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Proximate Composition of Larvae, Prepupae and Adult in Black Soldier Fly (*Hermetia illucens*)

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ABSTRACT

The black soldier fly (*Hermetia illucens* L.) is an insect species and able to convert low-value substrates is highly nutrient feed. Most of the aquaculture industry uses fish meal as a protein source in the diet of fish however fish meal was high in demand, price, and limited hence as an alternative the supplement protein should possess certain content to meet the role of fish meal in aquafeed products. Besides, a lack of research and studies on using BSF as a substitute in fish feed was noticed. This project aims to analyze the proximate composition in larvae, prepupae and adults were freeze-dried at -20°C, ground, and analyzed for proximate composition such as crude protein, crude lipid, ash, crude fiber, and moisture. Data were analyzed using one-way ANOVA to identify the significant difference in proximate composition in the three stages of BSF which are larvae, prepupae, and adult. As a result, the proximate composition revealed that BSF larvae possessed 32.3% crude protein, 26.7% crude lipid, 9.0% ash, 10.1% crude fiber, and 6.9% moisture while BSF prepupae contain 27.0% crude protein, 4.2% crude lipid, 10.6% ash, 8.6% crude fiber and 37.4% moisture also BSF adult have 52.6% crude protein, 15.6% crude lipid, 7.5% ash, 13.2% crude fiber and 25.6% moisture. BSF adult shows the highest crude protein content thus data was able to contribute more information on the nutritional value that potentially can be replaced in the diet of fish feed.

Keywords: *Hermetia illucens*, black soldier fly, proximate composition, feed, protein

INTRODUCTION

The increasing population worldwide is parallel with the demand for protein. In order to fulfill this need, animal cultures such as farming, and aquaculture industries are growing consistently in most of the world regions. To support these two industries, feed animal origin is frequently in demand and outsources from other animals and plants. At the same time, giving attention to the environmental issues as well as certain suitable raw materials are also needed (Allegretti et al., 2018; Dicke, 2018).

Recently, insects are found as new raw materials to be incorporated into animal feeds (Biasato et al., 2019; Gasco et al., 2019). Products from insects are proven to have a chemical composition and can meet the nutritional requirements of farm or cultured animals (Rumpold et al., 2013). This raw material is rich in crude protein, essential amino acids, and lipids containing fatty acids with anti-microbial properties (such as lauric acid) besides the other micronutrients (Gasco et al., 2018). One type of the proposed insect for this purpose is black soldier flies.

Black soldier fly (BSF) larvae are small harmless insects which used as raw materials in animal feeds for several agricultural and industrial applications (Smets et al., 2020). In terms of culturing, BSF requires non-selective and cheap rearing substrates such as plant debris and animal and domestic wastes. BSF is also able to decompose a variety of organic waste materials and convert it into valuable protein and fat-rich biomass (Gold et al., 2018).

MATERIALS AND METHODS

Sample preparation

An approval Ref: UAPREC/07/015 was obtained from Universiti Sultan Zainal Abidin (UniSZA) Plant and Animal Research Ethics Committee (UAPREC) before the study commenced. BSF larvae, prepupae, and adults were purchased alive from Green Soldier Biotech LPT and were delivered to UniSZA by postage. Upon arrival, all samples were stored in a deep freezer at -20°C. Prior to analyses, samples were freeze-dried using a CHRIST freeze dryer (Caligiani et al., 2019). Samples were ground using pestle and mortar and kept in zip lock bags. All analyses were conducted at UniSZA.

Determination of the proximate composition

Analysis of dry matter (DM), crude protein (CP), crude fiber (CF), crude lipid, ash, and NFE in experimental diets were conducted according to Analysis of the Association of Official Chemists (AOAC) as explained by (Komilus & Mufit, 2021). The Kjeldahl method was adopted for the determination of crude protein while the Soxhlet method was used for the determination of crude lipid. The sample was burnt to ash in a furnace at 550°C overnight. The ashes were placed in an oven at 105°C and the percentage of ash was determined after constant weight is achieved.

Crude protein

The percentage of crude protein in BSF samples was calculated according to the following formula (Komilus & Mufit, 2021):

$$\% N = \frac{A \times (T-B) \times 14.007 \times 100}{\text{Wt. of sample} \times 1000} \qquad \textbf{Eqn.1}$$

Where
T = Volume acid for sample
B = Volume acid for blank
A = Normality of HCl
F = Protein factor, 6.25

Crude fibre

The percentage of crude fibre in BSF samples was calculated according to the following formula (Komilus & Mufit, 2021):

$$\% \text{ Crude fibre} = \frac{[(W3 - W1)] - [(W4 - W5)]}{W2} \times 100 \qquad \textbf{Eqn.2}$$

Where
W1 = Weight of fibre bag (g)
W2 = Weight of sample (g)
W3 = Weight of crucible and fibre bag after digestion (g)
W4 = Weight of crucible and ash (g)
W5 = Weight of blank value of the empty fibre bag (g)
W6 = Weight of crucible (g)
W7 = Weight of crucible and ash of the empty fibre bag (g)

Crude lipid

The percentage of crude lipid in BSF samples was calculated according to the following formula (Komilus & Mufit, 2021):

$$\% \text{ Crude lipid} = \frac{W3 - W2}{W1} \times 100 \qquad \textbf{Eqn.3}$$

Where
W1 = Weight of sample (g)
W2 = Weight of extraction beaker (g)
W3 = Weight of extraction beaker + lipid (g)

Ash

The percentage of ash in BSF samples was calculated according to the following formula (Komilus & Mufit, 2021):

$$\% \text{ Ash} = \frac{\text{Difference in Wt. of ash}}{\text{Wt. of sample}} \times 100 \qquad \textbf{Eqn.4}$$

Difference in wt. of ash = W3 - W1

Where
W1 = Weight of empty crucible (g)
W2 = One gram of each sample taken in the crucible (g)
W3 = Cooled crucible + weighed (g)

Moisture

The percentage of moisture was calculated according to the following formula (Komilus & Mufit, 2021):

$$\% \text{ Moisture} = \frac{W1 - W2}{\text{Wt. of sample}} \times 100 \qquad \textbf{Eqn.5}$$

Where
W1 = Initial weight of crucible + sample (g)
W2 = Final weight of crucible + sample (g)
Note: Moisture-free samples were used for further analysis

Statistical analysis

Data were analyzed by one-way ANOVA to determine statistically significant differences in proximate composition in three stages of BSF. All of the required data were analyzed by using SPSS Windows 27.

RESULTS AND DISCUSSION

Proximate composition

This study was done to know the nutritional value in different life stages of black soldier fly (*Hermetia illucens*) larvae, prepupae, and adults. The standard procedures were used to determine the proximate composition of the black soldier fly and are presented in the graph below (Fig. 1).

Crude protein analysis

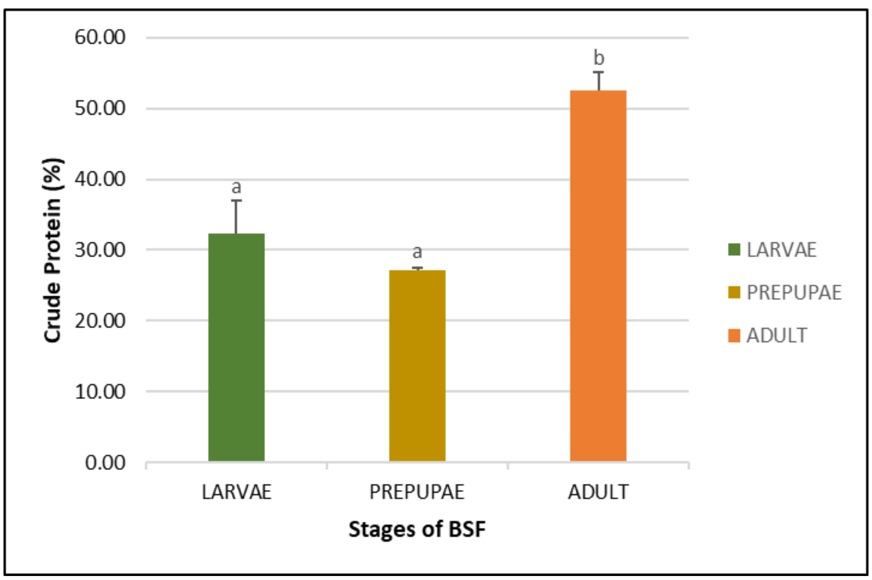

Fig. 1. Composition of crude protein.

The previous study had shown that BSF larvae and prepupae can be a replacement in the diet of fish feed (Barragan-Fonseca et al., 2017). In this study, the crude protein in the larvae is 32.3% and gradually decreased in prepupae to 27.0% and increased in adults of BSF with 52.6% of crude protein content. Fig. 1 shows no significant difference between larvae and prepupae but there is a significant difference for adult BSF. According to Huang et al. (2019), BSF larvae have 42.0% of crude protein content and almost the same as beetles which are 42.2% and followed by other insects such as *Tenebrio molitor* 38.3%, *Eristalis tenax* 40.9%, and cricket 32.6% crude protein. Therefore, BSF is an insect that is known as an 'energy' insect due to its richness of nutritional value such as protein and lipid.

Crude fibre analysis

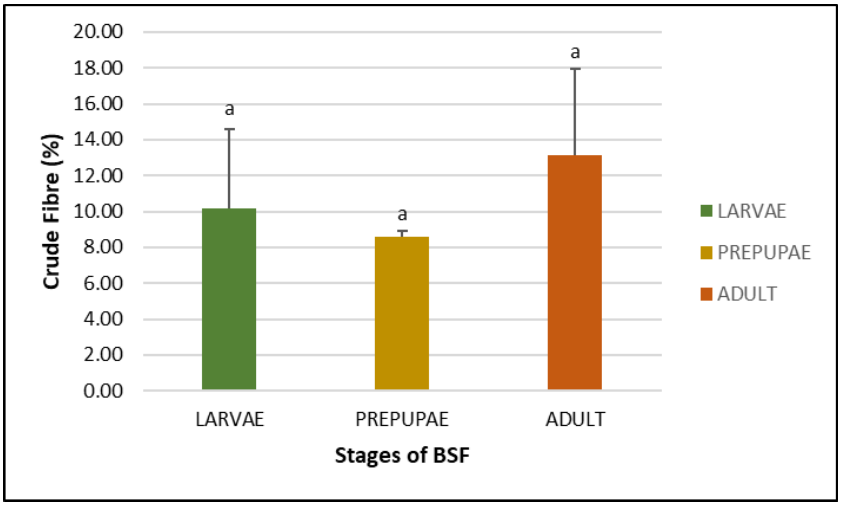

Fig. 2. Composition of crude fibre.

In this study, the crude fibre content in BSF prepupae is 8.6% and is higher compared to the previous study 5.37% (Ferdousi et al., 2022) and from Makkar et al. (2014) studies which is 7%. There are no significant differences in every stage of BSF as shown in Fig. 2. According to Ferdousi et al. (2022), there are few differences in crude fibre value due to the variation of rearing substrates, food sources, species, locality of the insects, and the method used during analysis.

Crude lipid analysis

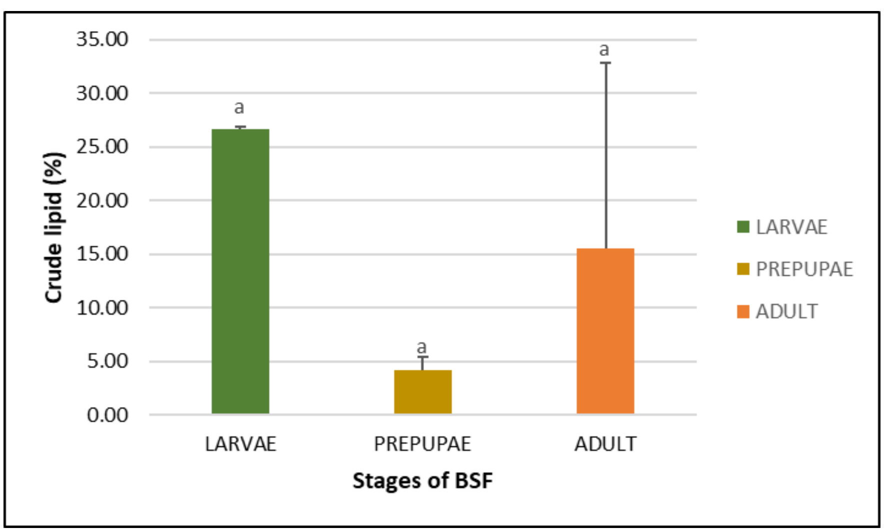

Fig. 3. Composition of crude lipid.

As BSF enter new life stages, the crude lipid content also differed (Liu et al., 2017) and in this study, the crude lipid content increased in BSF larvae as it reached its highest which is 26.7% while after entering the prepupae stage the crude lipid content decreased to 4.2% and increased back to 15.6% of crude lipid content when reached the adult stage. This fluctuation of crude lipid content may be due to adult BSF that requires energy for the reproduction of the species (Priyadarshana et al., 2021) and it shows no significant difference for every stage of BSF (Fig. 3).

Ash analysis

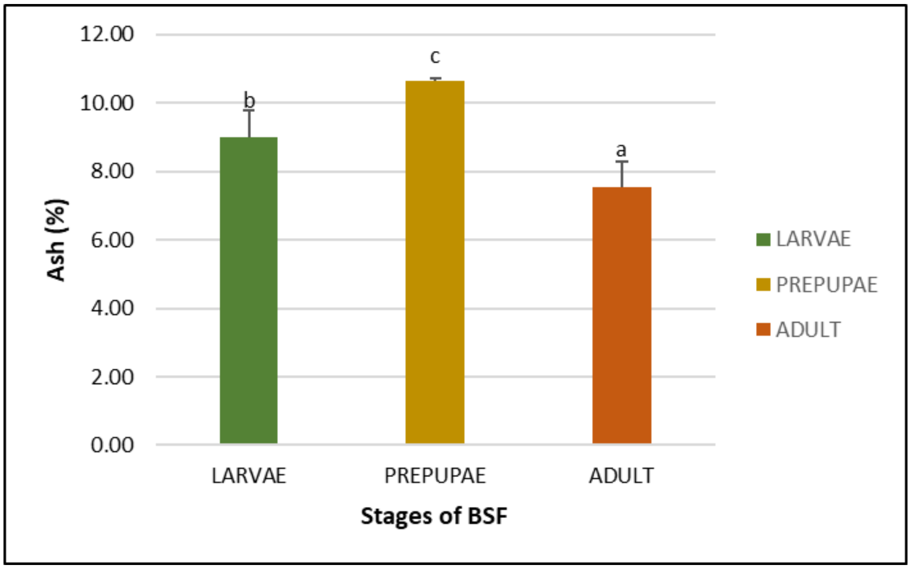

Fig. 4. Composition of ash.

The ash content in different life stages of BSF ranged between 7.5% - 10.6% and there is a significant difference in every stage of BSF (Fig. 4). From this study, prepupae of BSF has the highest ash content 10.6% compared to larvae 9.0% and adult 7.5%, indicating that prepupae BSF has high mineral content compared to larvae and adult of black soldier fly thus a higher level of minerals in the prepupae stage compared to larvae stage is due to cuticle formation during the prepupa period (Liu et al., 2017). Therefore, it is suggested that it is preferable to harvest BSF at the prepupae stage and make them as aquaculture feed (Priyadarshana et al., 2021).

Other minerals important for animal feed such as phosphorus, potassium, and magnesium, appear to be unaffected by the rearing substrate as the prepupae reared on bio-waste had a much higher ash content than those reared on restaurant waste, owing to a much higher level of calcium in the prepupae (Spranghers et al., 2017). Nonetheless, prepupae raised on energy-rich substrates with low ash and fibre content such as restaurant waste, appear to have very low ash content, making them more suitable as a feed ingredient (Spranghers et al., 2017).

Moisture analysis

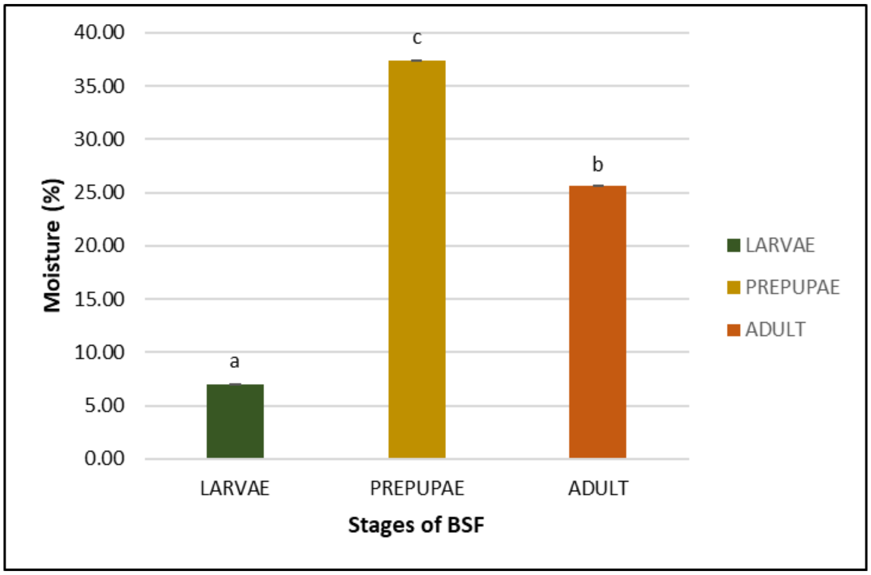

Fig. 5. Composition of moisture.

Freshly harvested prepupae contain about 50 - 60% of moisture content (Gangadhar et al., 2018). From this study, it was found that prepupae BSF has the highest moisture content 37.4%, followed by adults 25.6%, and 6.9% for BSF larvae after moisture analysis was done. Fig. 5 shows that there are significant differences in every stage of BSF. BSF development is dependent on feed quality (Nguyen et al., 2013) and also the amount of moisture in food affects larvae BSF. Humidity affects the decomposition of feeds as humid surroundings will slow down decomposition (Cheng et al., 2017).

CONCLUSION

This study has shown that different life stages of BSF have significant effects on nutrient composition. Results showed that there were statistically significant differences in nutrient composition among BSF which are larvae, prepupae, and adult ($p < 0.05$). This study suggests that adult BSF can be a replacement in diet formulation of aquafeed with high crude protein content in overcoming the high quantity of organic wastes throughout the world.

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