that is the gizzards capacity and energy requirements (North & Bell, 1990). The limiting factors cause quail stop eating when energy needs are fulfilled. Feed given during the study was in accordance with the needs of quail that have been specified in SNI (1995) with energy content of 2900 kcal/kg. Quail used in this study had the same age, namely four weeks so that the gizzards size was not much different. This caused the feed intake of all treatments was not different, except in R2 treatment (extract pupae powder with dose of 10%).

Quail Body Weight

The observation during the experiment found that the body weight gain of quails from the beginning to the end of experiment increased in quails fed with R2 treatment (extract of pupae powder at a dose of 10%) (Table 3). The provision of pupae powder in extract form as a force feeding enable all of the ingredients entered the body of quails through gastrointestinal tract without being wasted. Silkworm pupae powder in the form of polysaccharide extract contains active ingredients that can boost the immune system of the experimental quails. These results are consistent with the results reported by Ido *et al*. (2015), that the administration of extract of house fly pupae powder in red sea bream fish improved growth rate and immune system.

Total Number of Erythrocytes

The results showed that the number of erythrocyte in the group R1, R2, R3, R4, R5, and R6 were similar ($P>0.05$) (Table 4). Erythrocytes are blood cells that have a nucleus and play a role in carrying hemoglobin to bind oxygen throughout the body. The ranges of average erythrocytes number of laying quail studied were 2.97-3.46 x 10^6/mm^3. This value was still in the normal range. According to Sturkie & Griminger (1976), the normal number of erythrocytes is 2.30-3.86 x 10^6/mm^3. This indicates that the active ingredient contained in pupae powder extract, pupae powder residue and pupae powder in the form of polysaccharides silkrose did not

Table 2. Average feed consumption of quails during experiment (g/bird/wk)

Treatments	Weeks							
	1	2	3	4	5	6	7	8
R0	79.67±0.40	81.00±0.33	90.04±0.68	97.27 ±0.99	100.04±0.72	120.01±0.17	134.20±0.42	140.01±-0.52
R1	80.04±0.61	81.19±0.16	92.52±0.32	98.02±0.48	101.22±0.28	120.14±0.41	135.47±0.19	140.11±0.33
R2	81.36±0.13	85.29±0.19	94.52±0.46	100.28±0.21	130.16±0.37	140.44±0.18	149.12±0.13	154.70±0.32
R3	79.52±0.15	80.79±0.21	89.67±0.67	97.13±0.38	100.02±0.42	120.00±0.29	134.01±0.78	137.15±0.51
R4	79.86±0.58	82.71±0.62	90.19±0.81	97.45±0.37	100.17±0.28	121.46±0.61	134.27±0.43	140.08±0.62
R5	79.21±0.17	80.34±0.12	89.22±0.10	97.08±0.92	100.00±0.71	119.47±0.57	133.98±0.18	137.13±0.49
R6	79.19±0.35	80.21±0.67	89.16±0.15	97.01±0.21	99.71±0.34	119.15±0.13	133.12±0.68	136.05±0.86

Note: R0= ration without pupae powder extract (control), R1= ration + 1% pupae powder extract, R2= ration + 10% pupae powder extract, R3= ration + 1% residue of pupae powder extraction, R4= ration + 10% residue of pupae powder extraction, R5= ration + 1% pupae powder, and R6= ration + 10% pupae powder

Table 3. Average body weight gain of quails during experiment (g/bird)

Treatments	Weeks							
	1	2	3	4	5	6	7	8
R0	72.10±0.27	75.28±1.15	80.12±1.14	85 ±2.12	95.87± 0.53	109±0.42	124± 0.12	139±-1.11
R1	72.13±1.69	75.14±0.32	80.09±1.25	87.00±1.13	97.89±0.22	118±0.23	127±1.28	142±1.34
R2	72.13±0.48	75.07±0.19	80.14±1.09	87.30±0.49	98.58±0.14	121±1.47	138±0.45	149±2.12
R3	72.03±1.13	75.15±0.21	80.23±0.47	86.30±0.18	96.87±0.67	110±2.23	124±1.47	138±0.45
R4	71.89±1.25	75.02±0.11	79.45±0.56	86.00±1.23	96.45±0.72	105±2.12	125±1.13	139±1.18
R5	72.34±0.43	75.18±0.41	80.17±0.68	85.59±0.57	95.98±0.54	115±0.61	130±1.26	138±0.67
R6	71.45±0.34	75.11±0.27	80.00±1.11	85.47±0.81	96.43±2.13	117±1.14	128±1.16	137±0.45

Note: R0= ration without pupae powder extract (control), R1= ration + 1% pupae powder extract, R2= ration + 10% pupae powder extract, R3= ration + 1% residue of pupae powder extraction, R4= ration + 10% residue of pupae powder extraction, R5= ration + 1% pupae powder, and R6= ration + 10% pupae powder

Table 4. Mean of erythrocytes, hemoglobin, hematocrit, and leukocytes number in quail blood during experiment

	Treatments							
	R0	R1	R2	R3	R4	R5	R6	Standard*
Erythrocyte number (10^6/mm^3)	3.24±0.27	3.39±0.32	3.05±0.38	3.46±0.29	3.22± 0.20	2.97±0.35	3.39±0.16	2-3.86
Hemoglobin level (g%)	9.94±1.69	9.75±2.60	12.21±2.42	7.57±1.57	12.23±1.88	10.83±2.23	10.03±1.66	7-13
Hematocrit value (%)	33.56±1.74	32.09±1.30	35.18±1.53	29.42±2.31	33.34±2.30	33.94±2.29	31.13±2.12	30-37
Leukocyte number (10^3/mm^3)	3.77±0.46^a	6.93±0.09^a	14.22±0.04^b	4.13±1.85^a	6.44±1.32^a	3.55±0.41^a	3.97±0.98^a	20-40

Note: Means in the same row with different superscripts differ significantly (P<0.05). *Based on Sturkie & Griminger (1976).
R0= ration without pupae powder extract (control), R1= ration + 1% pupae powder extract, R2= ration + 10% pupae powder extract, R3= ration + 1% residue of pupae powder extraction, R4= ration + 10% residue of pupae powder extraction, R5= ration + 1% pupae powder, and R6= ration + 10% pupae powder.

inhibit the formation of erythrocytes, thus laying quail conditions were healthy.

Level of Hemoglobin

The results showed that the hemoglobin concentrations in the quails fed with ration R1, R2, R3, R4, R5, and R6 were not different (P>0.05) (Table 4). Hemoglobin closely related to the erythrocyte and hematocrit. Hemoglobin is a simple protein, red color poster on erythrocytes, and function in the binding of oxygen. Hemoglobin concentrations of the experimental quails ranged from 7.57% to 12.23 g%. These hemoglobin values are still in the normal range. According to Sturkie & Griminger (1976), the normal hemoglobin level is 12.30 g%.

Value of Hematocrit

The results of the variance analysis showed that the hematocrit values of the experimental quails fed with ration R1, R2, R3, R4, R5, and R6 were not different (P>0.05) (Table 4). Hematocrit value is a term that means the percentage (by volume) of blood that consists of red blood cells after being centrifuged (Fradson, 1992). Hematocrit value is influenced by the number and size of red blood cells (Sturkie & Griminger, 1976). Hematocrit values in this study ranged from 29.42%-

35.18%. The hematocrit values were in the normal range. According to Sturkie & Griminger (1976), the normal hematocrit value in quail is 37%.

Hematocrit values indicate the total number of erythrocytes in the blood, thus becoming one of the indicators of the determination of the blood ability to carry oxygen (O2) which are commonly known as Oxygen Carrying Capacity (Maheswaran *et al*., 2008).

Decrease in hematocrit value can be caused by several other factors, namely the level of stress by environmental influences and nutrition, dehydration and parasites in the blood (Challenger *et al*., 2001). During the maintenance of experimental laying quail, ambient temperature tended to be high, ranging from 31-33°C. Heat stress can occur when the ambient temperature exceeds 32°C (Hemid *et al*., 2010). The condition causes laying quail consume more water. The high consumption of drinking water causes the water concentration in the blood increases, thereby decreasing the percentage of blood (hematocrit) (Tamzil *et al*., 2013). It can also be caused by the high levels of yolk precursors (William *et al*., 2004), and due to the increased concentrations of estrogen in the blood (Wagner *et al*., 2008), an assumption that illustrates the decline in quail's hematocrit concentrations with higher egg production.

Number of Leukocytes

Further test results indicated that averages numbers of leukocytes of the control group were similar to those of R1, R3, R4, R5, and R6 groups (Table 4). However, the R2 group quails had significantly ($P<0.05$) higher leukocyte numbers as compared to the other treatments ($14.22 \times 10^3/mm^3$). The increased number of leukocyte occurred at the 24 h after observation and decreased at 48 h ($9.86 \times 10^3/mm^3$) (Figure 1). The ranges of average number of leukocytes in the control (R0), R1, R2, R3, R4, R5, and R6 were 3.55 to $14.22 \times 10^3/mm^3$ and this range was lower but still under the normal range. According to Sturkie & Griminger (1976), the normal leukocyte count in quail ranges from 20 to $40 \times 10^3/mm^3$.

The number of leukocytes in this study tended to be lower compared to a standard leukocyte count in quail. This lower number of leukocyte was due to the state of heat stress because the ambient temperature during the experiment was in the range of 31-33°C. The decrease in the leukocytes number in the experimental laying quail in this study was approximately 50% when compared to standard quail leukocytes that were not in stress conditions (Table 3). Tamzil *et al*. (2014) reported that the levels of leukocytes in chickens decreased by 40% at the time of heat stress. Heat stress decreased the number of leukocytes so that the immune system became lower (Kusnadi, 2009; Usama *et al*., 2013). Muhammad (2013) stated that during stress the number of leukocytes and lymphocytes decreased as well as improving the ratio of H/L, which is one important indicator of stress in laying quail, especially during the laying period. Blood sampling in this research was performed at 7 weeks of age at the beginning of laying eggs. Female quails start laying eggs at the age of 6 weeks (Sujana *et al*., 2012). Blood sampling was conducted at 12, 24, and 48 h after giving the treatment.

Leukocyte is an active unit to provide fast and powerful defense against any material that causes infection (Guyton & Hall, 1997). Leukocytes play a role in responding the immune. The use of silkworm pupa powder was able to maintain the number of leukocytes in laying quails for functioning the immune system, because the silkworm pupae powder contained polysaccharides silkrose which could boost immune function. Polysaccharides silkrose of silkworm pupae could significantly increase macrophage phagocytosis and increase hemolysin antibody and lymphocyte transformation. This indicates that polysaccharide of silkworm pupae clearly indicate the presence of non-specific immunity (innate immunity) (Sun *et al*., 2007). Ethanol extraction could improve the content of polysaccharides found in insect pupae (Liu & Zhang, 2006). Polysaccharides silkrose contained in the 10% of pupae powder extract was assumed can increase the number of laying quail leukocytes which were experiencing stress on the production period. According to Hrabcakova *et al*. (2014), a lot of factors that affect the levels of leukocytes in poultry i.e., genetic (species and strains), exposure to stress (stress may be temperature, environment uncomfortable cage, handling), system maintenance, feed, production period (starter, grower, layer) and the lighting program.

Leukocyte Differentiation

In this study, the number of heterophile, lymphocytes, and monocytes were observed at 12, 24, and 48 h. This was done to document the effect of treatment on the immune responses by counting white blood cell types. The results of the variance analysis showed that R1, R2, R3, R4, R5, and R6 groups did not have significant different number of heterophile in laying quail blood (Table 5). The average number of heterophile

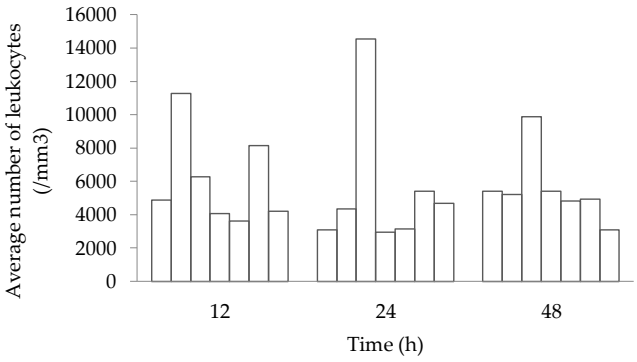

Figure 1. Average number of leukocytes in the experimental of quail blood at different sampling time. ⊡R0 ⊠R1 ⊠R2 ⊟R3 ⊠R4 ⊠R5 ⊟R6. R0= ration without pupae powder extract (control), R1= ration + 1% pupae powder extract, R2= ration + 10% pupae powder extract, R3= ration + 1% residue of pupae powder extraction, R4= ration + 10% residue of pupae powder extraction, R5= ration + 1% pupae powder, and R6= ration + 10% pupae powder.

in quail blood in the study ranged between 22.33% and 41.55%. The number of heterophile in this study was quite high, indicating quail treated R1, R2, R3, R4, R5, and R6 had good immune responses. Sturkie & Griminger (1976) stated that the percentage of normal quail heterophile ranged between 20%-30%. In this study, the number of heterophile increased at the 12th hour and then decreased until the 48 h of observation in the groups R2, R3, R4, and R5 (Figure 2).

The results of variance analysis showed that groups R1, R2, R3, R4, R5, and R6 did not have significantly different in number of lymphocytes in the quail blood studied. The range of lymphocytes in this study was 46.89% to 75.55% (Table 5). Lymphocyte values in this study were quite high, indicating that quail in the groups of R1, R2, R3, R4, R5, and R6 had a good immune desist. Sturkie & Griminger (1976) stated that the percentage of quail lymphocytes normally ranged between 30%-66%. In this study, the number of lymphocytes increased until the 24 h, although decreased in the 48 h (Figure 2). Lymphocytes have very important roles in the immune system (Melvin *et al*., 1993).

The results of variance analysis showed that treatment of R1, R2, R3, R4, R5, and R6 did not affect the number of monocytes in the quail blood. Monocytes in this study ranged 0.33%-3.67%. This indicated that there was no an acute infection in quail under the treatment of R1, R2, R3, R4, R5, and R6. This situation indicated the status of quail was in a healthy state. Sturkie & Griminger (1976) states that the percentage of quail monocytes normally range between 0%-8.1%. In this study, the number of monocytes increased in the 12th h up to 48th h, in the treatment of R1 and R2 (Figure 2). Polysaccharides silkrose that enters the body is regarded

Table 5. Percentage of leukocytes differentiation of laying quail during experiment

	Treatments							
	R0	R1	R2	R3	R4	R5	R6	Standard*
Heterophiles	37.33±0.27	37.80±0.06	30.79±0.42	36.00±0.41	36.53±0.46	40.34±0.10	40.22±0.45	20-30
Lymphocytes	61.34±0.28	60.20±0.12	64.55±0.83	62.00±0.43	61.58±0.64	57.00±0.31	59.45±0.40	30-66
Monocytes	1.33±0.33	2.00±0.09	3.67±0.52	2.00±0.47	1.89±0.38	2.66±0.11	0.33±0.33	0-8.1
Basophiles	-	-	0.33±0.33	-	-	-	-	0-2
Eosinophiles	-	-	0.66±0.43	-	-	-	-	0-3
H/L	0.61±0.15	0.63±0.10	0.48±0.06	0.58±0.17	0.60±0.12	0.70±0.18	0.68±0.15	0.34-0.43

Note: *Based on Sturkie & Griminger (1976). R0= ration without pupae powder extract (control), R1= ration + 1% pupae powder extract, R2= ration + 10% pupae powder extract, R3= ration + 1% residue of pupae powder extraction, R4= ration + 10% residue of pupae powder extraction, R5= ration + 1% pupae powder, and R6= ration + 10% pupae powder.

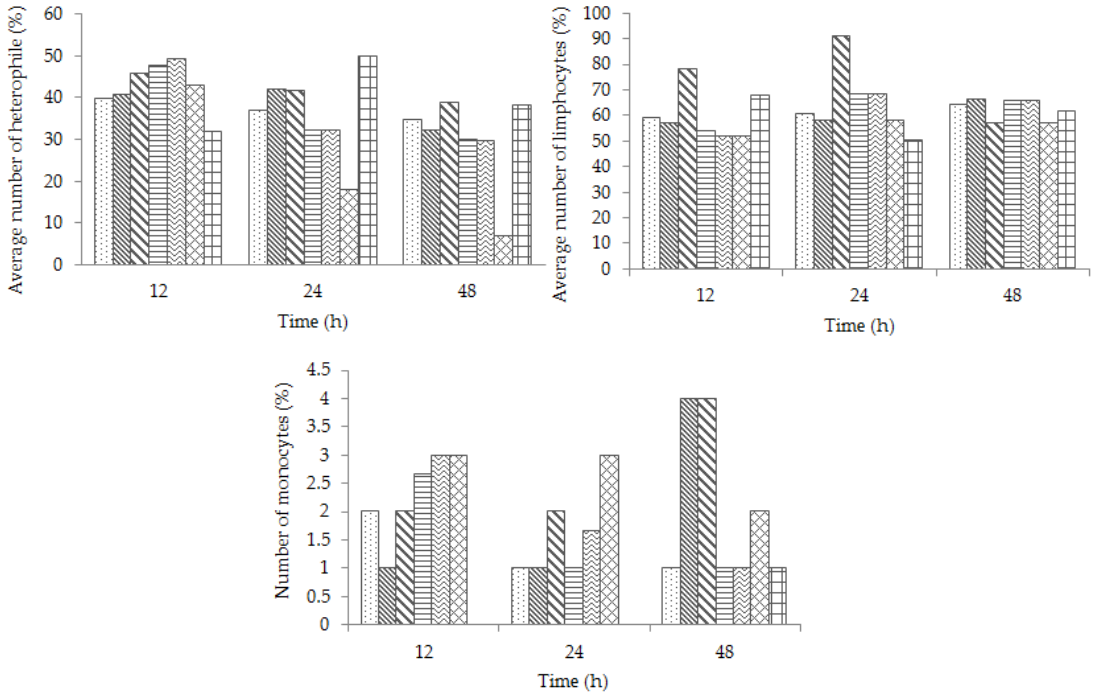

Figure 2. Leukocytes differentiation in the experimental of quail blood heterophiles, limphocytes, and monocytes at different sampling time. The treatments were R0= ration without pupae powder extract (control), R1= ration + 1% pupae powder extract, R2= ration + 10% pupae powder extract, R3= ration + 1% residue of pupae powder extraction, R4= ration + 10% residue of pupae powder extraction, R5= ration + 1% pupae powder, and R6= ration + 10% pupae powder. ⊡ R0 ⊠ R1 ⊠ R2 ⊟ R3 ⊠ R4 ⊠ R5 ⊟ R6.

as a foreign body, so it will attract monocyte through its phagositic capabilities. Monocytes are an essential component of the innate immunity (Parihar *et al*., 2010).

CONCLUSION

Total leukocyte of laying quail in this study significantly increased in quail treated with the addition of extract pupae powder with dose of 10% in the ration. The increase of leukocytes number occurred at the 24 h, and then decreased at 48 h after observation. The addition of silkworm pupae powder extracts containing polysaccharides' silkrose in the ration could increase the number of leukocytes in laying quail at heat stress conditions.

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