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NUTRIENT PROFILE OF BLACK SOLDIER FLY LARVAE (*Hermetia illucens*): EFFECT OF FEEDING SUBSTRATE AND HARVEST TIME

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ABSTRACT

The objective of this study was to asses the effect of different feeding substrates (FS), harvesting times (HT), and the interaction between FS and HT on the chemical composition of black soldier fly larvae (BSFL). The experiment used a 4 x 2 factorial arrangement with two main factors, FS (T1, T2, T3, T4), and HT (15-d and 20-d). Thus, there were eight treatment combinations, all together with five replications. The results show that there was no interaction (P>0.05) between FS and HT on dry matter (DM), crude protein (CP), crude lipid (CL), phosphorus (P), gross energy (GE), and amino acid contents. The ash content of BSFL grown on T2 media and harvested on day 15 was higher (P<0.05) than those grown on T2 media and harvested on day 20. The calcium (Ca) content of BSFL grown on all media and harvested on day 20 was higher (P<0.05) than those harvested on day 15. In conclusion, combining fruit wastes and tofu by-products produced BSFL with high CP content but low CL, ash, Ca, and P contents. In addition, BSFL grown on all substrates media and harvested on day 15 had better CP, Ca, and P contents. The dispensable amino acid of BSFLs fed with T3 diets was the best. The lowest body weight gain was produced by feeding a substrate containing a high percentage of rice bran. The findings indicate that the best nutrient composition of BSFL as animal feed would be achieved in early harvest time (15-d) and grown in heterogeneous feeding substrates.

Keywords: black soldier fly larvae, growing media, maggot, nutrient, proximate

INTRODUCTION

Protein sources for poultry diets generally come from both plant protein sources (PPS) and animal protein sources (APS). Generally, the utilization of PPS in poultry diet formulations is comparatively higher than APS. The Indonesian poultry feed industry still depends on PPS and APS from overseas, including meat and bone meal, corn gluten meal, and soybean meal (SBM). Argentina, Brazil, the USA, Paraguay, and India are the countries that produce and supply SBM for Indonesia (Natalia *et al.* 2019). Regarding meat and bone meal, Indonesia imports this ingredient from Australia, New Zealand and Canada. As reported by Dimiyati (2021), the volume of imports of PPS increased from 57.30% in 2015 to 84% in 2020. Meanwhile, the volume of imported meat and bone meal range from 0.63 to 1.05 million tons per year, so the economic value reaches IDR5.5-9.2 trillion (Dimiyati 2021). The price of these feed ingredients frequently fluctuates, depending on the exchange value of the US Dollar towards IDR.

Recently, the limited availability of SBM in the international market triggered the increase in prices of SBM. This limited availability of SBM in the international market was caused by the increased importation of SBM by China. Meanwhile, local soybeans are available but highly limited, so it is not a feasible option for feed millers. An increase in SBM prices will certainly have an impact on increasing the local compound feed prices.

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The cost of feed ingredients reached 83-89% of the total cost of broiler production, and 84 to 89% of the total cost of the laying hens (Natalia *et al.* 2019). Therefore, efforts to find alternative proteins must be made to overcome the issue of dependence on imported protein sources, as to finally reduce production costs. The black soldier fly (BSF) (*Hermetia illucens*) is one of the organisms studied for its characteristics and nutrient content. This fly originated in America and subsequently spread to subtropical and tropical regions of the world (Čičková *et al.* 2015). Indonesia's tropical climate is ideal for cultivating black soldier fly larvae (BSFL). Regarding cultivation, BSFLs are very easy to develop on a mass-production scale and do not require special equipment. Patterson *et al.* (2021) reported that the BSFL meal could be included in the laying hen's diets up to 16% without any negative effects on growth performance and egg production.

The crude protein (CP) content in BSFL was quite high, around 12.9 to 78.8%, with crude lipid content of 29 to 32%, 4.8 to 5.1% calcium (Ca), and 0.60 to 0.63% phosphorus (P) (Bondari & Sheppard, 1987; Bosch *et al.* 2014; Hopkins *et al.* 2021; Lu *et al.* 2022). The amino acid content of the BSFL, especially leucine, lysine, and valine, was superior to other protein sources such as SBM and fish meal, while methionine and tryptophan of BSFL were almost similar to that of SBM (Lu *et al.* 2020).

However, the nutritional value, survival, and performance of BSFL are influenced by various factors, such as the type and composition of the growing substrates and harvesting times (Barragan-Fonseca *et al.* 2017; Ewald *et al.* 2020; Broeckx *et al.* 2021; Lan *et al.* 2022). The growing media generally used for BSFL production are municipal waste, agro-industrial waste, and manure and feces (Supriyatna *et al.* 2016a; Dortmans *et al.* 2017; Broeckx *et al.* 2021). Dortmans *et al.* (2017) and Broeckx *et al.* (2021) reported that BSFL grew well in a substrate rich in protein and available carbohydrates. On the other hand, Tschirner and Simon (2015) found that the BSFLs fed with organic substrates high in fiber produced BSFL with higher CP content (52.3%) compared to those fed with substrates high in protein content with BSFL CP content of only 40%. In addition, the larva may consume the waste easily when the waste has undergone some microbial decomposition process and is in the form of pasta or liquid.

Most published data regarding the chemical composition of BSFL was obtained from single substrate or two different substrates (Tschirner & Simon 2015; Supriyatna *et al.* 2016a; Lan *et al.* 2022). The production of BSFL from a combination of substrates is limited. In addition, the chemical composition of BSFL that has been reported is mostly obtained from BSFL harvested on day 20. The published data of BSFL chemical composition harvested on day 15 is highly limited. Based on the above explanation, research has been conducted to evaluate the effect of different feeding substrates (FS) and harvesting times (HT) on the chemical composition and growth performance of BSFL.

MATERIALS AND METHODS

Feed Ingredients

The main ingredients used in this experiment are fruit wastes (banana and papaya) and vegetable wastes (mustard greens and water crest) obtained from a local traditional market, tofu by-products purchased from a local tofu market, and liquid palm sugar, sago (*Putak* meal), and rice bran obtained from a local distributor.

Experimental Design and Diet Formulation

The experiment was designed using a 4 x 2 factorial design, with the first main factor being feeding substrates (FS: T1, T2, T3, and T4) and the second main factor being harvested time (HT: 15 days and 20 days). Each treatment was replicated five times (10 kg/replication). The experimental diets were as follows:

Table 1 Growing media of black soldier fly larvae (BSFL)

Ingredients	Feeding Substrate			
	T1	T2	T3	T4
	…………………..% as fed..........................			
Fruits wastes (50% banana and 50% papaya)	24.4	24.4	-	-
Vegetable wastes (50% water crest and 50% mustard greens)	-	-	75	-
Tofu by-product	61	7	-	92.4
Rice bran	7	56	17.4	-
Sago meal	-	5	-	-
Effective Micro-organism (EM4)	0.3	0.3	0.3	0.3
Liquid palm sugar	0.3	0.3	0.3	0.3
Clean Water	7	7	7	7
Total	100	100	100	100
Calculated analysis (% as fed)				
Crude protein (%)	16.07	11.43	4.03	22.81
Crude lipid (%)	3.54	4.31	2.65	4.56
Crude fiber (%)	8.11	10.64	15.56	6.56
Ash (%)	3.70	8.87	17.19	3.67
Calcium (Ca, %)	5.53	4.51	27.54	8.32
Phosphorus (P, %)	2.76	2.91	2.18	0.22

Black Soldier Fly Larvae (BSFL) Production

BSFL was produced at three main stages, including (i) fermentation, (ii) BSF catching and mating, and (iii) the growing period. 1) *Fermentation:* All ground ingredients, free from harmful materials and inorganic elements, were weighed according to feeding substrate formulation. The ingredients were mixed until homogenous with a moisture level of 70 to 80% and fermented for seven days in a room. 2) *BSF catching, mating, and egg deposition:* on day seven, the fermented substrates were moved into a round plastic container and placed in a sheltered cavity to invite the BSF to mate and lay eggs. 3) *Growing period:* Once the larvae have appeared, the feeding substrates containing larvae were removed to the BSF housing for growing. 4) The BSF larvae (BSFL) were fed and grown till days 15 and 20 during the experiment. The feed given to the BSFL is 50 g/day per replication (container). The determination of feed given to BSFL per day (50 g) is referred to Supriyatna *et al.* (2016b). The authors reported that the feed needed for the best growing of one larva was 100 mg/day on a dry weight basis.

For the growing period, the BSFL used for growth performance data was placed separately from the BSFL for chemical composition data. There were 60 larvae per replication used for growth performance data.

Sample preparation

Black soldier fly larvae (BSFL) were harvested on day 15 (Figure 1a) and day 20 (Figure 1d). The BSFL from each treatment was removed from the leftover growing media, mixed with water, and then screened. The BSFL was then weighed to obtain the total wet weight. Then the BSFL was oven-dried (at 60° C, Memmert) for four days, crushed with a stone mortal, ground with a sample mill (0.5 mm screen size), and sub-sampled, packed, and labeled (Figure 1). The BSFL sample was sent to the laboratory for chemical analysis.

Figure 1 Sample Preparation of Black Soldier Fly Larvae. (a) Black Soldier Fly Larvae (BSFL) (15-days-old); (b) Oven-Dried BSFL (15-days-old); (c) Ground BSFL (15-days-old); (d) BSFL (20-days-old); (e) Oven-Dried BSFL (20-days-old); (f) Ground BSFL (20-days-old);

Chemical Analysis

The dried BSFL samples were analyzed for their dry matter (DM), crude protein (CP, crude lipid (CL), crude fiber (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), gross energy (GE), Ca, P, and amino acids. All the chemical analyses were performed at BPT Ciawi Bogor Laboratory. The DM content was determined by using AOAC method No. 930.15 (AOAC 2005). The nitrogen content was analyzed using AOAC 2001.1 (AOAC 2005), while AOAC 942.5 (Van Soest Method; AOAC 2005) was used to determine NDF and ADF contents. The starch content of sago was analyzed using the titration method. An automatic PARR Bomb Calorimeter was used to measure the GE level. The Ca content was analyzed using an Atomic Absorption Spectrophotometer (AAS, Flame Varian 220). The Ca analysis was conducted as described by Nalle *et al.* (2021): the sample was weighed and put in the muffle furnace for three hours (550^{o} C). Then, the ash was destructed using acid, solubilized, and pipetted to measure at AAS with the wavelength of 422,7 nm (Nalle *et al.* 2021). The spectrophotometer method (UV VIS Agilent Cary 100) was used to determine the content of P. The wavelength of the spectrophotometer used was 400 nm (AOAC 2012). The amino acid content was determined using High-Performance Liquid Chromatography (HPLC, ICI Instrument/Shimadzu SCL-10A/Shimadzu CBM 20A) as described by Nalle *et al.* (2019).

Statistical Analysis

The chemical composition of dried BSFL samples obtained from this experiment was analyzed using the two-way analysis of variance (ANOVA) referring to the General Linear Model procedure of SAS (SAS OnDemand). Meanwhile, body weight gain data were analyzed using the one-way analysis of variance (ANOVA) referring to the General Linear Model procedure of SAS (SAS On Demand).

RESULTS AND DISCUSSION

Chemical Composition of Black Soldier Fly Larvae

Table 2 describes the chemical composition of BSFL fed with different feeding substrates and harvested at different times. Statistical analysis showed that no interaction ($p > 0.05$) between FS, and HT was observed in the parameter of DM, CP, CL, P, and GE. There was an interaction ($p < 0.05$) between feeding substrate (FS), harvesting time (HT) for ash, and Ca content of BSFL. The ash content of BSFL grown in T2 media and harvested on day 15 was higher ($p < 0.05$) than those grown in T2 media and harvested on day 20. The ash content of BSFL reared in T1, T3, and T4 media and harvested on day 15 and day 20 was similar ($p > 0.05$). The present result partly agreed with Liu *et al.* (2017), who reported that there the ash content of late pupae was higher (10.2%) than the ash content of 14-day-old BSFL (8.3%). The range of BSFL ash content in the present study was 9.60 to 17.6%, which was in the range reported by Seyedalmoosavi *et al.* (2022).

The difference ($p < 0.05$) in Ca content was only found between BSFL grown in T3 media harvested on day 15 and those grown in T3 media and harvested on day 20. The tendency of higher Ca content in 20-day-old BSFL was probably due to the bone and teeth of BSFL having grown well. In a review by Seyedalmoosavi *et al.* (2022), it was reported that BSFL required minerals for the skeleton formation and the formation of other structural tissues (e.g., teeth). Furthermore, it was explained that although protein and chitin are the main components of the exoskeleton of most insect species, BSFL has a so-called mineralized exoskeleton which explains the high Ca content.

Statistical analysis showed that except for DM content, the main factor, FS affected ($p < 0.05$ to $p < 0.001$) the chemical composition of BSFL meal. The CP content of BSFL fed with T1 experimental diet was similar ($p > 0.05$) to the CP content of BSFL from the T2 diet; however, the CP content of T2 was higher ($p < 0.05$) than the CP content of BSFL fed with T3 and T4 diets. It is interesting to note that the T3 feeding substrate with the lowest CP content (Table 1) produced the BSFL with lower CP content ($p < 0.05$) compared to T1 and T2 treatment diets (Table 2). This could probably be due to the presence of higher antitrypsin and lectin contents in the tofu by-product used in the T3 treatment diet, which inhibits CP digestibility. Isanga and Zhang (2008) explained that tofu by-products contained 30 to 50% trypsin inhibitor and caused a negative effect on CP digestibility. Another possibility was that the lower mineral and vitamin contents in the T3 diet led to low nutrient metabolism.

It is interesting to note also that of the CP content of BSFL from T3 diets (high in fiber but low in protein) had similar ($p > 0.05$) CP content with BSFL grown in T4 diets (high protein substrates and low in fiber). The present result agreed with Tschirner and Simon (2015), who reported that the BSFLs given organic materials rich in fiber produced high CP BSFL. According to Tschirner and Simon (2015), the larva may consume the waste easily when the waste has been degraded by microbes and in the form of pasta. The present result indicated that the BSFL was tolerable to a high-fiber diet and could digest the fiber. Kim *et al.* (2011) reported that the BSFL digestion system contained several micro-organisms that produced lignin-cellulase, which degrades lignin into simple sugar. Supriyatna and Ukit (2016) reported that BSFL could degrade organic wastes because of the presence of cellulolytic produced by cellulolytic bacteria, especially *Bacillus sp.*

The range of CP content of BSFL obtained in the present study was 32.7 to 42.9% (as fed). The CP content of BSFL in the present study was lower than the CP content of BSFL reported by Tschirner and Simon (2015), i.e., 37 to 52.3%. The difference was probably due to the difference in the method, including the type of growing media and the harvested time. Growing media used by Wardhana (2016) included palm kernel meal, cow feces, pig feces, chicken excreta, fruit wastes, and other organic wastes. In addition, the BSFL was harvested on day 25. The growing media used by Tschirner and Simon (2015) was a mixture of middling (control group), dried distillers' grains with soluble (protein group), and dried sugar beet pulp (fiber group).

Regarding the second main factor, the CP content of BSFL harvested on day 15 was comparable ($p > 0.05$) to those harvested on day 20. The FS x HT interaction did not affect ($p > 0.05$) the CP content of BSFL.

It was expected that the CL content of BSFL fed with T4 and T2 diets should be higher than the CL content of BSFL grown in the T1 and T3 diets (Table 1 and Table 2) because of their high CL content in the diets. The reality showed that the CL of BSFL fed with the T3 diets was higher ($p < 0.05$) than in those fed with the T2 and T4 diets, while the CL content of T1, T2, and T4 was similar (p > 0.05). Thus, this study proved that the CL content of BSFL did not relate to the CL content of the grown substrates.

The CL of BSFL harvested on day 20 was higher (*p* < 0.05) than those harvested on day 15. This result indicated that as the age of BSFL increased, the CL content of BSL increased. No interaction (*p* > 0.05) between FS and HT was found in the CL content of BSFL in all treatment diets.

The ash content of BSFL fed with T4 diets was higher (*p* < 0.05) than those fed with T1, T2, and T3 diets. The Ash content of BSFL was similar (*p* > 0.05) between T1 and T2 when harvested on day 20. The BSFL grown in T2 diets and harvested on day 15 had higher (*p* < 0.05) ash content compared to those who were grown in the same feeding substrate but harvested on day 20. On the other hand, the BSFL fed with T3 and T4 diets and harvested on day 20 had higher (*p* < 0.05) respective ash content than those that were grown in the same feeding substrate but harvested on day 20. The comparison was difficult to be made due to the limitation of published data related to the present study.

Table 2 shows the ash content of BSFL grown in the T4 diets was higher (P<0.05) than the ash content of BSFL grown in T3. This was an unacceptable result since the ash content of T4 diets was very low (Table 01) but produced BSFL with high ash content. The ash content of the T4 and T1 diets was quite similar (Table 1), but these media produced BSFL high in ash content (Table 2). The present study indicated that the ash content of BSFL was not affected by the ash content of the grown substrates.

The Ca content of the BSFL fed with T3 and T4 diets were higher (*p* < 0.05) than those grown in T1 and T2 diets. This could be due to the higher Ca content of the T3 and T4 diets than the T1 and T2 diets (Table 1). The P content of BSFLs fed with T3 was lower (*p* < 0.05) than those fed with T1, T2, and T4. The lowest P content of BSFL in T3 diets is related to the lowest P content of T3 diets (Table 1).

Table 2 Chemical composition of black soldier fly larvae (BSFL) fed with different feeding substrates and harvested at different times

Feeding Substrate	Harvesting time (day)	Dry Matter	Crude Protein	Crude Lipid	Ash	Ca	P	Gross Energy
		……………………….% as fed					…………….……	(MJ/kg DM)
T1	15	93.4	39.6	29.9	10.7[cd]	1.65[d]	1.42	23.7
	20	94.8	36.9	36.1	9.83[d]	2.16[cd]	1.00	24.9
T2	15	94.1	42.9	20.7	13.2[b]	2.92[bc]	1.27	21.6
	20	92.4	40.0	33.0	9.60[d]	2.21[cd]	0.94	24.4
T3	15	94.5	32.7	36.9	12.9[bc]	3.68[b]	0.87	23.9
	20	94.4	33.6	35.9	14.4[b]	5.06[a]	0.83	23.5
T4	15	94.7	37.8	28.3	16.4[a]	4.87[a]	1.20	22.3
	20	93.8	34.5	30.8	17.6[a]	5.09[a]	1.16	22.2
SEM		0.617	1.75	2.99	0.815	0.295	0.086	0.67
Main Effects								
Feeding Substrate (FS)								
T1		94.1	38.3[ab]	32.9[ab]	10.2[c]	1.91[b]	1.21[a]	24.3[a]
T2		93.5	41.5[a]	26.8[b]	11.4[c]	2.57[b]	1.11[a]	23.0[ab]
T3		94.5	36.2[c]	36.4[a]	13.7[b]	4.37[a]	0.85[b]	23.7[a]
T4		94.3	36.1[bc]	29.5[b]	16.9[a]	4.98[a]	1.18[a]	22.2[b]
SEM		0.437	1.24	2.12	0.576	0.209	0.061	0.484
Harvesting Time (HT, day)								
15d		94.2	38.25	28.9[b]	13.3	3.28	1.19[a]	22.9
20d		94.0	36.27	33.9[a]	12.9	3.63	0.98[b]	23.8
SEM		0.309	0.876	1.50	0.407	0.148	0.043	0.25
Probability P> F								
FS		NS	***	*	***	***	**	*
HT		NS	NS	*	NS	NS	**	NS
FS x FA		NS	NS	NS	*	*	NS	NS

Notes: Different superscripts at the same column indicate significant differences (P < 0.05); * = significantly different at P < 0.05; *** = significantly different at P < 0.001; NS = not significantly different (P > 0.05); SEM = Standard Error of Mean.

Table 3 describes the indispensable amino acid (IAA) profile of BSFL fed with different feeding substrates and harvested at different times. Except for isoleucine, the interaction between FS and HT did not affect ($p > 0.05$) the indispensable amino acid content of BSFL. The content of isoleucine in BSFs fed with T3 and T4 diets and harvested on day 20 was similar ($p>0.05$) to those fed with the same feeding substrates but harvested on day 15. There was a tendency that as the age increased, the IAA content of BSFL increased. However, the statistical analysis showed that only the content of isoleucine in BSFL fed wth T1 and T2 diets and harvested on day 20 was higher ($p < 0.05$) than those fed with the same feeding substrates but harvested on day 15. This result partially indicated that the isoleucine content of BSFL increased as the age of BSFL increased.

Feeding substrate affected ($p < 0.05$ to <0.01) the content of leucine, phenylalanine, and valine content of BSFL. The leucine content of the BSFLs fed with T2 diet was lower ($p < 0.05$) than those fed with three other treatment diets. The difference in leucine and phenylalanine content of BSFL was probably due to the difference in leucine and phenylalanine content and the digestibility of feeding substrates. The phenylalanine content of BSFL grown in T4 feeding substrates was higher ($p < 0.05$) than those grown in T3 diets. No differences ($p > 0.05$) in valine content were observed in BSFLs fed with T1 and T2 diets and between T3 and T4.

Regarding the second main factor, the statistical analysis showed that the content of leucine, lysine, phenylalanine, and valine of 20d BSFL was higher ($p < 0.05$) than the respective content of leucine, lysine, phenylalanine, and valine of 15d BSFL. These results indicated that it is better to harvest the BSFL on day 20 because the indispensable amino acid profile of 20-day-old BSFL is better than 15-day-old BSFL. In addition, the results indicated that as the age of BSFL increased, the indispensable amino acid content increased. The comparison was difficult to be made because of the limitation of published data related to the present study.

Table 3 Indispensable amino acid (IAA) profile of black soldier fly larvae (BSFL) fed with different feeding substrates and harvested at different times

Feeding Substrate	Harvesting time (day)	Arg	His	Isoleu	Leu	Lys	Meth	Phen	Thr	Val
		………………………….% as fed…………………………………………								
T1	15	1.97	1.20	1.51[b]	3.03	2.57	0.46	1.47	1.48	2.29
	20	2.31	1.29	1.69[a]	3.23	2.65	0.51	1.51	1.48	2.39
T2	15	2.03	1.19	1.49[b]	3.01	2.57	0.55	1.38	1.66	2.25
	20	2.08	1.27	1.75[a]	3.18	2.64	0.51	1.50	1.49	2.39
T3	15	2.13	1.19	1.62[ab]	3.05	2.53	0.59	1.37	1.57	2.11
	20	2.23	1.25	1.69[a]	3.20	2.64	0.64	1.54	1.67	2.29
T4	15	2.21	1.36	1.63[a]	3.22	2.53	0.59	1.55	1.71	2.16
	20	2.15	1.26	1.65[a]	3.20	2.59	0.54	1.54	1.58	2.17
SEM		0.103	0.040	0.043	0.042	0.047	0.056	0.033	0.07	0.05
Main Effects										
Feeding Substrate (FS)										
T1		2.14	1.25	1.61	3.13[a]	2.61	0.49	1.49[ab]	1.48	2.34[a]
T2		2.05	1.23	1.62	3.09[b]	2.61	0.53	1.44[b]	1.58	2.32[a]
T3		2.18	1.22	1.66	3.13[a]	2.59	0.61	1.45[b]	1.62	2.20[b]
T4		2.18	1.31	1.64	3.22[a]	2.56	0.57	1.55[a]	1.64	2.17[b]
SEM		0.072	0.029	0.030	0.290	0.033	0.039	0.023	0.052	0.033
Harvesting Time (HT, day)										
15d		2.09	1.24	1.57[b]	3.08[b]	2.55[b]	0.55	1.44[b]	1.60	2.21[b]
20d		2.19	1.27	1.69[a]	3.21[a]	2.63[a]	0.55	1.52[a]	1.56	2.31[a]
SEM		0.051	0.020	0.021	0.021	0.023	0.028	0.016	0.036	0.023
Probability P> F										
FS		NS	NS	NS	*	NS	NS	*	NS	*
HT		NS	NS	***	***	*	NS	**	NS	**
FS x FA		NS	NS	*	NS	NS	NS	NS	NS	NS

Notes: Different superscripts at the same row indicate significant differences (P < 0.05); * = significantly different at P < 0.05; *** = significantly different at P < 0.001; NS = not significantly different (P > 0.05); SEM = Standard Error of Mean. Arg = Arginine; His = Histidine; Iso = Isoleucine; Leu = Leucine; Lys = Lysine; Meth = Methionine; Phen = Phenylalanine; Thr = Threonine; Val = Valine.

Regarding the dispensable amino acids (DAA), published data showed that the DAA contents are now considered important in poultry diet formulation. The calculation of DAA such as alanine, aspartic acid, glutamic acid, glycine, serine, and proline in poultry diet had been reported by previous researchers (Awad *et al.* 2015; Siegert & Rodehutscord, 2018; Hofmann *et al.* 2020). According to Awad *et al.* (2015), in which low-protein diets fortified with DAA (glycine, glutamic acid, proline, alanine, and aspartic acid) produce similar body weight gain with the group of birds which were given the balanced nutrition diet (positive control). They also reported that the addition of glycine improved the feed conversion ratio of birds. Thus, it is essential to have a database of the dispensable amino acid profile for each feed ingredient, including BSFL, used in poultry diets. Table 4 describes the profile of DAA of BSFL fed with different feeding substrates and harvested at different times.

Table 4 shows no interaction ($p > 0.05$) between FS and HT was found for the DAA content of BSFL. Regarding the first main factor (FS), the significance ($p < 0.01$ to 0.001) was in the aspartic acid, cysteine, glutamic acid, and proline of BSFL. The aspartic acid and glutamic acid content of BSFLs fed with T3 diets were higher ($p < 0.05$) than the aspartic acid and glutamic acid content of BSFLs fed with T1, T2, and T4 diets. The cysteine content of BSFLs with fed T3, and T4 diets were higher ($p < 0.05$) than those fed with T1 and T2 diets. The present results indicated the feeding substrate containing a high percentage of vegetable wastes (T3) produced the best dispensable amino acid profiles.

Regarding the second main factor, the statistical analysis showed that the content of aspartic acid and serine of 20d BSFL was higher ($p < 0.05$) than the content of aspartic acid, glycine, glutamic acid, and proline of 15d BSFL. These results indicated that it is better to harvest the BSFL on day 20 because the indispensable amino acid profile of 20-day-old BSFL were better than 15-day-old BSFL. In addition, the results indicated that as the age of BSFL increased, 15-day-old indispensable amino acid content increased. The comparison was difficult to be made because of the limitation of published data related to the present study.

Table 4 Dispensable amino acid (DAA) profile of black soldier fly larvae (BSFL) fed with different feeding substrates and harvested at different times

Feeding Substrate	Harvesting time (day)	Ala	Asp	Cys	Glu	Tyr	Ser	Gly	Pro
		…………………..% as fed……………………………..							
T1	15	0.66	3.51	0.30	4.85	1.11	0.62	1.11	1.08
	20	0.71	3.81	0.31	4.92	1.37	0.71	1.13	1.13
T2	15	0.37	3.06	0.36	4.87	1.07	0.58	1.04	1.13
	20	0.72	3.40	0.32	4.90	1.36	0.75	1.14	1.16
T3	15	0.72	3.53	0.44	4.75	1.16	0.72	1.18	1.42
	20	0.86	3.85	0.49	4.76	1.212	0.78	1.17	1.24
T4	15	0.81	3.87	0.44	5.01	1.15	0.75	1.19	1.38
	20	0.78	4.13	0.41	5.16	1.22	0.77	1.23	1.24
SEM		0.044	0.083	0.035	0.077	0.137	0.052	0.0471	0.063
Main Effects									
Feeding Substrate (FS)									
T1		0.73	3.23[c]	0.34[b]	4.89[b]	1.22	0.67	1.09	1.14[b]
T2		0.69	3.66[b]	0.31[b]	4.89[b]	1.24	0.67	1.12	1.11[b]
T3		0.79	4.00[a]	0.43[a]	5.09[a]	1.19	0.76	1.21	1.31[a]
T4		0.79	3.69[b]	0.47[a]	4.76[b]	1.19	0.75	1.18	1.33[a]
SEM		0.031	0.059	0.025	0.054	0.097	0.036	0.033	0.045
Harvesting Time (HT, day)									
15d		0.73	3.49[b]	0.39	4.87	1.12	0.67[b]	1.13	1.25
20d		0.77	3.79[a]	0.38	4.94	1.29	0.75[a]	1.17	1.19
SEM		0.022	0.041	0.017	0.038	0.068	0.026	0.023	0.031
Probability P> F									
FS		NS	***	***	**	NS	NS	NS	**
HT		NS	***	NS	NS	NS	*	NS	NS
FS x FA		NS	NS	NS	NS	NS	NS	NS	NS

Notes: Different superscripts at the same row indicate significant differences (P < 0.05); * = significantly different at P < 0.05; *** = significantly different at P < 0.001; NS = not significantly different (P > 0.05); SEM = Standard Error of Mean. Ala = alanine; Asp = aspartic acid; Cys = Cysteine; Glu = glutamic acid; Tir = Tyrosine; Ser = Serine; Gly = glycine; Pro = proline

The IAA and DAA content observed in the present study were lower than those reported by the previous researchers (Lu *et al.* 2022). The difference was probably due to the difference in methodology, especially for feeding substrate and harvested times.

Body Weight Gain (BWG) of Black Soldier Fly Larvae

Statistical analysis showed that the body weight gain (BWG) of BSFL harvested on day 20 was affected ($p < 0.05$) by the feeding substrates. This is in line with Kinasih *et al.* (2018), who reported that the body weight of BSFL was affected by the feeding substrates. Body weight gain of 15d-BSFL was not measured, so it is not presented in this article. As can be seen in Table 5, the BWG of BSFL fed with T2 diets (CP 11.43% and CF 10.64%) ($p < 0.05$) were lower than the BWG of BSFL fed with T1, T3, and T4 diets during the experiment. The difference in BWG may be due to the difference in nutrient composition, palatability, and digestibility of the feeding substrates.

It is interesting to note that the BWG of BSFL fed with T3 diets (CP 4.03% and CF 15.56%) (Table 5) was higher ($p < 0.05$) than the BWG of BSFL fed with T2 (CP11.43% and CF 10.64). Even though the feed intake was not recorded, the high BWG of BSFLs fed with T3 diets was probably due to the high feed intake in this treatment diet. The feed intake was not documented in this experiment because the media used was semi-liquid, so it was difficult to measure. The separation of BSFL and media should have been conducted by water so it would affect the weight of the feed. However, the palatability of the feeding substrate was conducted by observing the leftover of each treatment. The comparison was difficult to be made due to the limitation of publication related to the present research.

The BWG of BSFL fed with T3 diets (CP 4.03% and CF 15.56%) was similar ($p > 0.05$) to those fed with T4 (CP 22.81%; CF 6.56%) and T1 (CP 16.07%; CF 8.11%) diets. The reasons behind these results were: i) the BSFL could degrade fiber so the nutrients needed for growing were fulfilled; ii) the antinutrient level, especially protease inhibitors, in T3 diets was probably lower than in T1 and T4 diets. So, the nutrient digestibility in the T3 diets might be higher than in the T1 and T4 diets. As can be seen in Table 1, T1 and T4 diets contain a high amount of tofu by-products. The tofu by-product was made by soybean seeds, so the by-product may contain protease inhibitors that could inhibit protein digestibility since soybean contain trypsin inhibitory (Aviles-Gaxiola *et al.* 2018). As a consequence, the amount of nutrients absorbed would be low as well, which finally led to a low growth rate. Beniers (2021) reported that trypsin was located in the posterior part of the BSFL midgut, and the trypsin inhibitor may decrease its activity. However, the present result was not in agreement with Kinasih *et al.* (2018), who reported that BSFLs fed with tofu dreg and chicken feed had higher development time compared to those given horse manure and vegetable wastes. The difference was probably due to the difference in methodology.

Supriyatna and Ukit (2016) reported that the BSFL were able to digest organic wastes cellulolytic enzyme, which was produced by cellulolytic bacteria, especially *Bacillus* spp. in the BSFL digestive organ. Felicia and Suhartono (2021) reported that the whole body of BSFL contained proteolytic, amylolytic, chitinolytic, and cellulolytic activities. These enzymes play an important role in nutrient digestion (protein, starch, chitin, and cellulose). Kim *et al.* (2011) extracted gut BSFL and found several enzymes which were high amylase, lipase, and protease activities, trypsin-like protease activity of leucine arylamidase, α-galactosidase, β-galactosidase, α-mannosidase, and α-fucosidase.

Table 5 Growth performance of 20-day-old black soldier fly larvae (BSFL) (g) fed different organic substrates

Variable	Treatment				SEM	*p-value*
	T1	T2	T3	T4		
Initial body weight (g)	0.071	0.074	0.074	0.064		
Final body weight (g)	0.191	0.155	0.201	0.165		
Body weight gain (g)	0.122^{a}	0.082^{b}	0.127^{a}	0.102^{ab}	0.008	0.013

Notes: Different superscripts at the same row indicate significant differences (P < 0.05); SEM = Standard Error of Mean.

The present result did not agree with Spranghers (2020), who reported that the BWG of BSFL will increase as the dietary protein content increases. The difference was probably due to the difference in the feed ingredient used. Each feed ingredient contains a different level of nutrients and anti-nutrients. The experimental diet used by Spranghers (2020) was a chicken diet that was low in anti-nutritional factors. In this experiment, the authors used a self-mixing diet which consists of mostly organic wastes containing high anti-nutrients. The author also found that the shortage of lysine and methionine did not decrease the growth rate of BSFL.

CONCLUSION

Different feeding substrates and harvesting times produced different chemical compositions and amino acid profiles of black soldier fly larvae. The combination of fruit wastes and tofu by-products produced BSFL with high crude protein but low crude lipid, ash, calcium, and phosphorus. The use of tofu by-products as feeding substrates produced BSFL with the lowest crude protein content. Black soldier fly larvae fed with nearly all treatment diets and harvested on day 15 had higher crude protein, calcium, and phosphorus content. The dispensable amino acid profile of Black Soldier Fly Larvae fed with the substrates containing a high percentage of vegetable waste was the best. Feeding substrates containing a high amount of rice bran produced the lowest body weight gain.

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