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The Effects of Diet Formulation on the Growth and Protein Content of the Black Soldier Fly Larvae

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ABSTRACT

The purpose of this research was to compare the diet formulation for rearing the black soldier fly larvae based on the growth and protein content. Five diet formulas were used to rear the BSF larvae as follows: diet 1 (pork-based diet+ mixed with the basal diet formula adapted from Lastari et al.), diet 2 (chicken-based diet+ mixed with the basal diet), diet 3 (fish-based diet+ mixed with the basal diet), diet 4(pork + chicken + fish + mixed with the basal diet) and diet 5 (basal diet as a control diet). The prepupae of BSF in each diet formula were measured growth and analyzed protein content, amino acidcontent, and food safety standards. The resultshaveshownthat the prepupae of BSF as rearing in diet formula 4 had the maximum average width, length and weight of 5.44±1.26 mm, 17.08±1.32 mm and 0.34±1.12 g, respectively. Moreover, protein content in the powder of BSF prepupae was the highest at 48.24% when compared with others, while the diet formula 5 as a control was 44.38%. Fifteen types of amino acids were found, with the highest amino acid content being Glutamic acid, Aspartic acid and Alanine, with values of 4.68, 3.57 and 3.47 mg/ 100mg, respectively. In terms of food safety standards (chemical residues, microorganisms and heavy metal residues), they were lower than the standard values.

Keywords: Diet formulation; Protein content; Growth; Black soldier fly larvae

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Introduction

The Black Soldier Fly (BSF), *Hermetia illucens* (L.), is an insect in the Stratiomyidae family and belongs to the same group as houseflies. However, unlike houseflies, BSF do not attack human home environments and are not carriers of diseases and pests on farms. BSF originated in America and then spread to subtropical and tropical regions of the world [1]. BFS is a beneficial insect in the environment and animal feed as they are able to feed on various organic substrates of vegetables and animals. BSF has a short lifecycle of about six to seven weeks. Five main stages can be distinguished in the BSF lifecycle: egg, larval, prepupal, pupal and adult. Several pieces of research about the benefits of BSF, the research [2] found that BSF has a high content of protein and fat, making it a potential replacement for protein-rich ingredients in poultry diets.

Nowadays, the government has encouraged farmers to rear the BSF larvae, which are used to eat and decompose organic waste in households [3]. More importantly, especially during the prepupae stage of BSF, it is used as a source of supplementary protein or as a substitute in animal feed forducks, chickens and fish [4]. The research [5] has found that BSF larvae contain high protein (45-65%), high fat (26-35%) and energy 2,900 Kcal/kg. It also contains omega 3, 6 and 9 as well as contains 28.9-50.7% of the Lauric acidthat found in breast milk, which helps inhibit the growth of various pathogens. Additionally, it also contains vitamins, minerals and anti-oxidants.

The development of diet formulas is important for rearing BSF larvae in order to increase their nutritional value. The BSF larvae can serve as a cost-effective replacement for expensive protein sources. The research [6] focused on the development of snacks made from Nakhon native farm rice supplemented with cricket powder protein. It was found that cricket powder had a high chemical composition, especially in protein content (60.4% by weight) and fat (16.92% by weight). Therefore, this research aimed to study and compare different diet formulas for rearing the black soldier fly larvae on the growth and protein content. The food used to rear the BSF larvae is either agricultural waste or household waste for reuse. This research provides essential information for developing high-protein BSF larvae that can be utilized as a replacement or supplement in pet food, currently an expensive commodity. Additionally, it extends the application of local wisdom in utilizing insects as a biological feed source with high nutritional value, promoting the cost-effective use of natural resources for maximum benefit.

Materials and Methods

1.Insect for experiments

The BSF larvae are obtained by using food to attract the BSF to lay eggs, and these eggs are then reared in the diet formula [7]. The process involves combining 500 grams of

rice bran, 5 grams of brown sugar, and 5 milliliters of EM, then slowly adding water and mixing well. The young BSF larvae were reared in round plastic containers with a diameter of 45 cm and a height of 12 cm until the larvae are approximately 7 days old, at which point they are used in further experiments.

2. The study of the suitable diet for the growth of the BSF larvae

The 7 days olds of BSF larvae were reared in a total of 5 diet formulars and supplemented with the diet formular [8] as follows: 25% fermented rice bran + 25% fermented coconut residue + 25% fermented tofu residue + 25% fermented palm kernel meal. Protein and fat content were analyzed at 44.38 and 17.01%, respectively. The diet for rearing the BSF larvae was obtained from chemical-free farms. The diet formula for rearing the BSF larvae was as follows:

Diet 1: Morning glory (5%) + Carrot (5%) + Pumpkin (5%) + Watermelon rind (5%) + Pineapple rind (5%) + Banana rind (5%) + Pork (35%) + Mix with the based diet formula [8](35%)

Diet 2: Morning glory (5%) + Carrot (5%) + Pumpkin (5%) + Watermelon rind (5%) + Pineapple rind (5%) + Banana rind (5%) + Chicken (35%) + Mix with the based diet formula [8] (35%)

Diet 3: Morning glory (5%) + Carrot (5%) + Pumpkin (5%) + Watermelon rind (5%) + Pineapple rind (5%) + Banana rind (5%) + Fish (35%) + Mix with the based diet formula [8] (35%)

Diet 4: Morning glory (5%) + Carrot (5%) + Pumpkin (5%) + Watermelon rind (5%) + Pineapple rind (5%) + Banana rind (5%) + Pork, Chicken, Fish (35%) + Mix with the based diet formula [8] (35%)

Diet 5:The diet formula as a based diet formula adapted from Lastari et al. [8] (100%) (control) Table 1

Table 1 Diet formula as suitable for rearing the BSF larvae

Diet formula	Ingredient (%)										
	Morning glory	Carrot	Pumpkin	Water melon	Pine apple	Banana	Pork	Chicken	Fish	Pork, Chicken, Fish	Lestari et al., (2020)
1	5	5	5	5	5	5	35				35
2	5	5	5	5	5	5		35			35
3	5	5	5	5	5	5			35		35
4	5	5	5	5	5	5				35	35
5											100

Diet formula 4 : the ratio between pork : chicken : fish is 11.66 : 11.66 : 11.66

1. Study on the growth of the BSF larvae

The prepupae of BSF were randomly collected from each diet formulas, comprising 30% of the total number of BSF larvae. The growth of the prepupae of BSF was studied by measuring the width, length and weight under a stereo microscope according to the method [9].

2. Analysis of the protein content and amino acids of the powder of prepupae of BSF

A total of 100 of the prepupae of BSF from each diet formula were washed with distilled water. Then they were dried in an insect incubator at 60°C for 1-2 days, subsequently they were finely ground by a centrifuge through a sieve with a diameter of 80 mesh. After that, the powder of prepupae of BSF was analyzed for total crude protein by the Kjeldahl method [10]. After analyzing and obtaining the maximum protein content, the powder of prepupae of BSF was further analyzed for amino acid content using Inhouse method (HPLC-precolumn-AccQ*Tag) according to the method [11].

3. Study on food safety standard of the powder of prepupae of BSF

The high protein content of the powder of BSF prepupae was analyzed for food safety standards (TIS 9017 2550) as follows: Pesticide residue analysis referring to the method [12], Microbial standard analysis referring to the method [13] and analysis of heavy metal residue referring to the method [14].

4. Data analysis

Data on the BSF larvae were analyzed using the SPSS program, and analysis of variance (ANOVA) and mean comparisons were performed using Duncan's multiple range test (DMRT).

Results and Discussion

1. Comparison of the growth of the prepupae of BSF in reared different of diet Formulas

The results found that the prepupae of BSF as reared in diet formula 4 had the greatest average width, length, and weight of 5.44$\pm$1.26 mm, 17.08$\pm$1.32 mm and 0.34$\pm$1.12 g, respectively. The prepupae of BSF as reared in diet formula 5 (control) had average width, length and weight of 4.09$\pm$1.76 mm, 16.05$\pm$1.52 mm and 0.20$\pm$2.04 g respectively. (Table 2, Figure 1). Diet is the main factor affecting the growth of BSF larvae. The protein in the diet affected the formation

of cells and tissues of the body. The results found that diet formula 4 consisted of pork, chicken, and fish, which are protein sources, resulting in lager body size than those fed in other diet formulas. Diet formula 4 contained various protein sources such as pork, chicken, and fish, which the BSF larvae fed more types of protein sources. As a result, the amount of protein accumulated in the BSF larvae increases. According to the research [15], the BSF larvae fed a protein diet, resulting in an increase in body size. The average weight was 0.23±0.01 g, the length was 0.53±0.46 mm, and the width 4.35±1.54 mm when compared with the size of BSF larvae fed with carbohydrates, fruits and vegetables.

Table 2 The average length, width, and weight of BSF larvae reared in different diet formulas

Diet formula	Length (mm)	Width (mm)	Weight (g)
Diet 1	4.35±1.54^{b}	16.38±1.04^{b}	0.22±1.16^{b}
Diet 2	4.28±1.28^{b}	16.58±1.26^{b}	0.22±1.08^{b}
Diet 3	4.39±2.08^{b}	16.44±1.54^{b}	0.23±1.24^{b}
Diet 4	5.44±1.26^{a}	17.08±1.32^{a}	0.34±1.12^{a}
Diet 5 (control)	4.09±1.76^{b}	16.05±1.52^{b}	0.20±2.04^{b}
F-test	*	*	*
C.V. (%)	79.32	83.54	86.38

* = significance at p<0.05

Mean values in the same column with the same letter are not significantly different according to DMRT

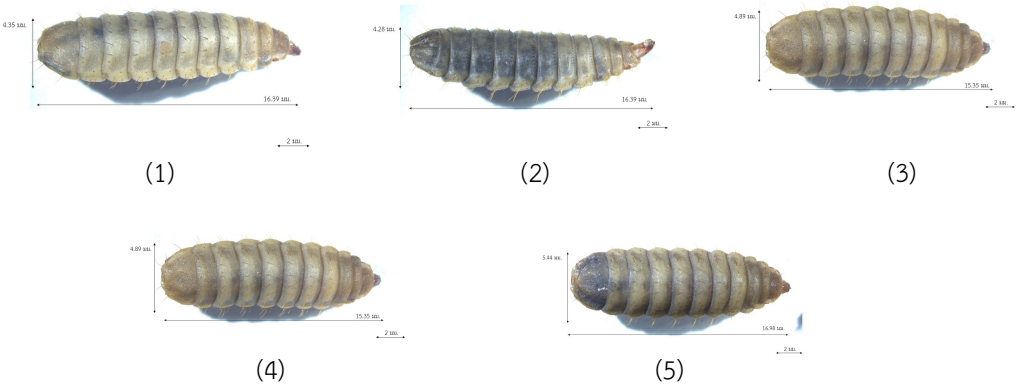

Figure 1 Width and length of prepupae of BSF reared in different diet formulas
(1) Diet 1 (2) Diet 2 (3) Diet 3 (4) Diet 4 and (5) Diet 5

2. Comparison of the protein content of the powder of prepupae of BSF reared in different diet formulas

From the comparison of the protein content of the powder of BSF prepupae reared in different diet formulas, it was found that the diet formula 4 consisted of Morning glory (5%) + Carrot (5%) + Pumpkin (5%) + Watermelon rind (5%) + Pineapple rind (5%) + Banana rind (5%) + Pork, Chicken, Fish (35%) + Mix with the based diet formula [8] had the highest protein content compared to diet formula 1-3 and diet formula 5 as a comparative control. The protein contentin the powder of BSF prepupae was 48.24%, while diet formula 1-3 had the protein content of 46.72, 46.66, and 46.42%, respectively. The diet formula 5, used as a control, had protein content of 44.38% (Table 3). The powder of BSF prepupae in diet formula 4 has the highest protein content because they were fed 3 protein sources (pork, chicken, and fish). The data from [16] showed that in 100 grams of pork, chicken, and fish, the protein content was analyzed as 27, 25 and 22 grams, respectively. When mixed together, the protein content increases. Thus, the BSF larvae accumulate a large amount of protein. According to the research [17], the diet used to rear BSF larvae will affect the amount of nutrients in the larvae. The diet with protein and fat resulting in the BSF larvae had more protein and fat nutrient accumulation. If the larvae BSF were used as fish food, fish have a high protein content of 70.9-74.1% and fat content of 20.3-22.8%. From the research of [18], it was described that protein was a type of nutrient that was important for animal growth. Amino acids, the smallest units of protein, play a crucial role in promoting cell and tissue growth.The experiment results indicate that the protein source used to rear the BSF larvae are pork, chicken, and fish, which serve as the main sources of protein. According to the research [19] using various protein sources significantly impacts animal growth compared to feeding protein alone, leading to increased body size and weight.

Table 3 The protein content of prepupae of BSF reared in different diet formula

Diet formula	Protein content (%)
Diet 1	46.72[a]
Diet 2	46.66[a]
Diet 3	46.42[a]
Diet 4	48.24[b]
Diet 5 (control)	44.38[c]
F-test	*
C.V. (%)	89.38

*= significance at $p<0.05$

Mean values in the same column with the same letter are not significantly different according to DMRT

3. The analysis of amount and type of amino acids

From the analysis of the amount and type of amino acids in the powder of BSF prepupae from diet 4, it was found that there were 15 types of amino acids and the amount of each amino acid. The types of amino acids included Aspartic acid, Serine, Glutamic acid, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Lysine, Isoleucine, Leucine, and Phenylalanine. The maximum amounts of amino acids were Glutamic acid, Aspartic acid, and Alanine, with values of 4.68, 3.57, and 3.47 mg/ 100mg, respectively. (Table 4). From the table 4, the amino acids can be divided into 2 groups; 1. essential amino acids (Threonine, Histidine, Valine, Lysine, Isoleucine, Leucine, and Phenylalanine), and 2. non-essential amino acids (Aspartic acid, Serine, Glutamic acid, Glycine, Arginine, Alanine, Proline, and Tyrosine). The research [20] described that Glutamic acid is a non-essential amino acid that is important in the nervous system. It acts as an intermediate in the transmission of nerve signals and facilitate metabolic processes in the body. Additionally, it enhances muscle firmness and strength and aids the immune system.

Table 4 Amount and type of amino acids of in the powder of prepupae of BSF reared in diet formula 4

Amino acids	mg/100 mg
Aspartic acid	3.57
Serine	1.68
Glutamic acid	4.68
Glycine	1.76
Histidine	0.74
Arginine	1.63
Threonine	1.45
Alanine	3.47
Proline	2.34
Tyrosine	1.66
Valine	2.27
Lysine	2.22
Isoleucine	1.60
Leucine	2.60
Phenylalanine	1.30

4. Food safety standard of the powder of prepupae of BSF

The results of the powder of BSF prepupae fed with diet formula 4 were analyzed according to the food safety standards, including chemical residues, microorganism, and heavy metal residues. The results showed the values lower than the standard thresholds

(Table 5 and 6). This was attributed to the diet used for feeding the BSF prepupae, and obtained from non-toxic food sources or organic farming. Consequently, the food safety standard of the powder of BSF prepupae was lower than the standard value. The food safety standard defines chemical residue obtained from farmer's plots without the use of chemicals.

Table 5 chemical residue detected in the powder of prepupae of BSF rearedin diet formula 4

Test item (group)	Test results	Standard (mg/kg)
Organochlorine		
DDT	0.05±0.15	0.5
Heptachlor	0.02±0.01	0.2
Mehtoxychlor	0.08±0.06	0.2
Dieldrin	0.08±0.33	0.5
Organophosphate		
Malathion	0.08±0.05	2.0
Chlorpyrifos	0.05±0.12	0.5
Dimethoate	0.02±0.03	0.2
Dichlorvos	0.08±0.33	0.1
EPN	0.02±0.08	0.2
Carbamate		
Carbaryl	0.01±0.03	2.0
Carbofuran	0.08±0.14	0.2
Methomyl	0.06±0.33	0.1
Methiocarb	0.03±0.01	0.3

MRL (TAS 9002-2016)

Table 6 The food safety standard of the powder of prepupae of BSF reared in diet formula 4

Test item	Test results	Standard (mg/kg)
Microorganism		
Salmonella spp.	No detected	No detected
Escherichia Coli	0.5 cfu/g	< 10 cfu/g
Coliform	50 cfu/g	< 300 cfu/g
Enterobacteriaceae	100 cfu/g	< 300 cfu/g
Total Plate Count	1×10^{2} cfu/g	< 1×10^{4} cfu/g
Yeast & Mold Count	10 cfu/g	< 1×10^{2} cfu/g
Heavy metals		
Cadmium (Cd)	0.1 ppm	< 2 ppm
Lead (Pb)	0.2 ppm	< 5 ppm
Mercury (Hg)	0.02 ppm	< 0.33 ppm
Arsenic (As)	0.1 ppm	< 2 ppm
Fluorine (F)	20 ppm	< 150 ppm
Carbamate		
Carbaryl	0.01±0.03	2.0
Carbofuran	0.08±0.14	0.2
Methomyl	0.06±0.33	0.1
Methiocarb	0.03±0.01	0.3

(Source: [21])

Conclusions

The prepupae of BSF reared on the diet formula 4, supplemented with pork, chicken, and fish protein, had the highest growth including width, length, and average weight. The powder of BSF prepupae was analyzed for protein content, and the highest protein content was 48.24%. A total of 15 types of amino acids were found, with the maximum amounts observed for Glutamic acid, Aspartic acid, and Alanine. In addition, the value of food safety standards for pesticide residue, microorganisms, and heavy metals were below the standard levels for animal feed. Therefore, the prepupae of BSF reared on diet formula 4 are safe and can be utilized in various fields, such as animal feed. It may be used as a supplement or a substitute for protein, representing a sustainable feeding option involving insects as a biological food source with high nutritional value and promoting the cost-effective utilization of natural resources for maximum benefits.

References

[1] Wang, Y. S. & Shelomi, M. (2017). Review of Black Soldier Fly (*Hermetia illucens*) as Animal Feed and Human Food. *Foods, 6,* 91-114.

[2] El-Hack, M. E. A., et. al. (2020). Black Soldier Fly (*Hermetia illucens*) Meal as a Promising Feed Ingredient for Poultry: A Comprehensive Review. *Agriculture*, 10*(8), 339.*

[3] Amrul, N. F., et. al. (2022). A Review of organic waste treatment using black soldier fly (*Hermetia illucens*). *Sustainability*, 14(8), *4565.*

[4] Sharanabasappa, D., et. al. (2019). Biology of black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) on muskmelon fruit. *Indian Journal of Entomology,* 81(1), 153-155.

[5] Abduh, M. Y., et. al. (2018). Factors affecting the bioconversion of Philippine tung seed by black soldier fly larvae for the production of protein and oil-rich biomass. *Journal of Asia-Pacific Entomology,* 21, 836- 842.

[6] Chooklin, S. (2020). *Development of Extruded Snack from Indigenous Upland Rice of Nakhon Fortified Cricket Protein Powder with Twin Screw Extruder to Commercial Product* (Research reports). Nakhon Si Thammarat: Rajamangala University of Technology Srivijaya. (in Thai)

[7] Photarin, S., et. al. (2021). *Promotion and Development of Farmer Potential through Environmental Management Innovations.* Bangkok: National Research Council of Thailand (NRCT). (in Thai)

[8] Lestari, A., et. al. (2020). Maggot Black Soldier Fly (*Hermetia illucens*) Nutritional Content Using Various Culture Media. *Journal Peternakan Integratif*, 8(3), 202-211.

[9] Burana, K. & Jamjanya, T. (2010). Seasonal Distribution, Rearing Methods and Nutritional Value of Black Soldier Fly (*Hermetia illucens* L.). In *The 11th Graduate Research Conference* (p. 603-609). 12 February, 2010, Khonkaen, Thailand. (in Thai)

[10] AOAC. (2000). *Official methods of AOAC international.* 17th ed. Maryland: The Association of Official Analytical Chemists.

[11] Subroto, et al. (2020). The Analysis Techniques of Amino Acid and Protein in Food and Agricultural Products. *International Journal of Scientific and Technology Research*, 9(10), 29-36.

[12] Wattanasoontorn, P. & Amornsanguansin, J. (2016). Detections of Pesticide and Herbicides Residues in Soils Samples from Paddy Fields in Chainat Province. *Academic Journal Uttaradit Rajabhat University,* 11(2), 246-258. (in Thai)

[13] Tiangthip, R., et. al. (2021). Investigation of Microbial Quality in Ready-to-Eat Food from Cafeterias of Thammasat University at Rangsit Centre. *Thai Science and Technology Journal,* 29(6), 1021-1031. (in Thai)

[14] Chuaduangpui, P. & Nooklum, R. (2015). *Heavy (Pb, Cd, Cu and Zn) contents in some*

Economic Marine Organisms in Fishing Ground along The Coast of Langu District, Satun Province, (Master thesis, Prince of Songkla University). (in Thai)

[15] Rasdi, F. L. M., et. al. (2022). Growth and Development of Black Soldier Fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) Larvae Grown on Carbohydrate, Protein and Fruit-Based Waste Substrates. *Malaysian Applied Biology,* 51(6), 57-64.

[16] McDonald, P., et al. (1995). *Animal Nutrition*. 5th ed. Harlow: England.

[17] Kröncke, N & Benning, R. (2023). Influence of Dietary Protein Content on the Nutritional Composition of Mealworm Larvae (*Tenebrio molitor* L.). *Insects,* 14(3),261.

[18] Hege, K. (2023) The Role of Nutrition for the Growth and Health of Animals. *Journal of Animal Research and Nutrition*, 8(1), 1-2.

[19] Abro, M. R. et al. (2012). Effect of Various Protein Source Feed Ingredients on the Growth Performance of Broiler. *International Journal of Medicinal Plant Research*, 1(4), 38-44.

[20] Zolotarev, V., et. al. (2009). Effect of free dietary glutamate on gastric secretion in dogs. *Annals of the New York Academy* of *Sciences*, 1170(1), 87-90.

[21] Department of Livestock Development. (2013). *Good Practice for Pet Food.* Bangkok: Ministry of Agriculture and Cooperatives. (in Thai)