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# Blood Profile of Soybean Meal Substitution with Black Soldier Fly Larvae in New Zealand White Rabbit

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**Abstract** | Meat consumption in Indonesia is dominated by high-cholesterol meat that can lead to several cardiovascular diseases. Rabbits (*Oryctolagus cuniculus* Linnaeus, 1758), known as low cholesterol meat, can be alternatively chosen for daily consumption. Soybean meal (SBM), as the primary protein source in rabbit ration, has some disadvantages. Protein value in Black Soldier Fly Larvae -BSFL (*Hermetia illucens* Linnaeus, 1758) is equal to SBM and better in the amino acid profile. However, the chitin contained in BSFL may cause subclinical inflammation by blood parameter detection. Thus, this study investigated the BSFL substitution effect on rabbit blood traits. This study was performed using 28 wk old weaned male New Zealand White rabbits (1300 g  $\pm$  130 g) that were randomly divided into control group (T0) and treatment group (T1, T2, T3), which respectively substituted 0 %, 10 %, 20 %, and 30 % SBM with BSFL. Blood samples were collected from the right ear marginal vein. The result showed an increasing trend of leukocytes and neutrophils in the treatment group ( $P > 0.05$ ) that implicated the inflammation occurrence. The body responded to chitin as Pathogen Associated Molecular Pattern (PAMP) to trigger the inflammation. BSFL can substitute SBM. However, it needs further study to eliminate the inflammation response caused by chitin in BSFL.

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## Introduction

Beef and chicken, which have high-level cholesterol, are mainly consumed in Indonesia and can lead to several kinds of cardiovascular disease, including stroke and heart failure (D.J.P.K.H., 2017; Lloyd-Jones, 2010; Zotte and Szendrő, 2011). In Indonesia, beef and chicken consumption was about 0.538 kg

capita<sup>-1</sup> mo<sup>-1</sup> while cardiovascular-related disease increased from 25.8 % in 2013 to 34.1 % in 2018. This increase might be related to the consumption of high-level cholesterol meat, including beef and chicken that contain (60 to 114) mg 100 g<sup>-1</sup> cholesterol. On the other hand, rabbits (*Oryctolagus cuniculus* Linnaeus, 1758) include 56.4 mg 100 g<sup>-1</sup> cholesterol, an alternative daily meat consumption since they have

lower cholesterol meat. In addition, rabbits have a high reproductive rate and can be massively produced in a short period (Khan *et al.*, 2016). The gestation time takes about 30 d to 31 d and can reach up to 12 litter sizes at birth (Megan *et al.*, 2015).

Increasing the rabbit productivity needs intensified treatment, including giving high nutritious feed containing several ingredients that meet the daily rabbit nutrition requirement, such as pelleted ration (Rahman *et al.*, 2015). The intensified rabbit farms utilize soybean meal (SBM) as the main protein source in ration due to high protein value and its low price (Mauer *et al.*, 2016). However, in Indonesia, SBM is fully imported mainly from America and causes fluctuating prices of SBM due to the Rupiah (IDR) to Dollars (USD) exchange rate. These facts lead to the unstable rabbit meat price that finally may disrupt national food security (Bantacut, 2017). Thus it is necessary to find an alternative protein source to substitute SBMs.

The protein value in Black Soldier Fly Larvae-BSFL (*Hermetia illucens* Linnaeus, 1758) is equal to SBM and even better in the amino acid profile (Makkar *et al.*, 2014). Previous research has reported the increased productivity in layer hen and broiler (*Gallus gallus* Linnaeus, 1758), while another research found the decreased productivity and digestibility after being fed by BSFL (Cuttrignelli *et al.*, 2017; Mauer *et al.*, 2016; Mwaniki *et al.*, 2018). Chitin was assumed to be the factor that caused this result due to chitin inhibiting protein absorption. Furthermore, it was also reported that chitin which formed the exoskeleton of BSLF, caused subclinical inflammation. However, the symptoms could only be observed using hematology parameters, including the white blood cells (WBC) profile (Burton and Zaccane, 2007).

As one of the blood profile parameters, WBCs have an important role in protecting the body from foreign pathogenic and harmful agents through the inflammation process (Moore *et al.*, 2015), thus determining the WBCs population would show the body condition regarding inflammation after consuming alternative ration. Furthermore, red blood cells (RBCs) and hemoglobin might also show physiological change after consuming alternative percentages regarding the capacity to transport the digested nutrients. Therefore, determining the blood profile such as RBCs, hemoglobin (Hb), and WBCs

would reveal the effect of SBMs substitutions with BSLF meal (SBMs-BSLFM).

## Materials and Methods

### *Experimental design and diet preparation*

This study was performed on a local rabbit farm in Meteseh, Tembalang District, Semarang, Indonesia, under ethical clearance approval from Sultan Agung Islamic University with approval number 629/X/2019. The SBM and BSLF were purchased from the Animal Science Laboratory of Diponegoro University, Semarang, Indonesia. A total of 20 weaned male New Zealand White rabbits, 8 wk old, weighing  $1\,300\text{ g} \pm 130\text{ g}$  ( $\text{cv} = 10\%$ ), purchased from a local farmer, were randomly divided into one control group (T0) and three treatment groups (T1, T2, T3), which respectively substituted 0 %, 10 %, 20 %, and 30 % SBMs with BSFL M. Rabbits were reared for 10 wk and fed ad libitum in battery cages under 26 °C to 31 °C temperature, 70 % humidity.

### *Blood sample collection*

The whole blood samples were collected at the end of rearing time using a 3 cc syringe from the marginal vein of the right ear. The blood samples were put into an EDTA-contained blood tube to prevent clotting and transferred in a cool box to the laboratory for analysis. Red Blood Cells (RBCs), White Blood Cells (WBCs), Haemoglobin (Hb), and differentiated WBCs were analyzed using BC-3600 Mindray automated cell counter (Shenzhen Mindray Bio-Medical Electronics®).

### *Statistical analysis*

One way Analysis of Variance (ANOVA) was performed by IBM SPSS (V. 24.0) to determine the difference between treatments (Adinurani, 2016).

## Results and Discussion

This study showed no significant result in every blood profile parameter ( $P > 0.05$ ) as shown in Table 1. However, a slight increase trend of WBCs was shown in BSLFM substitution.

Black soldier fly larvae are one of the potential protein sources to substitute SBM in rabbit ration due to their high protein and amino acid profile value. Chitin mainly forms the exoskeleton of insects, inhibits protein absorption, and causes subclinical

**Table 1:** Blood profile of rabbit in BSLF substitution.

	T0	T1	T2	T3	Sig
RBCs (x10 <sup>3</sup> )	4.83 ± 1.51	5.64 ± 0.18	6.10 ± 0.47	5.93 ± 0.36	0.30
Hb (g dl <sup>-1</sup> )	10.70 ± 3.38	12.63 ± 0.77	13.23 ± 0.56	13.20 ± 0.69	0.33
WBCs (x10 <sup>3</sup> )	7.16 ± 4.65	7.36 ± 3.93	10.63 ± 5.15	12.56 ± 5.00	0.46
Neutrophils (%)	63.66 ± 5.85	69.00 ± 6.08	59.67 ± 11.71	67.67 ± 2.51	0.44
Lymphocytes (%)	29.67 ± 4.72	25.00 ± 5.19	34.67 ± 10.11	26.67 ± 2.08	0.31
Mononocytes (%)	6.67 ± 1.15	6.00 ± 1.00	5.67 ± 2.88	5.67 ± 0.57	0.86

**RBCs:** Red blood cells; **Hb:** Hemoglobin; **WBCs:** White blood cells.

inflammation and some health disorders, including asthma and allergy, that cannot be seen visually in the animal (Burton and Zaccane, 2007).

This study revealed that the RBCs and Haemoglobin were in the normal physiological range. On the other side, the treatment groups showed the overpopulation of leukocytes (Moore *et al.*, 2015). The elevated WBCs number indicated inflammation in response to the feed ingredients contained in the ration (Ahluwalia *et al.*, 2013). White blood cells generally play an essential role in removing harmful foreign substances that might be included in feed ingredients, and their number would increase along with the existence of these substances (Chmielewski and Strzelec, 2018; Moore *et al.*, 2015). The inflammation process can be triggered by several factors, including pathogen or damage molecule pattern exposure.

Chitin which is the peritrophic matrix of insects, was reported to be one of the pathogen-associated molecular patterns (PAMP) that would trigger the initial inflammatory response in animals once they were exposed to the body (Da Silva *et al.*, 2009; Klauser *et al.*, 2013; Mogensen, 2009). This complex process would induce some pro-inflammatory cytokines production, such as Interleukin-6 (IL-6), Tumor necrotic factor  $\alpha$  (TNF- $\alpha$ ) that will recruit more leukocytes to remove the PAMP-related chitin from the body (Da Silva *et al.*, 2009; Koller *et al.*, 2011). At the same time, IL-6 and TNF- $\alpha$  production caused by inflammation can reduce rabbit appetite leading to lower productivity (Broom and Kogut, 2017; Sieske *et al.*, 2019). However, symptoms of inflammation were not observed visually during the rearing period.

The limitation of this research was not investigating the pro-inflammatory cytokine, including IL-6 and TNF  $\alpha$  level, that would confirm the inflammation occurs in rabbits fed BSLF substitution.

## Conclusions and Recommendations

Substitution of soybean meals with black soldier fly larvae meals showed the normal condition of blood profile parameters. However, the increasing trend of leucocytes can indicate that inflammation occurs in the rabbit body, leading to reduced productivity. The chitin contained in BSLF needs further consideration due to triggering the inflammation process.

## Novelty Statement

BSLF as an alternative protein source became a significant concern due to its high protein profile and sustainability. However, the study regarding BSLF as a protein source in the ration is limited to poultry and fishery. In addition, there is less study to analyze the effect of BSLF in rabbits; thus, this study investigated the impact of SBMs substitutions with BSLF (SBMs-BSLF) on the rabbit blood profile.

## Author's Contribution

**AP:** Conceptualized and designed research, elaborated intellectual content, carried out experimental studies, data acquisition, data analysis, statistical analysis and revised manuscript.

**CMSL:** Performed literature search and reviewed manuscript.

**SS:** Analyzed data and reviewed manuscript.

**MFMA, MZ and MIM:** Elaborated intellectual content, performed literature search, reviewed manuscript and guarantor.

**AN:** Elaborated academic content, completed literature search, reviewed manuscript.

All authors read and approved the final manuscript

## Conflict of interest

The authors declare that there is no conflict of interest

regarding the publication of this article

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