THE EFFECT OF ORGANIC WASTE COMBINATION IN FEED MEDIUM ON THE GROWTH AND DEVELOPMENT OF *Hermetia illucens* LARVAE

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ABSTRACT

This study aims to determine the effect of a combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste on: 1) the growth and; 2) development of BSF larvae. The study was conducted using a completely randomized design (CRD) at the Biology Garden of the Faculty of Mathematics and Natural Sciences, Yogyakarta State University. The independent variables used were combinations of feed media with fermented tofu pulp, cabbage waste, coconut pulp, and rice bran in 3 treatments. The dependent variables observed were growth, including biomass increase and individual larval growth, as well as BSF larval development. The control variables of the study were container size, initial BSF larva biomass, and BSF larva age, which was 10 days after hatching. Observations of individual growth and BSF larva development were conducted once a week. BSF larva biomass measurements were conducted every 2 weeks using the gravimetric method. Data analysis for this study used ANOVA and Duncan's Multiple Range Test (DMRT). The combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste had a significant effect ($P<0.05$) on the increase in biomass and individual growth of BSF larvae, as well as on the speed of BSF larvae development into pupae.

Keywords: *Hermetia illucens*, BSF Larvae, Waste, Development, Growth

Introduction

Organic waste used as a medium for BSF (Black Soldier Fly) larvae contains various nutrients, one of which is protein that affects BSF larvae production (Rachmawati *et al.*, 2010). In addition to being a solution to reduce waste that has the potential to pollute the environment, the use of organic waste such as tofu pulp, coconut pulp, cabbage waste, and rice bran has the potential to have a positive impact on BSF larvae cultivation. Organic waste in the form of tofu pulp, coconut pulp, cabbage waste, and rice bran has different nutritional contents, so when combined, there is an opportunity for waste with low nutritional content to be used as BSF larvae feed. The right combination of organic waste in the form of tofu pulp, coconut pulp, cabbage waste, and rice bran is expected to produce high-quality BSF larvae in terms of size and content, thereby increasing the economic value of the BSF larvae cultivation process.

BSF larvae are organisms that act as decomposing agents because they consume organic materials (Mudeng *et al.*, 2018). BSF larvae are an alternative feed for livestock that is high in protein (Cicilia & Susila, 2018). The right composition of organic waste will provide maximum nutrition for BSF larvae growth, resulting in good quality BSF larvae. A medium with the appropriate nutrient content will support the life of BSF larvae (Wahyuni *et al.*, 2020). An inappropriate medium will have a negative impact on the life of BSF larvae. The medium in which maggots live greatly affects their survival rate (Hassanah *et al.*, 2023). Larval growth is influenced by nutritional content, especially the protein content in the feed provided, as protein supports the molting process, which is fundamental to larval growth. If BSF larvae lacks protein, the molting process will be disrupted, and larval growth will be inhibited (Gold *et al*., 2019). Development is an increase in the complexity of body structure and function to support the life of living things.

Tofu pulp is used as a material for making tempeh and as animal feed by the community [3]. Tofu pulp is widely used as feed because it is high in carbohydrates and protein (Syahputra and Ardani, 2019). Coconut pulp is an industrial waste product that is highly nutritious for animal feed. Coconut pulp has a protein content of 11.35% (Maulana *et al*., 2021). Rice bran is a by-product of rice milling that is commonly used as animal feed because it is affordable. Rice bran is used as animal feed because it contains nutrients such as protein, fiber, and carbohydrates that are beneficial to livestock. Cabbage waste that is simply discarded can cause environmental pollution. To reduce environmental pollution caused by vegetable waste such as cabbage, this waste is used as an animal feed mixture. Cabbage waste contains nutrients that can be added to animal feed.

Feed in the form of organic waste often has a pungent odor and a short shelf life due to the decomposition process. To overcome this, one step that can be taken is to ferment the food. The fermentation process can increase the nutrient content of the material and extend its shelf life (Yamin, 2008). The moisture content of the medium will affect the growth of BSF larvae. The BSF larval medium must be sufficiently moist, with a water content between 60% and 90%, to be digestible by BSF larvae (Dortmans *et al*, 2021). Feeding that is neither too wet nor too dry will support faster maggot growth (Syahputra *et al*., 2023). This study is expected to benefit readers, especially those who will cultivate BSF larvae using a medium of tofu pulp, coconut pulp, rice bran, and cabbage waste. This study aims to determine the effect of a combination of tofu pulp, coconut pulp, rice bran, and cabbage waste on the growth and development of BSF larvae.

Methods

Materials

BSF larvae, tofu pulp, coconut pulp, rice bran, cabbage waste, magic chalk, water, molasses, and Yakult.

Working Procedures

Preparation Stage for BSF Larvae and Larvae Culture Media

The containers used in this study were plastic containers measuring 33 x 27 x 11 cm3 according to the number of treatments and replicates. The containers were arranged on shelves in the Biology Garden of the Faculty of Mathematics and Natural Sciences, UNY, then covered with plastic wrap and labeled according to the treatment. Three grams of BSF larvae eggs were hatched in plastic containers until they were 10 days old. The hatching of BSF larvae eggs was carried out using a medium consisting of tofu pulp and rice bran. The medium used in this study was made from tofu pulp, coconut pulp, rice bran, and cabbage waste. The coconut pulp and rice bran to be used as a mixture in the medium were fermented for 3 days before being used as a medium for BSF larvae. The medium for BSF larvae was fermented using a mixture of molasses and Yakult. The combination of treatment media used includes:

Control : 100% tofu pulp
P1 : 62.5% tofu pulp + 6.25% coconut pulp + 25% rice bran + 6.25% cabbage waste
P2 : 25% tofu pulp + 25% coconut pulp + 25% rice bran + 25% cabbage waste
P3 : 6.25% tofu pulp + 25% coconut pulp + 6.25% rice bran + 62.5% cabbage waste

BSF larvae that are 10 days old are separated from the hatching medium using a sieve, then placed in each container at a weight of 8 grams. Containers filled with treatment medium and BSF larvae are placed on a rack and covered with plastic wrap. The containers containing BSF larvae and each treatment were labeled according to the treatment and covered with magic chalk on each side to prevent external interference.

Observation of BSF Larvae Growth and Development

The data observed in this study consisted of data on the increase in BSF larva biomass, as well as the individual growth and development of BSF larvae. Individual growth measurements of larvae included the length of BSF larvae, which was measured using graph paper. The development of BSF larvae was observed qualitatively by observing the morphology of the larvae. Larval biomass measurements were performed using the gravimetric method. BSF larval biomass was calculated using the following formula:

$$M = \frac{G}{g} \times m$$

with:
M : BSF larva biomass
G : Total biomass of BSF larvae and media
g : Biomass of BSF larva samples and media
m : Biomass of BSF larva samples

Temperature and Humidity Monitoring

Temperature observations are conducted using a thermometer, air humidity observations are conducted using a hygrometer, and media moisture observations are conducted using a soil moisture sensor.

Data Analysis Techniques

The data obtained was tabulated in tables and graphs to make it easier for readers to understand and draw conclusions. The tabulated data was analyzed using descriptive tests. The data was analyzed using ANOVA tests with the help of SPSS software. If significant results were obtained with a significant level ≤ 5%, further testing was conducted using the DMRT (Duncan's Multiple Range Test).

Results and Discussion

The effect of the combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste on BSF larval biomass was analyzed by calculating the average BSF larval biomass at the beginning and end of the study, as shown in Figure 1.

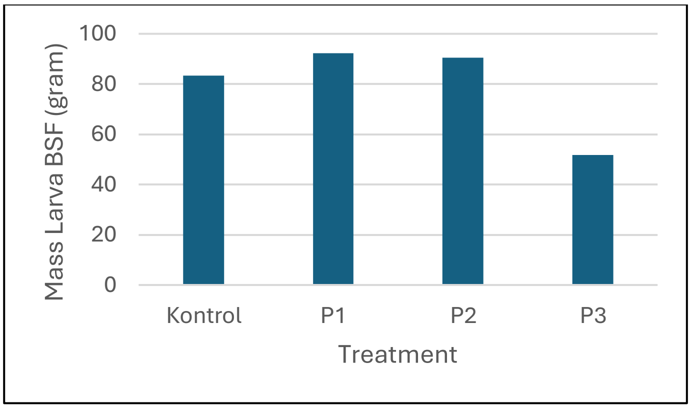

Figure 1. Graph of BSF Larva Biomass Growth

Based on the One Way ANOVA test analysis, the results show that the significance value of 0.011 < 0.05. This means that the combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste has a significant effect on the increase in BSF larvae biomass in each treatment. The increase in BSF biomass in treatment 3 was significantly different from treatment 1, treatment 2, and the control. The increase in BSF larvae biomass in treatment 1, treatment 2, and the control was not significantly different. Based on the results of the DMRT analysis, the best treatment was P1.

The highest increase in BSF larva biomass was found in treatment 1 at 92.27 grams, followed by treatment 2 at 90.54 grams, and treatment 3 at 51.87 grams. The increase in biomass in the control data was 83.36 grams. The results of the one-way ANOVA test showed that the combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste had a significant effect on the increase in BSF larva biomass. The increase in BSF larva biomass was greatly influenced by the nutritional content of the BSF larva culture medium. This is in line with research stating that BSF larvae maintenance is greatly influenced by the BSF larvae growth medium (Raharjo *et al.*, 2016). The nutritional content of the BSF larvae growth medium focuses on the protein content in the BSF larvae growth medium. The protein content in each treatment has different levels, so that the treatment has a significant effect on the increase in BSF larvae biomass.

The increase in BSF biomass in treatment 3 was significantly different from treatment 1, treatment 2, and the control. The increase in BSF larva biomass in treatments 1, 2, and the control did not differ significantly. This was because the media in treatments 1, 2, and the control had a higher protein content than treatment 3. This is in accordance with the theory that tofu pulp is widely used as feed because it has a high carbohydrate and protein content (Syahputra and Ardani, 2019). The control data used 100% tofu pulp media, and the BSF larvae grown on this media experienced an increase in biomass. This indicates that tofu pulp can be used as a growth medium for BSF larvae.

Treatment 2 had no significant difference in results compared to treatment 1. The protein content in the treatment 2 media combination had a positive effect on the increase in BSF larva biomass. The media combination in treatment 3 contained lower protein than treatments 1 and 2 because the largest component of the media was cabbage waste. The protein content in cabbage waste tends to be low, so that the increase in BSF larva biomass in treatment 3 was not as high as in treatments 1 and 2. If the BSF larva culture medium lacks protein content, the growth and development of BSF larvae will be inhibited and become suboptimal because protein plays a role in the formation of new BSF larva cells. The increase in biomass in the control data and treatments 1, 2, and 3 showed an increase. This indicates that the addition of materials such as tofu pulp, coconut pulp, rice bran, and cabbage waste has a positive effect on the increase in larval biomass.

The growth that occurs in BSF larvae tends to be rapid if the food and nutritional requirements of the larvae are met. One of the reasons for this is that during the larval stage, living creatures eat continuously. The reason larvae always feel hungry and eat continuously is because of the release of serotonin from the brain's feedback neurons, which regulate nerve activity in various types of cells in the antenna lobe. The modulation that occurs in the larval brain ultimately sends signals in the form of aroma stimulation from food, causing the larvae to feel hungry (Vogt *et al.*, 2021).

Individual growth of BSF larvae was observed by recording the length measurements of individual BSF larvae. Observations were made once a week by measuring the length of three BSF larvae samples from each replicate in each treatment. Individual growth data of BSF larvae are presented in Figure 2.

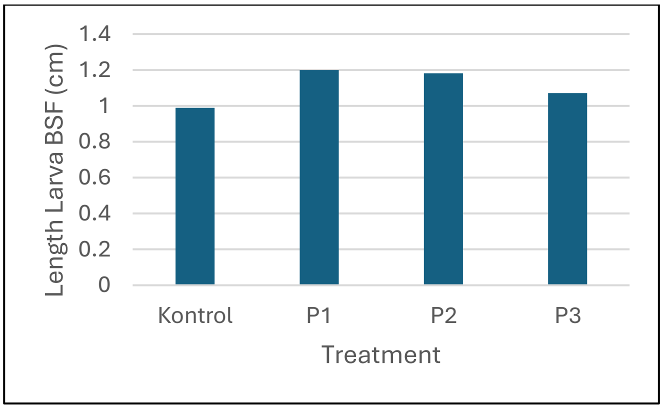

Figure 2. Graph of Individual Growth of BSF Larvae

The highest individual growth of BSF larvae was found in treatment 1 with a length of 1.20 cm. Treatment 2 had a length of 1.18 cm, and treatment 3 had a length of 1.07 cm. In the control data, the average increase in BSF larva length was 0.99 cm. The results of the one-way ANOVA test showed that the combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste had a significant effect on the individual growth of BSF larvae. The different protein levels in each treatment medium had a significant effect on the growth of BSF larvae length. The best growth in length was found in treatment 1 compared to treatments 2 and 3. This was because the composition of tofu pulp in treatment 1 was greater than in treatments 2 and 3. Tofu pulp is high in protein, which supports the growth of BSF larvae length.

The individual growth of BSF larvae in treatments 1 and 2 differed significantly from the control treatment. The individual growth of BSF larvae in treatments 1, 2, and 3 did not differ significantly. This was due to differences in the nutritional content of the BSF larval growth media. BSF larvae in the control data were grown on a 100% tofu pulp medium, which had the highest protein content of 13.07%, but the highest BSF larvae length growth was found in treatment 1, which was a combination of mixed feed consisting of tofu pulp, coconut pulp, rice bran, and cabbage waste with a protein content of 9.43%. This is because the treatment consisting of a combination of feed from various organic wastes has more complete nutrients to support BSF larvae growth.

Protein plays an important role in supporting BSF larval growth, but other nutrients also play an important role in larval growth. A feed combination consisting of various organic wastes will provide more diverse nutrients than feed from a single type of material, even if it has a high protein content. Media containing a lot of organic material greatly affects the growth of BSF larvae (Maulana *et al*., 2021). Treatments 1, 2, and 3 were treatments with a combination of feed consisting of various types of organic waste, but the best growth was still found in treatment 1, which had the highest protein content among the three treatments, so protein still plays an important role in the growth of BSF larvae.

Food that enters the mouth of BSF larvae is digested mechanically and chemically with the help of amylases and maltase enzymes. In addition to mechanically breaking down food, BSF larvae also have enzymes in their mouths that can break down food. The enzymes found in the mouth of the larva include amylase and maltase (Fauzi and Muharram, 2019). Food that has been broken down in the mouth is transported to the larva's intestine through the esophagus. The posterior part of the midgut functions as a place for digestion and absorption of food because it contains digestive enzymes (Gold *et al*., 2019).

Protein breakdown is carried out by protease enzymes consisting of pepsin and trypsin. Food transported from the esophagus enters the endo peritrophic section of the larva. Food is catalyzed using digestive enzymes until it is smaller in size and can enter the peritrophic matrix and move to the ectoperitrophic, which is located within the peritrophic matrix (Fauzi and Muharram, 2019). The digestion of nutrients in the form of proteins that have been simplified into amino acids by protease enzymes will then be absorbed through the walls of the small intestine, which have a structure called villi and microvilli (Tettamanti *et al.*, 2022). After being absorbed by the small intestine cells, amino acids and other nutrients enter the bloodstream through the blood capillaries so that they can be distributed throughout the larvae's body and provide energy for growth and development. The remaining undigested organic material is directed to the rear intestine towards the anus and excreted as feces.

Microorganisms found in the digestive system of BSF larvae include *Streptococcus sp., Bacillus sp., Micrococcus sp.,* and *Aerobacter aerogens* (Wardana, 2016). The presence of microbiota in the digestive system of BSF larvae is more commonly found in the posterior intestine than in the anterior intestine. Microbes found in the intestine of larvae can undergo decomposition so that they can be digested and metabolized by BSF larvae through a diffusion process which is then transferred to the haemolymph tissue of the larvae (Bonelli, 2017). Based on this theory, it can be concluded that the protein content in the larvae's body does not entirely come from protein that enters the larvae's body through food alone, but also from other nutrients that are converted into protein and from microorganisms that are digested in the BSF larvae's body.

Larval growth is also influenced by the process of molting. The molting process in larvae occurs when the larvae shed their old skin and replaces it with a new, larger skin to support the growth of BSF larvae. The molting process begins with the larvae preparing by increasing the hemolymph pressure in the old skin. Then, the molting hormone, ecdysone, is produced by the prothoracic glands in the larva's body (Aprizal, 2009). Epidermal cells form the cuticle and store flat endocuticle in a certain part, and its size can increase in length during the intermolt phase. Proteins and chitin found on the apical surface of epidermal cells are directed towards microvilli, and the cuticle emerges through a collection of chitin microfibrils and secreted proteins (Kaleka *et al*., 2019).

The old cuticle on the larva will remain intact and attached after the new cuticle is formed. Protease secretion occurs when the larva's cuticle is about to detach, then these enzymes will work together to digest chitin and protein, resulting in N-acetyl glucosamine and amino acids. The fluid resulting from the molting process will be reabsorbed into the hemolymph. The reabsorption process can occur through the new cuticle and epidermis or through the intestine in the absorption process in the posterior intestine (Kaleka *et al*., 2019). Once the reabsorption process is complete, the BSF larva will emerge from the old skin by pushing its body. BSF larvae that lack protein nutrients will experience a disrupted molting process and will form a weak new skin. Stressful conditions for BSF larvae also disrupt the molting process. Observations of treatments 1, 2, and 3 found remnants of old skin from the molting process in BSF larvae. This indicates that BSF larvae increase in size due to the molting process.

The observation of BSF larvae development in the first week was conducted on February 9, 2024. The observation of BSF larvae in treatment 1 in the first week showed a yellowish color, as shown in Figure 3 (a). In the second week, the size of the BSF larvae in treatment 1 was wide and the color of the BSF larvae was yellow and slightly light brown. The BSF larvae were actively moving and consuming the feed provided. In the third week, the BSF larvae was the largest compared to the other treatments, the color of some BSF larvae began to turn blackish brown, and the color of the medium became slightly dark brown, between the colors of the medium in treatments 2 and 3. The larvae were still actively moving and consuming the feed provided. In the fourth week of observation, BSF larvae were the largest among the other treatments, with a dark brown color and the highest number of pupae compared to the other treatments.

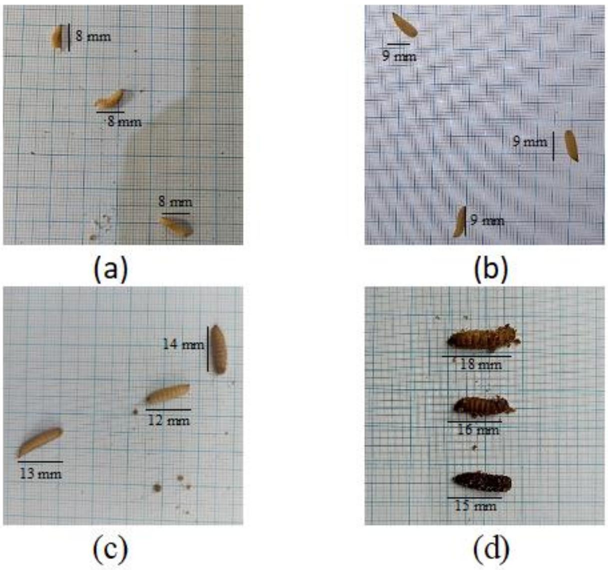

Figure 3. Morphology of BSF Larvae Treatment 1 in the First Week (a), Second Week (b), Third Week (c), and Fourth Week (d)

The movement of BSF larvae becomes passive, indicating that the larvae have entered the prepupa stage. BSF larvae are very active, but after entering the prepupa stage, they become less active and will slow down or become inactive (Mangisah *et al*., 2022). Larvae that have entered the pupa stage are jet black and no longer move. The highest number of pupae at the end of the study was found in treatment 1. Pupae in treatment 1 was first found on February 28, 2024, which was the earliest time of pupa emergence compared to other treatments. This is because the combination of feed and protein content in the feed provided had an optimal effect on the development of BSF larvae. Insects require protein, which is converted into amino acids that play a role in the insect development process (Dalangin *et al*., 2020).

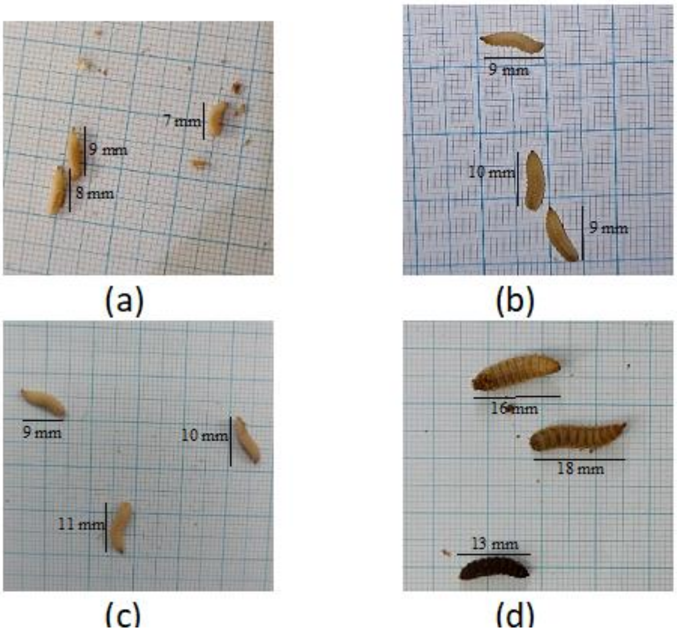

Figure 4. Morphology of BSF Larvae Treatment 2 in the First Week (a), Second Week (b), Third Week (c), and Fourth Week (d)

First week observation: BSF larvae were yellowish in color. Second week: BSF larvae were long and no wider than treatments 1 and 3, with a slightly brownish yellow color. Third week observation: the larvae were longer than treatment 1, with a yellowish body color and some brownish coloration, and the medium color was the lightest compared to other treatments. In the fourth week, the BSF larvae grew longer and slightly wider, with a brownish-black color, and one pupa was found in all replicates. The cause of the different colors of the maggot media between treatments was due to different feed compositions. Observations in the fourth week showed that the BSF larvae began to enter the prepupa phase, but only one pupa was found at the end of the study. The pupa was found at the end of the study on March 1, 2024. The feed combination influenced the speed at which BSF larvae entered the prepupa phase and turned into pupae. The nutritional content of the BSF larvae's living medium greatly influenced the quality of the larvae produced in the cultivation process (Fauzi and Sari, 2018).

First week observation: BSF larvae were yellowish in color. In the second week, BSF larvae were larger than those in other treatments and were yellowish in color. BSF larvae were active and consumed the feed provided. Third week observation: BSF larvae were smaller than treatment 1 and treatment 2, yellowish in color, and some larvae began to turn brownish, while the medium color was darker than treatment 1 and treatment 2. In the fourth week, the size of the BSF larvae was smaller than treatments 1 and 2, most of the BSF larvae were brownish-yellow, and some BSF larvae were found to be blackish, and one pupa was found in each replicate. The pupa was found at the end of the study on March 1, 2024. Most BSF larvae were yellowish-brown or had not yet entered the prepupa phase due to insufficient nutrients to support larval development. The smooth texture of the feed made it easier for the larvae to absorb and caused the feed to run out quickly, resulting in uneven larval growth and only a few larvae entering the prepupa phase. Insufficient nutritional content will cause the BSF larvae to change to the next phase more slowly. If the BSF larvae's living medium lacks protein content, the growth and development of BSF larvae will be inhibited and become suboptimal because protein plays a role in the formation of new BSF larvae cells (Fajri *et al*., 2021).

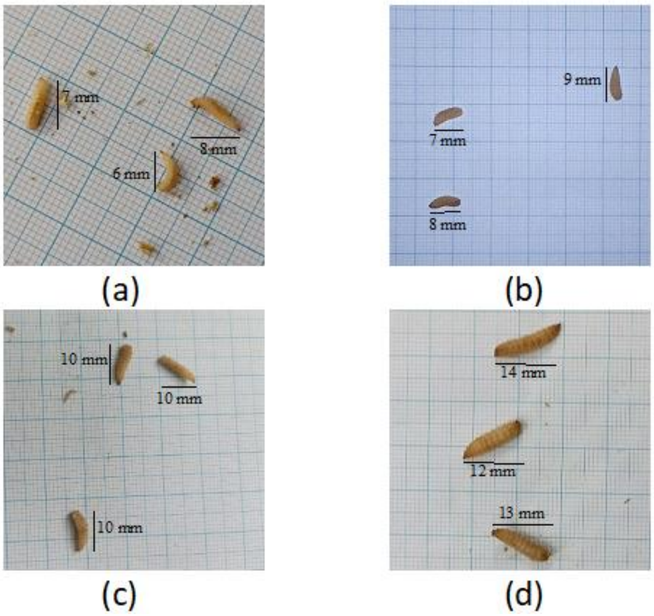

Figure 5. Morphology of BSF Larvae Treatment 3 in the First Week (a), Second Week (b), Third Week (c), and Fourth Week (d)

Based on observations of environmental parameters such as air temperature, air humidity, and media moisture, the average air temperature during the study was 30°C. The average air humidity was 60.1% and the average moisture content of the BSF larvae media was 61.8%. These environmental parameters are considered optimal conditions for BSF larvae growth. This is in accordance with the theory that states that the optimal temperature for BSF larvae growth is around 27-30°C and that BSF larvae will die if the temperature in their living environment exceeds 36°C (Septiawati *et al*., 2021). The BSF larvae medium must be sufficiently moist with a water content of between 60% and 90% to be digestible by BSF larvae.

Conclusion

Based on research, the combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste has a significant effect on the increase in biomass and individual growth of BSF larvae. The best growth was found in treatment 1 with a combination of 62.5% tofu pulp + 6.25% coconut pulp + 25% rice bran + 6.25% cabbage waste. The combination of fermented tofu pulp, coconut pulp, rice bran, and cabbage waste also affected the rate of BSF larvae development into pupae. The best rate of change of BSF larvae into the pupal phase and the highest number of pupae were found in treatment 1 with a combination of 62.5% tofu pulp + 6.25% coconut pulp + 25% rice bran + 6.25% cabbage waste. Further research is recommended on the quality of pupae produced from feeding a combination of tofu pulp, coconut pulp, rice bran, and cabbage waste. It is recommended to conduct research on the protein content in BSF larvae fed with different living media treatments. The use of tofu pulp as a mixture of BSF larvae cultivation media is highly recommended because it contains high protein and produces high-quality BSF larvae, both in terms of size and the speed of the BSF larvae's transformation into pupae.

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