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Comparative Evaluation of *Hermetia Illucens* Larvae Reared on Different Substrates: Effect on Growth (The Yield, Nutritional Properties and Bioconversion) Rate

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ABSTRACT

Media usage for Black Soldier Flies Larvae (BSFL) growth has not been standardized yet, and many research has tried to elucidate different substrates to produce optimal BSFL growth. This study aimed to analyse the effect of different media with different metabolizable energy and nutrient content, such as tofu waste (high metabolizable energy), *Azolla microphylla* (moderate metabolizable energy), and layer manure (low metabolizable energy), on the yield and nutritional properties of 19-d-old Black Soldier Fly Larvae (BSFL). We grew BSFL from egg to 19-d-old larvae and analysed the BSFL yields by total and individual weight. Nutritional properties were analysed by using proximate, including water content, ash, crude protein, crude fiber, and crude fat. The results showed that the growth media of BSFL on tofu waste, *Azolla microphylla*, and layer manure showed significant differences in total weight and individual weight ($p<0.05$). Tofu waste produced the highest total weight (280.75±4.99 g). The nutrient content (water, ash, crude protein, crude fat, and crude fiber) of BSFL grown on tofu waste is the best for yield production. Tofu waste also showed excellent performance in nutrient content except for the percentage of protein compared to Azolla, which was the highest among media, and tofu waste as a growth media for BSFL produced lower protein content compared to Azolla. The fiber content of media might influence the protein content. In conclusion, we suggest using high metabolizable energy for BSFL growth media to obtain good yield and high nutrient properties.

Keywords: Animal feed, Edible insect larvae, Metabolizable energy, Production, Protein source

Introduction

The increase of livestock business globally showed that the livestock product needed by consumers and the demand is getting higher. Feed is one of the factors that influence success in a farm and has a very important role in terms of quantity, quality, and continuous availability of livestock industries. Feed costs can contribute up to 60-70% of total production costs in poultry industries (Jeon *et al.*, 2011; Anggitasari *et al.*, 2016), while feed costs in ruminant industries are of about 70-80% of total production costs (Priyanti *et al.*, 2012). The problems that generally occur are fluctuations in feed prices and limited stock, which farmers often feel. Farmers try to use unconventional products such as agricultural waste, but these products are usually low-quality, such as having high fiber and low protein content and needing treatment and technology to increase these feedstuff quality (Balehegn *et al.*, 2020). The other alternative is using industrial waste products, which is usually expensive. Nowadays, farmers and researchers try an alternative that can overcome this problem by utilizing edible insect larvae of Black Soldier Fly as a feed ingredient (Natsir *et al.*, 2020).

Black Soldier Fly larvae (BSFL) are larvae of the *Hermetia illucens* fly originating from North America and are included in one type of substitute feed ingredient, which has a relatively high protein content of 40-50% (Wardhana 2016). Another advantage of BSFL is that they have anti-fungal content, and interactions between the digestive tract microbes of BSFL can produce bioactive components that can increase the robustness of livestock when it consumed the larvae. (Faridah and Cahyono 2020). The BSFL also determined has immunomodulator activity as livestock feed (Lee *et al.*, 2018); de Souza Vilela *et al.*, 2021; Zhang *et al.*, 2021). Furthermore, Giannetto *et al.* (2020) said that the BSFL can convert agricultural, industrial, and livestock waste to valuable biomass.

The quality of larvae significantly influenced by the substrates media (Oonincx *et al.*, 2015). The need for BSFL growth media and its feed has yet to be standardized and is still in the exploration stage. In general, BSFL will grow well in sufficient crude protein conditions. The growing medium that is often used when cultivating BSFL has crude protein ranging from 7-20% (Dafri *et al.,* 2022). However, research on growth media based on the metabolic energy of substrate is limited. Farmers often use growth media, including vegetable waste, fruit or household organic waste, hospital food waste, and agricultural waste such as tofu waste. Utilizing other biomass, such as *Azolla microphylla* and chicken manure, is a potential medium for the growth of BSF larvae. These growth media contain different nutrients and physical characteristics, especially the metabolic energy and crude protein. Tofu waste has a crude protein (CP) content of around 21% and high energy with an average of 5.055 kcal/g (high ME) (Efendi, 2013; Muhtaromah *et al.*, 2021). *Azolla microphylla* has a protein range of 24% and moderate metabolic energy of 2.160 kcal/kg (Maharani *et al.,* 2013), while layer manure contains CP is around 17% and low metabolizable energy is 1.100 kcal/kg (Maharani *et al.*, 2013; Pamungkas *et al.*, 2012). This study aimed to analyze the effect of tofu waste media, *Azolla microphylla*, and chicken manure on the production and nutrient content of BSFL.

Materials and Methods

The materials used in the BSFL cultivation included a plastic bio pond measuring 19 x 19 x 10 cm and 24 x 25 x 8 cm, 5 mm sieve mesh for support and 2 mm size for filters, nets/gauze, scales, 5 g of BSFL eggs, tofu waste, *Azolla microphylla*, laying hen manure, water, and concentrate feed. The materials used in the proximate of substrate and larvae include ovens, analytical scales, aluminium cups, desiccators, clamping pliers, furnaces, Soxhlet's, 50 mL fat flasks, filter paper, Kjeldahl flasks, distillers, 250 cc Erlenmeyer, 50 cc burettes 0.1 scale, and hot plates, glass funnels, glass spatulas, dropping pipettes, beakers, volume pipettes, measuring pipettes, pip pumps, and baking trays, dried and mashed BSF larvae, technical hexane, H_2SO_4, NaOH, K_2SO_4, distilled water, borax acid, HCl, mm indicator, distilled water, and alcohol.

Growth media preparation

The growth media used is squeezed first to reduce its water content (50% moisture content) (Purnamasari *et al.,* 2021). We used a completely Randomized Design (CRD) using three growing media/substrate (Tofu waste; *Azolla microphylla*: and layer manure) and each media was conducted in four replications. The growth medium used was analyzed proximately to determine its nutrient content and the result is shown in Table 1.

Hatching and cultivation of BSF larvae

The hatching process used 5 bio ponds to produce 5-d old larvae which will be used for growing on different media. In the center of the hatching bio pond, we put a sieve mesh with a size of 5 mm as a support for placing gauze as a base for laying BSFL eggs. In one bio pond, we used 1 g of BSFL eggs and 1 kg of chicken concentrate feed which has been mixed with water until the water content becomes 75% as a hatching medium (Purnamasari *et al.,* 2019). The hatching process requires a cover such as a gauze so that green flies or house flies do not enter the bio pond. This process lasts for 5 d and then the larvae was collected by sieving using a 2 mm sieve mesh. Furthermore, larvae were moved and grew in different bio ponds with different media as treatments (tofu waste, *Azolla microphylla*, and layer manure). The 60 g of 5 days old BSFL were placed in the enlargement bio pond contained 60 g of media with water content about to 50% and the media was added as much as 60 g per bio pond every day until day 18 (Purnamasari *et al.*, 2020). On the 19^{th} day, BSFL were harvested by sieving the BSFL from the growth medium.

Data collection and analysis

Data on total production weight and individual weight are collected by weighing. The total weight calculation was carried out by weighing the entire BSFL per bio pond, while the individual larval weight calculation was carried out by weighing ten larvae taken randomly with two larvae from each side of bio pond and two larvae from the center of bio pond and then averaged for each repetition (Balhis *et al.*, 2022). Analysis of the nutrient content of BSFL in the form of water and ash content used the gravimetric method, crude protein used the Kjeldahl method, crude fiber used a Van Soest analysis, crude fat used the Soxlet method, according to SNI 01-2891-1992. Data analysis used one way ANOVA and was validated by normality and homogeneity tests (total weight and crude protein). Abnormal or inhomogeneous data were analyzed using Kruskall Wallis (individual weight, moisture content, ash content, crude fat and crude fiber) (Sheskin, 1996).

Table 1. Nutrient content of tofu waste, *Azolla microphylla*, chicken manure

Growth media	DM	Water content	Ash	OM	CF	CP	CFb	EM
	%							(kkal/kg)
Tofu waste	5.9	94.1	2.5	3.4	15.2	23.4	15.8	3.800
Azolla microphylla	33.5	66.5	20.2	13.3	1.4	20.4	12.6	2.860
Chicken manure	35.0	65.0	33.7	1.3	0.7	16.3	10.7	1.960

DM: dry matter, OM: organic matter, CF; crude fat, CP: crude protein, CFb: crude fiber, EM: metabolic energy.

Results and Discussion

The effect of growth media on BSFL production

The data showed that a significant difference ($p<0.05$) was found in the total weight and individual weight of BSFL (Table 2). The average yield of BSFL in several growth media showed varied results, where the provision of tofu waste (P1) showed the highest yield compared to *Azolla microphylla* (P2) and chicken manure (P3), both for the total and individual weight of BSF larvae. The BSFL growth media is very important for the quality of the BSFL produced. The components and percentage of nutrients contained in the growth media will produce BSFL with different nutrient content (Maulana *et al*., 2021).

Table 1 shows the metabolic energy (ME) content of tofu waste, *Azolla microphylla*, and chicken manure was 3.800, 2.860, and 1.960 kcal/kg respectively. EM is energy that livestock used for activities, including metabolism, production, reproduction, and others (Sudrajat and Kardaya, 2016). The higher EM in the media produced higher total weight and individual weight of BSFL. The tofu waste EM of about 3.800 kkal/kg produced a total weight of BSFL 280,75 g, and chicken manure with low EM (1.960 kkal/kg) produced around one-third of tofu waste performance (83.50 g). The components and percentage of nutrient content in the growth media also influence the production of BSFL (Fajri *et al.*, 2021).

Growth media that has a high protein and fat content can accelerate weight gain in BSFL (Dafri *et al.,* 2022). Growth media that has low nutrient components can result in low BSFL production because the nutritional components contained are limited or lacking for BSFL growth. The lack of nutrient components in the growth media causes BSFL to develop and takes longer to get high production (Masir *et al.*, 2020). Moreover, Monita *et al.* (2017) also revealed that the texture of the growth medium also affected the development of BSF larvae. BSFL like soft growth media rather than hard ones. Hard growth media is less palatable than soft; therefore, the tofu waste shows the highest production results because tofu waste has a soft texture so that it can optimize consumption for BSF larvae. Even though tofu waste has the highest fiber content among the media, at this fiber content level, BSFL can still digest it to optimize its growth performance. This might be because the cellulolytic microbiota in the BSFL gut, such as *Bacillus spp* and *Dysgonomous sp*., potentially help to digest fibers (Seyedalmoosavi *et al.*, 2022). The BSFL also utilizes various carbohydrates, such as glucose and xylose, to produce lipids (Li *et al.*, 2015). Nonetheless, the ability of BSFL to digest fiber requires more energy to digest higher fiber, which might cause lower production if growth media has high fiber content (Hasnol *et al.*, 2020).

The effect of growth media on the nutritional properties of BSF larvae

The different growth media showed significant differences ($p<0.05$) of water, ash, crude protein, crude fat, and crude fiber content of BSFL. The results of the average nutrient content of BSFL in several growth media showed varied results and are presented in Table 3. The results of the highest water content were produced from *Azolla microphylla* with an average of 76.00 ± 0.65%, followed by chicken manure with the lowest water content of 71.53 ± 1.07%, and tofu waste 58.11 ± 2.42%. Maulana *et al.* (2021) reported that the water content contained in these BSFL was obtained from the growth medium given to the BSF larvae. High water content in the growth media can cause anaerobic conditions, while too low water content can cause dry growth media conditions (Purnamasari *et al.,* 2021) and this causes the growth media to become hard and cannot be digested by BSFL (Monita *et al.*, 2017). We used standardized water content of the media about 50% while growing the larvae, and it resulted in different water content of larvae. The increment of the substrate's moisture content has a negative effect of on feed consumption, development time, body size and thickness of larvae. Its also affected the fresh weight of larvae (Dzepe *et al.*, 2019).

The results of the highest ash content were produced from chicken manure media which was 27.48 ± 0.97%. The ash content in the larvae indicates non-organic material produced after the process of burning non-organic materials (Purnamasari *et al.*, 2019). The ash content in BSFL is influenced by the ash content of the growth medium provided. The ash content in the growth media of chicken manure is higher than that of *Azolla microphylla* and tofu waste (Table 1). This is directly proportional to the results of the ash content of the BSFL produced.

The provision of growth media in the form of tofu waste, *Azolla microphllya,* and chicken manure produced a crude protein content of 50.57%, 60.55%, and 46.19%, respectively (Table 3). Table 1 shows that the protein content in the growth medium from tofu waste is higher when compared to *Azolla microphylla* or chicken manure, but BSFL which have the highest protein are fed *Azolla microphylla* followed by tofu waste and chicken manure. These results are also in line with the research of Shumo *et al.* (2019) which also shows that the protein contained in the growth medium does not have a positive effect on protein yield in

Table 2. Production of BSFL growth in different substrate

Variable	Treatments		
	Tofu waste	*Azolla microphylla*	Chicken manure
Total weight (g)	280.75±4.99[a]	103.00±4.39[b]	83.50±2.88[c]
Individual weight (g)	0.311±0.006[a]	0.122±0.003[b]	0.101±0.002[b]

[a,b,c] different superscripts on the same line showed a significant difference ($p<0.05$).

Table 3. Nutrient qualities of BSFL growth in different substrate

Variable	Treatments		
	Tofu waste	*Azolla microphylla*	Chicken manure
Water (%)	58.11±2.42^{c}	76.00±0.65^{a}	71.53±1.07^{b}
Ash (%)	9.36±0.26^{c}	22.73±0.83^{b}	27.48±0.97^{a}
Crude protein (%)	50.57±1.76^{b}	60.50 ±0.25^{a}	46.19±1.17^{c}
Crude fat (%)	16.94±0.50^{a}	3.07±0.11^{b}	1.32±0.23^{c}
Crude fi (%)	16.29±1.41^{a}	12.81±0.48^{b}	11.20±1.44^{b}

^{a,b,c} different superscripts on the same line was significant different ($p<0.05$).

BSF larvae, in this case, it can be caused by the growth medium has a low protein content but has a high organic matter content and high crude fiber. In BSF larvae, growth media containing crude fiber will be digested with the help of microbes so that it can increase the protein content in BSF larvae. Moreover, the type of fiber such as cellulose produced higher protein content than lignocelluse (Yakti *et al.*, 2023).

The results of the crude fat content showed that the administration of tofu waste media (P1) had the highest average yield of 16.94±0.50%. The results of crude fat in BSFL show that the high crude fat is affected by the crude fat from the growth medium, this is because Table 1 shows results that are directly proportional to the results of crude fat in BSFL (Table 3). Research by Aldi *et al.* (2018) also revealed that crude fat in feed can affect the yield of crude fat from BSF larvae.

The growth media of tofu waste produced BSFL with a crude fiber content of 16.29 ± 1.41%, this value was higher when compared to the growth media in the form of *Azolla microphylla* and chicken manure. The results of the crude fiber content test that has been carried out show that the high crude fiber content is also influenced by the crude fiber content in the growth medium. The results of the crude fiber content test that has been carried out show that the high crude fiber content is also affected by the crude fiber content in the growth medium. Nafisah *et al.* (2019) revealed that the crude fiber content comes from the exoskeleton, where the exoskeleton of the larvae contains chitin.

Bioconversion rate of growth media to BSFL nutrition properties

The usage of tofu waste for BSFL growth medium showed increased dry matter content (DM). Tofu waste DM only 5.9% increased sharply to 41.8%, and the organic matter and crude protein (23.4% to 50.57%) also had a high conversion rate, but crude fat dan crude fiber slightly increased (Figure 1). In contrast, BSFL bioconversion with growth media using *Azolla microphylla* and chicken manure decreased DM (Figures 2 and 3). BSFL larvae with Azolla microphylla media could convert media proteins by 20% to 60.55%, and BSFL using chicken manure converted protein from media as much as 16.23% to 46.19%. The conversion of crude fat and fiber also experienced a slight increase in all media tofu waste, *Azolla microphylla*, and chicken manure. Ash content from the bioconversion of tofu waste has increased while using *Azolla mycrophylla*, and chicken manure decreased. Tofu waste also produced the highest body weight and if considering the production and nutrition properties among media and larvae, tofu waste was the best media for growing BSFL.

The protein conversion rate (g protein required to produce 100 g of body weight) of BSFL reported to be 24.6% and takes only 21 d (Seyedalmoosavi *et al.*, 2022). The bioconversion ability of BSFL is also influenced by the ability to digest various kinds of media used. This is related to enzymes in the salivary gland and digestive tract where the main enzymes are amylase, protease (Seyedalmoosavi *et al.*, 2022), and lipase, but the interaction between digestive tract microbes also helps digest nutrient components that cannot be digested directly by BSFL, especially fiber digestion and provides nutrients such as vitamins, increases immune response, and as a competitor of pathogenic microbes, hormonal system and also behaviour (Zheng *et al.*, 2017; Kim *et al.*, 2011). Jeon *et al.* (2011) reported that the results of pyrosequencing analysis of the gastrointestinal microbiota of BSFL larvae can change and are influenced by the diet/substrate used. Moreover, polyphagous insects can synthesize a wide range of proteolytic enzymes for digesting the diverse proteins obtained from several substrates (Seyedalmoosavi *et al.*, 2022).

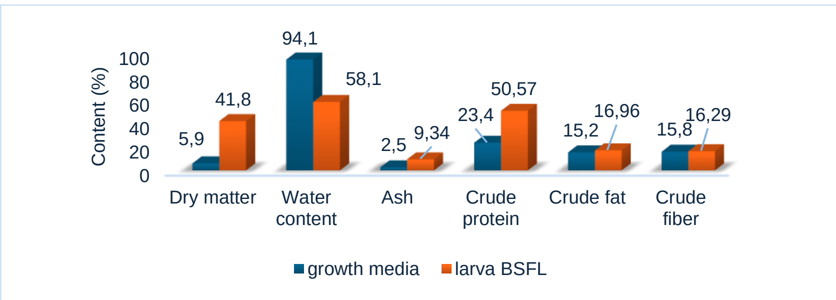

Figure 1. Nutrient content conversion rate of tofu waste to BSFL.

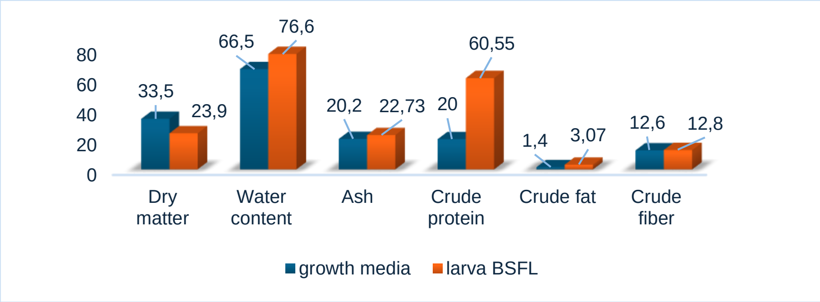

Figure 2. Nutrient content conversion rate of Azolla mocrophylla to BSFL.

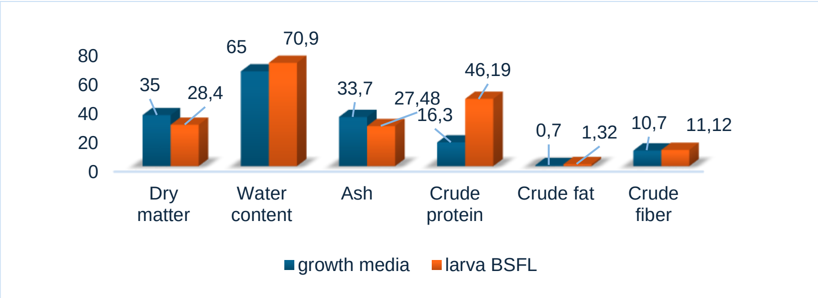

Figure 3. Nutrient content conversion rate of chicken manure to BSFL.

Conclusion

In conclusion, the application of different substrates influenced the yield and nutrient content of BSFL. High Metabolic energy in the substrate produced a high yield, including the total and individual weight of BSFL and high protein content. The nutrient composition of growth media influenced the nutrient composition of BSFL. The BSF bioconversion capacity to adjust low protein from substrate delivers a much higher protein, resulting in a 30-40% increase. The fiber content in substratemight be influence on protein yield of BSFL.

Conflict of interest

We have no conflicts of interest to disclose.

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Author's contribution

HK conduct the conceptualization, analyzed the data and write the manuscript, IT performed the experiment data analysis and write the manuscript, DEK write and layout the manuscript, MWJ write the manuscript, WM conduct the conceptualization and write the manuscript.

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