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Development of semi-artificial feed in the larva stage of the black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae)

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Abstract. *Anasya AD, Sugiarto, Mahajoeno E. 2022. Development of semi-artificial feed in the larva stage of the black soldier fly* Hermetia illucens *(Diptera: Stratiomyidae). Nusantara Bioscience 14: 188-194.* Alternative food sources in fish farming besides pelleted feed are generally used, but many have also used biotic materials, larvae of the black soldier fly (*Hermetia illucens* L.). Maggot stadia larvae have a high protein content of more than 19%, could be mass-produced, are low-priced, and has a fast growth time. Therefore, high levels of protein and nutrients in maggots can be increased through suitable semi-artificial formulations. The purpose of this study was to determine the nutritional value of the semi-artificial feed recipe given and to determine the effectiveness of the semi-artificial feed recipe in increasing the nutritional value and survival of larvae. Data analysis used qualitative analysis with descriptive analysis method and quantitative analysis by determining the amount of increased nutritional value of *H. illucens*. The research method was carried out in several stages, including (i) insect rearing obtained 2nd generation of tillers (F2); (ii) manufacture of semi-artificial feed recipes; (iii) calculation of insect survival; (iv) measurement of larval mass weight after treatment; (v) testing of nutritional content value includes water content, fat content and protein content of *H. illucens* after treatment. Results of the evaluation larval survival after treatment were effective enough 100%, larval instar life phase was between 28-32 days, while the highest increase in the nutritional value of larvae water content was 45.90%, fat content 7.25%, and protein content 45.95%, the average increase in mass weight of larvae was 12.50%.

Keywords: Artificial feed, black soldier fly, *Hermetia illucens*, larval survival, the nutritional value of maggot

INTRODUCTION

Indonesia has the potential for livelihood in the field of fisheries after agriculture. Indonesia occupies the number three position in world aquaculture fish production and is a fish supply country that meets the international fish market (FAO 2020). Many fish ponds are currently being developed for export and import purposes. Fish farmers require special fish feed to increase the weight and quality of fish. The feeds usually used are pellets, fish meals, essential amino acids, fatty acids, and other micronutrients. The high price of fish feed causes farmers to need other solutions to reduce feed costs, one of which is replacing pellets with artificial feeds that are high in protein and at low costs.

The feed that can be used as an alternative source of animal feed, especially fish is the black maggot soldier fly *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae). The selection of *H. illucens* as an insect substitute for animal feed because it has a high protein content and can be mass-produced, is rich in protein at every stage of its metamorphosis with good protein quality. In addition, it is more environmentally friendly and can be bred sustainably for diet protein. Therefore, *H. illucens* provides adequate nutritional value for animal feed and ensures optimal digestive problems and intestinal health in fish (English et al. 2021). According to (Riddick 2014), another aspect considered for using insect species for feed purposes is that they can be reared in bulk to provide large quantities at an affordable price.

Improving the quality of live insects, especially in the *H. illucens*, can be used accurately as a natural animal feed resource for aquaculture. The insect has a short life cycle, is included as a source of protein, and is rich in nutrients at each stage of the larval phase. The high protein content and the ability of *H. illucens* to be used as animal feed encourage the production of *H. illucens* of high quality. Using *H. illucens* as a feed ingredient must have guaranteed chemical safety (Lieven et al. 2021). The composition of the larval-rearing substrate is an important factor that must be considered because it has a bioaccumulative risk of various organic compounds in the larvae being bred, so semi-artificial feed for *H. illucens* is an alternative that can be developed.

Mass rearing of insects requires the development of artificial feeds that can meet nutritional needs and ensure good insect rearing. In addition, feed formulation greatly influences larvae's survival, developmental rate, and yield (Danieli et al. 2019). Therefore, although the semi-artificial feed is used to reproduce insects, it is necessary if insects are needed in large quantities regularly and continuously.

Semi-artificial feed is expected to produce rapid growth and development of larvae. It has a high nutritional value, and it is necessary to multiply insects so that good-quality larvae will be produced, such as high protein, low-fat content, and no bioaccumulative risk of organic compounds using feed semi-artificial. Maggot growth is largely determined by the medium in which the larvae grow. The type of *H. illucens* likes the distinctive aroma of the media, but not all media can be used to lay eggs for *H. illucens* (Tomberlin et al. 2018).

The objective of the study was to determine the nutritional value of the semi-artificial feed recipe to increase the nutritional value and survival of larvae. The survival, growth, and bioconversion ability of black soldier fly larvae are determined by the type of food consumed by the larvae (Lalander et al. 2019). Food has a very large role in insect nutrition, so semi-artificial feeding must be adjusted to the needs and nutrition of insects. Research on artificial feeds for insects, especially *H. illucens*, has not yet been widely developed. Given the excellent benefits of *H. illucens* for animal feed, especially in the fisheries sector, it encourages efforts to increase the nutritional value so that they are good for use as an alternative fish feed.

MATERIALS AND METHODS

Materials

The materials for making artificial feed include ground sweet corn, rice polish, soybean flour, chicken feather flour, DL-methionine, vitamins and premixes, limestone, salt, monocalcium phosphate, sodium bicarbonate, and tetracycline formaldehyde 37%. The materials used to analyze fat content include filter paper and hexane solvent. Analyzing protein content includes carrageenan, nitrogen, hydrochloric acid (HCl) solution, 15% phenol, HgO, aquadest, 50% NaOH, K_2SO_4 concentrated, gauze, and red metal indicator.

Procedures

This research was carried out from March to June 2022 at the Integrated Laboratory, Universitas Sebelas Maret, Surakarta, Indonesia. The research method was an experimental method using a Completely Randomized Design (CRD) with four treatments, each repeated twice. The treatment used is as follows: (i) diet 1 treatment was formulated according to standard specifications of feed sources that are often used for rearing larvae for the control feed formulations with a feed intake of 120 mg per day. The nutritional composition of this feed was used as the control because it was formulated according to the nutritional specifications needed by insects per day; (ii) treatment using rice polish by mixing 10% chicken feather flour; (iii) treatment resembles the ideal amino acids profile of *H. illucens*; and (iv) treatment with rice polish added 10% chicken feather flour, each component of which has been adjusted.

Population and sample

The population in this study was the black soldier fly (BSF) insect (*H. illucens*), obtained from around community farms in the Surakarta area. First, wild parent BSF insects (F0) were caught using insect nets (Hoffman et al. 2021). Then, BSF breeding would be carried out. The sample in this study was the larvae resulting from the mating of three pairs of brooders which would then be transferred to a 100 mL pill pot until the age of five days and then transferred to a 15x15x15 box until the age of 18 days before being transferred to the cage and became generation 1 (F1). Breeding was continued to obtain offspring until the second generation, and further testing using larvae in second-generation 2 (F2).

Insect propagation

Insect propagation initially, three pairs of F1 adult insects were taken randomly (Woods et al. 2019), later becoming brood stock, then transferred to cages of 30x30x30 cm^3 to produce eggs. After the eggs hatch, they are transferred to 100 mL pill pots. Then after reaching the age of five days, they are transferred to a 15x15x15 cm^3 box where the environment is very concerned with both temperature and humidity, then 100 larvae will be occupied in on nursery box (Figure 1). The propagation of the test insects was carried out in 8 boxes, with each being reared and repeated twice.

Preparation of semi-artificial feed

The manufacture of semi-artificial feed recipes that will be used adapts Woods et al. (2019) research by replacing additional feeds made compatible with *H. illucens* larvae, such as spray-dried blood meal replaced with rice polish because they have the same high protein and can be used properly as a safe food. In addition, pig brains are replaced with chicken feather flour, adding more DL-methionine tailored to the needs of *H. illucens* and changing the composition dose ingredient. Artificial feed comprises the needs for the growth and development of *H. illucens* (Table 1). The feed that will be used is wet feed during the larvae phase until it reaches the 4th larva stage, then it will be converted into dry feed until the pupa phase. According to Bekker et al. (2021), *H. illucens* grows and develops at 30-70% substrate humidity. Optimal moisture content for larval development, ultimate weight, feed conversion efficiency, and yield is found in substrates with moisture content in the range of 50-80% (Cheng et al. 2017).

The parameters observed in this study were the survival rate of larvae, larval weight, totals of the larval instar phase, and analysis of the nutrients contained in the larvae after being treated.

Calculating the survival rate of larvae

The survival rate of larvae can be seen from the ability of *H. illucens* to adapt to artificial feeds and can be calculated based on the percentage (%) of its effectiveness by reducing the standard feed. The survival rate, including the percentage of the number of live larvae and the number of larvae used during rearing, was calculated using Wirawan et al. (2021) as follows:

$$SR = \left(\frac{Nt}{No}\right) \times 100\%$$

Where:
NT: Number of live larvae
No: Number of initial larvae that the research carried out

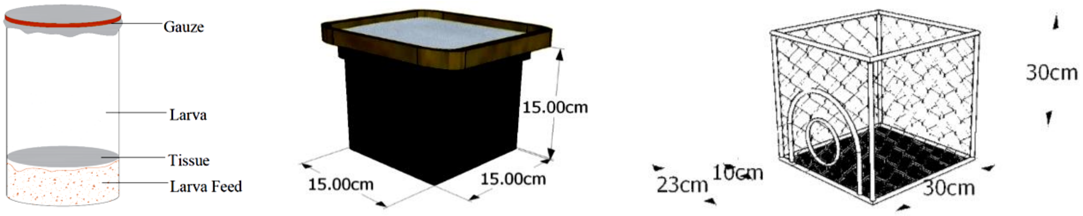

Figure 1. Insect propagation. A. 100 mL pill pot for five days old larvae, B. cage box for larvae, C. net cages for adult insect

Table 1. Preparation of semi-artificial feed recipes for *H. illucens*

Component	Diet 1 (control) %	Diet 2 %	Diet 3 %	Diet 4 %
Sweet corn	77.18	-	75.47	-
Rice polish	-	85.63	-	79.62
Soybean flour (46% crude protein)	18.62	0.22	20.12	4.43
Chicken feather flour	-	10.00	-	10.00
DL-methionine	0.07	0.07	0.29	0.27
Vitamin + premix	0.15	0.15	0.15	0.15
Limestone	1.82	1.87	1.81	3.13
Salt	0.25	0.21	0.25	0.21
Monocalcium phosphate	1.67	1.64	1.67	1.64
Sodium bicarbonate	0.24	0.21	0.24	0.21
Tetracycline formaldehyde 37%	0.01	0.01	0.01	0.01

Testing the weight of the larvae after being given treatment

The weight of the larvae was observed by measuring the weight of the larvae produced in the media for each treatment. Data collection on larval weight was obtained after the larvae were harvested at prepupa age; each treatment medium would be weight using a digital scale with a specification of 0.001 g. The result of the weight of larvae in each treatment medium was then recorded, and the total weight was calculated.

Larvae instar phase

The initial phase can indicate the feed media's success in meeting the nutritional needs of insects. The total instar phase of *H. illucens* shows the larval growth rate on the artificial feed given. Semi-artificial feed is expected to produce rapid growth and nutritional development and has high value. Therefore, feed nutrition is very influential on the total insect instar period.

Testing the nutritional content of larvae after treatment

After treatment, testing the nutritional status of *H. illucens* was done by analyzing the water, fat, and protein content contained in the larvae. The test method used in analyzing the moisture content by the oven (thermogravimetry), fat content using the micro-Soxhlet method (Zozo et al. 2022), and protein content using the Kjeldahl method (Maehre et al. 2018).

Analysis of water content

The principle of water content analysis is the distillation method, which is based on the direct measurement of the amount of water removed from the sample by evaporation (Deman et al. 2018). Water content is based on the difference by weight sample that determines the actual moisture before and after drying (Muchdar et al. 2021). Therefore, water in materials can be used as an index of stability during storage and a determinant of organoleptic. The water content analysis, according to Deman et al. (2018) procedure is as follows: (i) petri dish to be used is dried in the oven for 15 minutes, then cooled in a desiccator; after it has cooled, the weight will be calculated; (ii) the sample is weighed as 5 g put in a petri dish after that is dried in an oven for 6 hours at a temperature adjusted of 105°C; (iii) petri dish is cooled in a desiccator for 30 minutes and weighed again; (iv) petri dish dried in the oven again so that a constant weight can be obtained; (v) the following formula can calculate the percentage of water content.

$$\% = \frac{W_1 - W_2}{W} \times 100\%$$

Where:
W1: Weight of sample and petri dish before drying (g)
W2: Weight of sample and petri dish after drying (g)
W : Sample weight (g)

Analysis of fat content

Fat content analysis is defined as separating fat from the sample using a specific fat solvent. Analysis of the fat content profile was aimed at the essential fatty acid profile, especially the content of linoleic acid, which is important for the physiological process and the creation of linolenic acid (Adamkova et al. 2017). The procedure of analyzing fat content, according to Zozo et al. (2022), is as follows: i) 5 g homogenized sample was weighed and wrapped using filter paper, placed in a Soxhlet extraction device mounted above the condenser, and a fat flask below; ii) hexane solvent is used, and reflux is carried out until the solvent drops into the fat flask. Next, the solvent in the fat flask is distilled and collected; iii) the fat flask containing the extracted fat was then dried in an oven at 105°C for 5 hours; iv) the fat flask is then cooled in a desiccator for 20 minutes and weighed; v) the percentage of fat content can be calculated using the following formula.

$$\% = \frac{W_2 - W_1}{W} \times 100\%$$

Where:
W2: Final weight
W1: Initial weight
W: Sample weight

Analysis of protein content

Analysis of the protein content is the process of releasing nitrogen from protein in the material using sulfuric acid, which is carried out by heating. The Kjeldahl method is widely used to quantify insects' crude protein content ranging from 8 to 70% dry mass. The procedure evaluates the total Nitrogen (N) concentration, converted to a protein conversion factor (Levi and Jean 2017). The procedures for analyzing protein levels, according to Zozo et al. (2022), i) a 5 g sample is weighed, mashed, and put in a 30 mL Kjeldahl flask, added HgO concentrated; ii) destruction was carried out until a clear green color; iii) adding 60 mL of water and 50% NaOH solution before being transferred to a distillation flask; iv) distillate is accommodated in an Erlenmeyer flask which has previously been filled with 0.1 N and red metal indicator and then distilled accommodated; v) contents of the Erlenmeyer flask are titrated with 0.1 N NaOH until the yellow color is obtained; vi) the protein content is calculated based on the N content in the material by multiplying the conversion factor. The formula for calculating protein content is as follows.

$$\%N = \frac{(V1 - V2) \times N \times 14{,}007}{W\ (mg)} \times 6.25 \times P \times 100\%$$

Where:
%protein= % conversion factor (6.25)

Data analysis

Qualitative data analysis using descriptive and quantitative analysis was applied to determine the increase in the nutritional value of *H. illucens*. Quantitative analysis was used to determine the impact of changes in nutritional value due to the diet carried out with the amount of food given and the time required for larval growth after being given an artificial Diet, all compared in all treatments. Tests using the Tukey HSD test included calculating the survival rate of *H. illucens* and testing the weight of the larvae after being treated (F2). Meanwhile, the water content analysis, fat content analysis, protein content analysis, number of eggs, and total instar mass used analysis of difference test (significance was set p<0.05) were tested using ANOVA paired t-test. In addition, Duncan's distance test and statistical data analysis were calculated using the IBM SPSS version 22 variance to test the comparative hypothesis between samples and compare the highest increase in nutritional value in each treatment.

RESULTS AND DISCUSSION

The *H. illucens* is an insect with very good benefits, and it has a high nutritional and protein content, so that it can be used as an alternative to animal feed at a low price. Previous studies reported the nutritional content of *H. illucens* by providing feed from household waste for larval development and insect pupa stages. However, this study provides insight into changes in nutritional value and variations in the larval phase of *H. illucens*. The evidence of the information obtained supports insect breeders, fish farmers, researchers, and the entire animal feed industry, especially fish. In addition, research is valuable to improve mass breeding and development of livestock products using the *H. illucens* to meet future challenges in providing safe and viable protein as a priority for the global community. In this study, artificial feed contributed to a significant increase in larval weight of 12.50% and a short maggot life span compared to natural feeding. In addition, artificial feed increases the nutritional value of larvae with high protein and low-fat content, making it suitable for fish feed.

Larvae survival rate

The survival of insects is closely related to abiotic factors such as humidity. Temperature and relative humidity affected the survival of eggs. The relationship between development rate and temperature fits well with the linear relative humidity models (Mourao et al. 2021). The humidity in the study was 71%, *and H. illucens* had a high survival rate of 100% (Table 2). Environmental humidity is another important requirement for egg development and the survival rate of insects (Mourao et al. 2021). Humidity influences insect life. The optimum humidity of each insect varies according to each development's type and stage of life.

The higher humidity, the insect's body temperature increases; if the humidity decreases, the insect's body temperature will decrease. Humidity that's too high or too low can inhibit the activity and life of an insect, except for insects that can live in wet environments. The optimum humidity of each insect varies according to the development type and stage of life. In addition, humidity affects the evaporation of the insect's body preference for places to live and hide (Hasan et al. 2017). Calculation of the relationship between humidity and survival of larvae using the ANOVA was $1,6 \times (10)^{(-4)}$). At the same time, the Tukey HSD test, which was determined to be p<0.05, got a p-value of $2,54 \times (10)^{(-8)}$). It was concluded that humidity and feed nutrition significantly affected the survival rate of *H. illucens* in all treatments.

Humidity in this study tends to be stable. Efforts were made to stabilize the humidity by spraying with water using a spray in the study area during the day. The environmental humidity should not be too low or too high. Humidity that is too high encourages the growth of fungi and microorganisms. Air humidity that is too high or too low can inhibit the activity and life of an insect, except for insects that can live in wet environments.

Table 2. Percentage of survival *H. illucens*

Feed medium	RH (%)	Nt		$\overline{X}$	SR (%)
		1	2		
Diet 1	71	100	100		100
Diet 2	71	100	100		100
Diet 3	71	100	100		100
Diet 4	71	100	100		100
Σ		400	400		

Testing the weight of larvae after being treated

Testing the weight of the larvae after harvesting shows the success or failure of the given feed medium. The success index of larval weight is based on the high or low results of weighing the larvae after being harvested. The results showed that the weight of larvae (Table 3), the highest occurred in the Diet 3 of 20.85 g total weight, with an average weight of larvae per individual at 0.21 g. In comparison, the lowest weight occurred in Diet 2 at 18.45 g of total weight, with the weight of the larvae per individual at 0.18 g. The average weight of larvae in all Diet treatments was 19.34 g, while the average weight per individual was 0.19 g.

Meanwhile, according to calculations using ANOVA analysis where the significance is set at $p<0.05$, the p-value ANOVA is 0.00, while according to calculations using the Tukey HSD analysis with a significance set $p<0.05$, the results are 0.00, which means that the feed medium used is by the nutrients needed by *H. illucens*, besides that the nutrition of the feed has a significant effect on increasing the weight of the larvae after treatment. Based on level, Diet 1 and 4 feeds did not significantly affect larval weight after treatment. Diet 2 and 4 feeds did not significantly affect larval weight after treatment. Diet 3 significantly differs from Diet 1, 2, and 4. Diet treatments had a highly significant effect on the weight of larvae after treatment (Table 3).

The weight of the larvae after harvest indicates the ability of the larvae to accept the given feed medium and an indication of the success of the feed media used. The quality and availability of food affect the growth and development of insects. The stunted development and growth will cause individuals to have small sizes when the larvae to adult insects. Slower development leads to high mortality (Holmes et al. 2020). Differences in nutrition in the feed will cause various nutritional content of larvae. Weighed of *H. illucens* larvae is strongly influenced by the media they breed (Tschirner and Simon 2015). Media that has good quality and quantity has a good impact on the nutritional value of *H. illucens* produced, as well as accelerates its growth and development. Feed formulation affects larvae's survival, developmental level, and yield (Pacheco et al. 2022).

Larval instar stage

The fastest instar phase results occurred in Diet 3 with a total larval instar period of 28 days, while the longest larval instar period occurred in Diet 2 with a total larval instar period of 32 days (Figure 2). The relationship between the nutrition of the feed given and the total larval instar mass was calculated using the ANOVA paired t-test, which was set at $p<0.05$. The results were $1,5x\ (10)(^{-8})$, and it can be concluded that the nutrition of the feed given has a very significant effect on the total insect instar period. Nutrition has a very large influence on the length of the insect instar. The instar period is faster, allowing the nutritional needs to grow and develop to be met properly (Holmes et al. 2020).

The nutrients an organism absorbs from the diet are essential for development and determine how organisms can maximize their fitness. Alteration in diet quality during development has a wide-ranging effect on many life history characteristics (Chapman et al. 2013). A diet's two major nutritional components that contribute to development are proteins and carbohydrates. Protein provides essential amino acids necessary for viability. Imbalances in dietary amino acids can significantly affect development and the total instar stage. Carbohydrates provide energy for development and represent the mechanism by energy stored for the future (Nash and Tracey 2014).

Water content

The results of testing the water content in this study showed that the highest water content was found in Diet 3, with a total water content of 45.90%, while the lowest water content occurred in Diet 1, with a water content of 41.90%. Based on analysis using the ANOVA paired t-test obtained a significant result of 0.02. Furthermore, based on Duncan's Multiple Distance analysis at a significance level of 5%, the treatment of Diet 1 (control) was significantly different compared to diets 2, 3, and 4. While feeds 2, 3, and 4 had no significant difference (Table 4).

Figure 2. Chart total larval instar phase after treatment

Table 3. The average weight of larvae after being treated

Diet	Treatment (gr)		Average Weight (g)	Standard Deviation
	1	2		
Diet 1	19.40	19.20	19.30	0.14 b
Diet 2	18.70	18.20	18.45	0.35 a
Diet 3	21.30	20.40	20.85	0.64 c
Diet 4	19.00	18.50	18.75	0.35 ab

Note: Average value followed by the same letter in the same column shows that it is not significantly different according to Duncan's Multiple Distance Test at a 5% significance level

Table 4. Analysis of nutrient levels in *H. illucens* larvae

Feed medium	Water content	Fat content	Protein content
Diet 1	41.90±1.41 a	7.25±0.90 b	43.65±1.73 bc
Diet 2	44.20±0.57 b	7.00±1.80 b	41.74±0.54 ab
Diet 3	45.90±0.99 b	5.2±0.10 a	45.95±0.62 d
Diet 4	43.90±0.42 b	6.64±0.87 b	40.25±0.30 a

Note: Average value followed by the same letter in the same column shows that it is not significantly different according to Duncan's Multiple Distance Test at a 5% significance level

Water is the largest part of the body composition of living insects. Almost all reactions in the body of insects require fluids to carry out body metabolism. Nearly 30-60% of the insect body consists of water. The water content in the insect's body helps improve blood circulation in the insect's body. Insects are often dehydrated and freeze to reduce their body air conductivity (Takikawa et al. 2020). For the body's metabolism to run well, it takes a good intake of fluids in the form of feed containing minerals to replace the lost fluids properly. Food has a physiological impact on insects. Most insects are water content, stored in bonds to minimize water evaporation from the body. Therefore, the water content in the insect's body will be greater when compared to the water content in the food. According to Bekker et al. (2021), *H illucens* grow and develop in 30-70% substrate humidity. Substrate 50-80% water content, including optimum moisture content for larval development, final weight, feed conversion efficiency, and yield.

Fat content

Determination of the success of feed nutrition by testing proximate larvae using fat content analysis. Fats and oils are one of the groups belonging to the lipid group. These organic compounds have characteristics that are not soluble in water but in organic solvents such as ether, benzene, and chloroform. Fat is an energy reserve for periods of high energy intensity, such as flying or moving. The role of fat content in the formation of cell membrane structure. The phospholipid content of insects is usually less than 20%, varying according to the life stage and species of insects. Although the fatty acids profile in insects is influenced by the food eaten, cholesterol is the most abundant sterol in insects (Lenka and Anna 2016).

The results of the fatty acid analysis showed that the highest fat content of *H. illucens* maggot occurred in Diet 1 at 7,25%, while the lowest fat content was in Diet 3 with total fat of 5,20%. The calculation data using the ANOVA paired t-test was set at $p<0.05$ the p-value was 0.04. Duncan's multiple distance test was conducted to determine the difference in the increased fat content value in each treatment and to place a distance difference test on each data based on the notation that stated the difference occurred. It was shown that Diet 3 had a significantly different effect on fat content after treatment. Diets 1, 2, and 4 had no significant effect on fat content after treatment (Table 4). This study's low-fat content of *H. illucens* was due to the high water content of the larvae. According to Adamkova et al. (2017), insects that contain relatively large amounts of fat in fish feed are high, and it will cause liver damage in fish, causing death. The fat content allowed in fish feed is between 4-18%. Fat content in each treatment can be used as fish or other livestock feed because the fat content is not more than 18%. The low-fat content in *H. illucens* is due to the high water content in *H. illucens* maggots. Insects have high water, protein, and fat content. Fat content has the opposite relationship with water content; the higher the water content, the lower the fat content (Kroncke and Rainer 2022).

Protein content

Protein is needed in the insect body in large quantities because protein has a role as a neurotransmitter in the insect nervous system (Roriz and Joachim 2013). In addition, ultrastructural studies suggest that proteins are involved in sperm maturation in insects. Protein has an essential function in ovarian maturation in egg formation, so insects require large amounts of protein. *H. illucens* have significant protein and fat content and good amino acids (Xavier et al. 2018).

Analysis of protein content levels in this study was conducted to determine the increase in the nutritional value of *H. illucens* after being given treatment. Data from the analysis of protein levels in this study showed that the highest protein content occurred in Diet 3, with an average protein content of 45.95%. In contrast, the lowest protein content occurred in Diet 4, with an average of 40.25%. The analysis of protein content using ANOVA was determined to have a significant value of $p<0.05$; the results were 0.02. Continued the test with Duncan's multiple distances, and the significance was set at 5%. The treatment feed had a significant effect on all treatments. Diets 1, 2, and 4 showed significantly different protein content effects. At the same time, the Diet 3 feed gave a significantly different effect than other Diet treatments. Therefore, it can be concluded that all feeds in each treatment affected the larvae's protein content (Table 4).

The factor that supported the amount of protein in *H. illucens* is due to the addition of the composition of ingredients added in the feed media, such as DL-methionine and limestone, which is a source of protein and essential amino acids needed by insects. In addition, corn and rice polish have a high protein content. As a result, the *H. illucens* larvae have significant protein and good amino acids. The nutritional composition of the fatty acid and protein profile depends on the development stage, making it a good source of nutrients for animal feed.

The artificial feed given had a significant effect on water content and protein content in all treatments. The feed with the highest nutritional effect was found with basic ingredients of ground corn in the Diet 1 and 3 treatments with high protein content. Still, feed using rice polish is also good for *H. illucens* because the larvae have high water and high-fat content. Relatively low and has a protein content of more than 40%. All treatment feeds can be used and have a good effect on *H. illucens*. The low protein content in larva feed rice polish mixed with chicken feather flour has high protein but is not followed by a digestibility rate of only 5.58% (Nursinatrio and Rudy 2019).

In conclusion, semi-artificial feed in the study on all treatments gave good results on the survival rate of instar larvae to pupae reaching 100%. Feed that gave the greatest larval weight on Diet 3 with 20.85 gr when compared to larvae feed with other media. Diet 3 feed media gave the best number of instars, with a total of 38 days compared to other diets. A feed with increased nutritional value to larva water content occurred in Diet 3 with an average of 45,90% compared to Diet 1 as a control. Fat content analysis in all treatments was lowest on Diet 3 with 5,20% compared to control. The increase in protein levels was highest in Diet 3, with 45.95% compared to the control and all treatment media. Diet 3 feed medium gave a higher percentage increase in water and protein content compared to all treatments. Decreased fat content is a marker of increasing water content in larvae. The percentage of insect protein content is higher at each instar phase due to the presence of chitin. Diet 3 feed is considered the best for *H. illucens* larvae because it gives higher yields when compared to other diets.

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