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The Effect of Agro-Industrial Waste Mixtures on Productivity and Nutrient Content of Black Soldier Fly Larvae (*hermetia illucens*)

Muhammad Primo Arifki[*1], Ma'ruf Tafsin[2], Fatimah[3]

[*1]Natural Resources and Environment Management Study Program, Postgraduate School, Universitas Sumatera Utara, Medan, 20155, Indonesia

[2]Department of Animal Husbandry, Faculty of Agrotechnology, Universitas Sumatera Utara, Medan, 20155, Indonesia

[3]Department of Chemical Engineering, Faculty of Engineering, Universitas Sumatera Utara, Medan, 20155, Indonesia

[*]Corresponding Author: primo.arifki@gmail.com

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ABSTRACT

The feed media content plays an essential role in the growth of BSF larvae; appropriate feed content will provide optimal growth for BSF larvae. Agricultural industrial waste contains high levels of protein, fiber, and carbohydrates, which play an essential role in the growth and nutrition of BSF larvae. This study aims to determine the effect of providing agricultural waste as feed on the relative growth rate, rate of degradation of organic waste, and the nutritional content of BSF biomass. This research is an experimental study using various levels of experiments from the Laboratory of Standardization and Industrial Services Center in Medan, Indonesia, using the SNI 01-2891-1992 method, which consisted of ash content, carbohydrates, total fat, protein, and crude fiber. The results showed that the feed media's protein content would be more easily digested than the more complex carbohydrate and fiber content. It will increase protein content in feed media, giving a higher growth rate in larva BSF. The feed media with the highest protein content causes the feed media to be consumed more quickly, producing lower unconsumed feed residue and showing the highest degradation rate. The high protein content in the feed media can increase Proteobacteria bacteria and increase the protein bioconversion process in the intestines of BSF larvae.

Keywords: Black Soldier Fly, Bioconversion, Nutritional Content, Organic Waste

1. Introduction

The increase in public food consumption will be directly proportional to the increased volume of waste produced. Based on data from the Ministry of Environment and Forestry, 2020, Indonesia produced 32.82 million tons per year of waste (Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia, 2020). There was an increase of 12.62% in the volume of waste generated compared to 2019, and this will become a severe problem if it is not accompanied by technology and good waste management. One of the organic waste processing technologies that can be used is the bioconversion of waste using the Black Soldier Fly (*Hermetia illucens*) insect. This technology has advantages in the form of low installation costs, environmental friendly, high rate of conversion of organic waste, the use of various types of organic waste as a source of protein, can be run in tropical climates (Kim et al., 2021), low Greenhouse Gases (GHG) emissions (Salam et al., 2022) and can increase income for the community (Handayani, 2021). This technology uses organic waste as a substrate for growth in the larval phase. The larvae will break down some organic wastes using their mouths and digestive enzymes to be converted into nutrients in the larva's body (Singh & Kumari, 2019).

Several studies have been conducted to determine the effect of the type of substrate on the growth and nutrient content of the larvae (Raksasat et al., 2021), reported that a combination of water treatment waste and a palm kernel expeller with a ratio of 2:3 obtained the best larval weight of 46.99 mg/g due to the high protein content in the Palm Kernel Expeller (PKE). The high fiber content in PKE decreased larval weight in the ratio of water treatment waste and palm kernel expeller 0:5 of 39.9 mg/g. Barbi et al. (2020) reported that a combination of fruit with high fiber, polyphenol, and carbohydrate content in a mixture of exotic fruit and melon gave the best larval weight and larval nutrient content (Barbi et al., 2020).

Rahmi *et al.* (2020) reported that restaurant waste gave the best BSF larvae weight, length, and thickness compared to soybean and oil palm dregs (Rahmi et al., 2020). Yusuf *et al.* (2022) also reported that combining soybean dregs and coconut waste provided the highest protein content in BSF larvae at 48.60% compared to vegetable waste at 46.5% (Yusuf et al., 2022). In a study conducted (Syharizal et al., 2022), it was reported that palm kernel meal waste gave the most considerable larval weight of 673.67 g/4kg of the substrate and the highest protein content of 46.50% compared to the vegetable waste and coconut meal.

One type of waste that can potentially be used as a feed medium for BSF larvae is agricultural industrial waste. Agricultural industrial waste has an excess value of high protein, fiber, and carbohydrates, which are suitable for the growth of BSF larvae. According to (Iriondo-dehond, 2020), coffee cascara waste has a high protein and carbohydrate content, reaching > 12% and 45%. Furthermore, according to (Susanti, 2006), soybean waste has a high protein content of up to 16%. The protein content in industrial and agricultural waste is higher than in vegetable waste, which is only around 0.7-11% (Barbi et al., 2020). Agricultural and industrial waste also has other advantages in the form of abundant availability and ease of obtaining.

This study aims to determine the effect of giving agricultural waste as feed on the growth of BSF larvae and the rate of degradation of organic waste (Waste Reduction Index). Also, to determine the effect of using agricultural waste as feed on the nutritional content of BSF biomass. This study will use vegetable waste as a control or comparison medium with agricultural industrial waste media. Vegetable waste is used as a control medium because vegetable waste is generally used as a source of BSF feed by breeders.

2. Method

The research design aims to compare BSF, which is given different treatments based on the type of waste used as a growth medium. The independent variable in this study was the type of waste as a growth medium for larvae, which consisted of 4 treatments: 1) vegetable waste as the control group, 2) sugarcane bagasse, 3) coffee cascara, and 4) soybean waste. The dependent variables in this study were the relative growth rate and waste reduction index, as well as the nutritional content of the BSF biomass in the form of protein, fat, and ash content (Syharizal et al., 2022).

BSF's nutritional content was analyzed at the Laboratory of Standardization and Industrial Services Center (BARISTAN) in Medan City using the SNI 01-2891-1992 method. The weight, length, and thickness of BSF larvae were measured directly at the study site. Soybean dregs were obtained from one of the soymilk industries in Medan. Sugarcane bagasse is obtained from the PTPN II sugar processing industry. Cascara coffee is obtained from the coffee processing process in Takengon, Central Aceh. BSF seeds were obtained from one of the BSF breeders in the Medan Tembung sub-district, Medan City.

This study uses three repetitions with four treatments. Table 1 shows the content of waste in each treatment. The rate of degradation waste is calculated using the ratio of waste that is not consumed (Waste Reduction Index).

Table 1. Waste content in each treatment

Treatment	**Content (%)**			
	VW	**SD**	**SB**	**CC**
P0	100	-	-	-
P1	70	30	-	-
P2	-	30	70	-
P3	-	30	-	70

Description
VW : Vegetable Waste
SD : Soybean Dregs
SB : Sugarcane Bagasse
CC : Cascara Coffee

2.1. Research Procedure
The BSF eggs are placed in a hatching plastic container with gauze at this stage. The container is placed in an incubation room with a temperature of 27°C-28°C and humidity of 60-70% for four days. In the next stage, controlling larvae, eggs that have hatched are then transferred to enlargement media by being fed vegetable waste before treatment until the larvae are six days old (Kim et al., 2021).

Larvae that were six days old were then transferred to each growth medium. The total weight of the larvae in each medium reached 10g. The ratio of the amount of substrate in each medium is 150 mg/lv/day. Productivity measurement in the form of growth rate in wet products was carried out in the larval phase for 21 days with an interval of 3 days. The measured growth rates were weight and length of larvae, mortality, and waste degradation rates. Larvae that have turned into pupal phases aged 27 days are separated and cleaned using water. In the dry product in the form of pupa, the nutrient content in the BSF pupa biomass was measured (Prawanto, 2021).

3. Result And Discussion
3.1. Characteristics of Agro-industrial Waste
A proximate analysis of each agro-industrial waste was carried out to determine each feed medium's nutritional content. This study's proximate content analysis consisted of tests for ash content, moisture content, carbohydrates, total fat, protein, and crude fiber.

Table 2. Nutritional content in each waste

Parameter	Nutrional Content (%)			
	VW	SD	SB	CC
Protein	6,31	18,20	nd	nd
Fibre	84,07	57,42	69,68	77,68
Fat	6,85	18,20	0,03	7,88
Ashes	2,77	18.20	6,22	12,31
Carbohydrate	nd	4,16	24,07	2,12

Description
nd : not detected

Table 3. Nutritional content in each substrate

Parameter	Nutrional Content (%)			
	P0	P1	P2	P3
Protein	6,31	9,87	5,46	5,47
Fibre	84,07	76,08	66,00	71,60
Fat	6,85	10,25	5,48	10,98
Ashes	2,77	2,55	4,96	9,22
Carbohydrate	nd	1,25	18,10	2,73

Description
nd : not detected

Table 2 shows that the highest protein content was found in treatment P1 in a mixture containing vegetable waste and soybean dregs. This is due to the high protein content in soybean dregs and vegetable waste (table 2), while no protein content was detected in other waste. The highest fat content was found in the P3 treatment on a mixture of cascara coffee and soybean dregs. The results found that the high-fat content was due to vegetable waste, and coffee cascara had the highest fat content compared to other waste (table 3).

3.2. The Effect of Agro-Industry Waste on Average Length and Weight of BSF Larvae

The results show in Table 4 that treatments P0 (24,30 mm) and P1 (23,63 mm) gave the best larval lengths. However, the length of larvae produced by P1 was not significantly different (P>0.05) compared to treatment P0.

Table 4. Average larva length and weight in each treatment

Treatment	Average larva length (mm)	Average larva weight (g)
P0	24,30[b]	0,53[b]
P1	23,63[b]	0,52[b]
P2	17,43[a]	0,41[a]
P3	19,23[a]	0,40[a]

Figure 1 shows the growth in larval length in each treatment at the beginning of growth (3 days). It was found that P1 and P3 showed the highest growth in larval length, with an average larval length of 13.90 and 13.67 mm. In comparison, the lowest larval length growth at the beginning of growth was found in the P0 treatment of 13.05 mm.

At six days of observation, there was a significant increase in larval length growth in treatments P0 and P1, with an average larval length of 16.20 and 18.87 mm. Significant growth increases at P0 and P1 also occurred at 15 days of observation, and then the increase tended to be lower entering 18 days. In treatment P2 and P3, there was an increase in larval length growth, which was lower than P0 and P1 at observation 3-21 days. The highest average length of larvae was found in treatment P0, with a larval length of 24.30 mm, while the lowest larval length was obtained in treatment P2 at 17.43 mm.

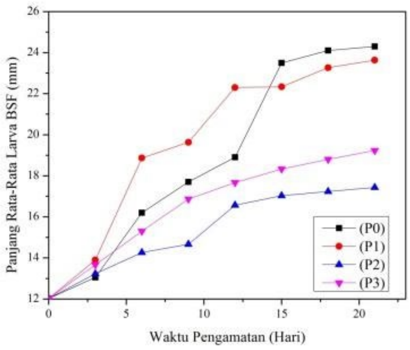

Figure 1. Growth average length of larvae in each treatment

The increase in larval length growth at the beginning of growth in treatments P1 and P3 could be due to the high total fat content in both treatments. The same results were obtained by (Li et al., 2014), where the feed medium with a high-fat content showed a better length of *Chrysomya megacephala* larvae at the start of growth (32-48 hours). These results are supported by research conducted by (Oddon et al., 2022), where the need for fat is greater at the start of the growth of BSF larvae. The highest length growth was found in treatments P0 and P1, where the two treatments had the highest protein content. The same results were reported by (Kieβling Franke, 2023), where the content of feed media with higher protein showed better growth of BSF larvae at a growth time of 8-18 days.

The results show in Table 3 that treatments P0 (0.53 g) and P1 (0,52) gave the best larvae weights, but from the results, it was also found that the weight of larvae produced in treatment P0 was not significantly different (P>0.05) compared to treatment P1.

The growth of the average weight of the larvae in each treatment obtained an average value of the larvae, which was not too significant at the beginning of the observation (3 days). At six days of observation, it can be seen that there was a significant increase in average larval weight in treatments P0 and P1. In the P0 treatment, it could be seen that there was a significant increase in the average weight of the larvae on the 12th to the 18th day, and then there was no change in the weight of the larvae at the end of the experiment. In treatment P1, there was a significant increase in the average weight of the larvae from day 15 until the end of the experiment. The highest average weight of larvae was obtained in treatment P0 of 0.53 g, while the lowest average weight of larvae was obtained in treatment P3 of 0.40 g.

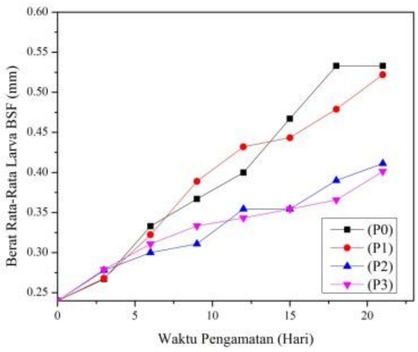

Figure 2. Growth average weight of larvae in each treatment

The highest average weight of larvae was found in treatments P0 and P1, possibly due to the two treatments having the highest protein content of 6.31 and 9.87%. The same results were reported by (Rahmi et al., 2020), where a high protein content in the feed media will result in a higher average weight of BSF larvae. The results of the study are supported by research conducted by (Holeh et al., 2022), where the higher protein content in the mixture of hotel and domestic waste shows the highest average weight of larvae compared to the mixture of hotel waste with lower protein content in the feed media. According to (Tschirner Simon, 2015), the protein content in the feed media will be more easily digested than the more complex carbohydrate and fiber content. It will result in better larval weight growth.

3.3. The Effect of Agro-Industry Waste on the Viability of BSF Larvae

From Table 5, it can be seen that treatment P2 gave the lowest larval survival value of 86.67%. The test results showed a significant difference (P<0.05) in feeding with treatment P2 on larval survival compared to other treatments.

Table 5. Viability of larvae

Parameter	Treatment			
	P0	**P1**	**P2**	**P3**
Moisture content in substrate (%)	68,60	75,35	71,01	90,05
Viability (%)	97,00[b]	96,67[b]	86,67[a]	94,00[b]

Table 5 shows that the highest larvae survival was obtained in the P0 treatment, 97.00%, and the lowest larval survival was obtained in the P2 treatment, 86.67%. According to (Bekker et al., 2021), water content plays an essential role in larval viability, where high water content causes lower larval viability. The results of this study are supported by research conducted (Yakti et al., 2023), where the high water content in the feed media causes a decrease in the viability of BSF larvae.

A different study (Broeckx et al., 2021) reported that the high water content in domestic food waste resulted in lower BSF larvae survival compared to chicken manure with lower water content. However, the results obtained in the P3 treatment had the highest water content of 90.05% and better larval survival than the P2 treatment of 71.01%. In addition to water content, several other factors influence larval survival, such as temperature, light intensity, and mineral content in the feed, which were not variables in the study. Further research is needed to determine other factors that play an essential role in larval survival.

3.4. The Effect of Agro-Industry Waste on the Specific Growth Rate

From Table 6, it can be seen that treatments P0 and P1 provide the best relative growth rate values, but from the results, it is found that there is no significant difference (P>0.05) in treatment P0 compared to P1.

Table 6. Specific growth rate larvae

Parameter	Treatment			
	P0	**P1**	**P2**	**P3**
SGR (g/day)	0,058[b]	0,055[b]	0,033[a]	0,032[a]

Table 6 shows the relative growth rate in each treatment; the highest relative growth rate was obtained in the P0 and P1 treatments of 0.058 and 0.055 g/day. Furthermore, the lowest relative growth rate was obtained in the P3 treatment of 0.32 g/day.The relatively high growth rates in the P0 and P1 treatments could be due to the high protein content in both treatments. High protein content will increase the average weight of the larvae. This is evidenced by the highest average weight of larvae obtained in treatments P0 and P1. At P2 and P3, relatively low growth rates were obtained due to the low protein content in the two treatment samples.

The high ash content also plays an essential role in increasing the relative growth rate, where the P0 and P1 treatments show lower ash content than P2 and P3. The results of this study are supported by research conducted (Fitriana et al., 2021), where the high protein content in chicken feed causes a higher growth rate for BSF larvae compared to vegetable and manure waste. The same research results were reported by (Meneguz et al., 2018), where the high protein content in the waste of the brewing and wine industry increased the growth rate of BSF larvae compared to the vegetable waste feed media.

3.5. The Effect of Agro-Industry Waste on the Waste Reduction Index

The most extensive waste reduction index (WRI) value was obtained in treatment P1 at 68.30%, as seen in Table 7; the lowest WRI value was obtained in treatment P3 at 22.80%. The residue produced comes from unconsumed feed and excretion. The composition of the feed will affect the speed of digestion of the BSF larvae; in the P0 and P1 treatments, which consist of vegetable waste and soybean waste, which contain high protein and fat, this causes the feed media to be consumed more quickly and produces lower unconsumed feed residue.

Table 7. Waste reduction index

Parameter	**Treatment**			
	P0	**P1**	**P2**	**P3**
WRI (%/day)	3,02	3,33	0,71	1,02

The P2 and P3 treatments consisted of bagasse and coffee cascara, which had high carbohydrate and fiber content. This causes a lower level of feed consumption and produces more significant feed residues than P0 and P1. The same results were obtained by (Meneguz et al., 2018), where the higher protein content in beer processing effluent increased WRI than vegetable waste with lower protein content. Broeckx et al. (2021) also reported that the high protein content in the feed media for domestic food waste resulted in a higher WRI value than vegetable waste, animal manure waste, and corn flour (Broeckx et al., 2021).

3.6. The Effect of Agro-Industry Waste on the Nutritional Content of Larvae

The nutritional content of BSF larvae is very dependent on the nutritional content of the feed media provided. Analysis of the nutritional content of BSF larvae consisted of fat, protein, ash, and water content. Analysis of nutrient content was carried out on fresh BSF larvae (Fresh Matter) at 21 days of BSF larvae.

The highest protein content was obtained in the P1 treatment of 12.2%. The lowest protein content was obtained in the P3 treatment of 7.06%. This could be due to the high protein content in the P1 treatment. The protein bioconversion process occurs in insects' gut through enzymatic processes with the help of protease enzymes. Protease enzymes can be produced by the larval gut or obtained through Bacteroides and Proteobacteria microbes from feed sources. Increasing protein in feed sources can increase Proteobacteria bacteria and increase the protein bioconversion process in the intestines of BSF larvae (Klammsteiner et al., 2020).

Table 8. Nutritional content of larvae

Parameter	**Nutrional Content (%)**			
	P0	**P1**	**P2**	**P3**
Protein	7,12	12,2	7,53	7,06
Fat	3,3	3,61	1,39	3,69
Ashes	3,21	2,75	2,9	2,8
Moisture	85	79	78,7	86,4

The results of this study are supported by research conducted (Kahar et al., 2020); here, the high protein content in soybean dregs causes a higher protein content in BSF larvae compared to vegetable dregs. A different study

(Syharizal et al., 2022) also reported that a high protein content in a mixture of Palm kernel meal resulted in a higher protein content in BSF larvae than in a mixture of coconut and cabbage waste.

Analysis of BSF larvae fat found that the highest fat content was found in the P3 treatment at 3.69%, and the lowest fat content was obtained in the P2 treatment at 1.39%. This could be due to the highest fat content obtained in the P3 treatment of 10.98%. The P3 treatment consisted of soybean and coffee dregs with the highest fat content compared to vegetable and sugar cane dregs. The bioconversion of fatty acids in the larvae consists of a glycolysis process by converting fatty acid compounds as triglycerides into Acetyl CoA compounds. These compounds are then converted into shorter fatty acids through an oxidation process and can be used as energy or accumulated in the bodies of BSF larvae (Hoc et al., 2020). The same results were reported by (Syharizal et al., 2022), where the high-fat content in the coconut dregs mixture caused a high-fat content in BSF larvae. Raksasat et al. (2021) reported that the ratio of Palm Kernel Expeller, which contains high fatty acids, resulted in a higher fat yield in BSF larvae (Raksasat et al., 2021).

4. Conclusion

The results showed that the soybean vegetable waste mixture had the highest relative growth rates of 0.058 and 0.55 g/day. The vegetable and soybean waste mixture shows the highest Waste Reduction Index value of 3.02%/day. The high protein content of a mixture of soybean dregs and vegetable waste produces the highest % protein composition in BSF larvae, namely 12.2%. The high-fat content in the mixture of soybean dregs and cascara coffee resulted in the highest fat composition in BSF larvae, namely 3.69%.

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