FINAL REPORT
DIPA BIOTROP 2018

THE USE OF *HERMETIA ILLUCENS* FOR SUSTAINABLE COCOA FARMING: COCOA POD HUSK BIOCONVERSION TO FEED SUPPLEMENT

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(SEAMEO BIOTROP)
2018

APPROVAL SHEET

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4.	Research Schedule	:	9 months
5.	Research Budget	:	Rp. ,-

Bogor, 01 December 2018

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ABSTRACT

As Indonesia is being the third largest cocoa producer in the world, an abundant amount of cocoa pod husk (CPH) is being discarded as agricultural waste. Conventional use of CPH is by composting it or use it directly as a sheep feed. The latter did not contribute towards the increase of daily weight gain of sheep due to low nutrient content. To give higher value of CPH, black soldier fly (BSF) (*Hermetia illucens*) is used as a bioconversion agent to convert CPH into a biomass of protein. There is a need to study the treatments of CPH to be able to be consumed by BSF larvae which then the larvae will be consumed by sheep through mixing the larvae into rations. The objectives of this study are to get the best CPH treatment to be bioconverted by BSF larvae and to measure its proximate composition. Values from the proximate analysis will be used as a basis to formulate feed rations and then be given to sheep in various concentrations. Finally, the sheep performances were measured.

Results showed that from six CPH treatments, BSF larvae fed with composted CPH preceded by administration of left-over food waste (LKS treatment) had the highest value of waste reduction index and second highest for relative growth rate and efficiency of conversion of ingested food. In terms of proximate analysis, BSF larvae fed with LKS treatment had higher crude protein and lower crude fat content compared to that of control so that it may replace soybean meal and increase digestibility, respectively. Five ration treatments (R1-R5) using various concentration of BSF larvae fed with LKS treatment were allocated to be given to 20 sheep. Randomized complete block design is used in this experiment with four replicates for each treatment. Values of average daily consumption, feed conversion ratio, % dry matter, and average weight gain of R2 over a month had no significant difference with control ration. R2 is the ration where BSF replaced 100% soybean meal. Therefore, BSF larvae could be a substitute of soybean meal as a protein source in feed. However, longer observation periods (up to 3 months) are needed to make a valid statement along with the measurement of rumen fermentation characteristics.

1. INTRODUCTION

1.1 Background

Waste has become a menace towards environment due to human over consumption. More attention should be given on organic waste, especially in developing countries like Indonesia because proportion of organic waste generated in Indonesia was over half of the total waste produced (Meidiana, 2012). Conventional business-as-usual method to deal with this waste is transporting it to the landfill but this method causes another problems such as greenhouse gases emission (Meidiana, 2012) and land shortage due to increased population.

One type of organic waste from intensively used ecosystems is cocoa pod husk. This waste is abundant because Indonesia is the third largest cocoa producer (Syamsiro *et al.*, 2012). Up to now, action taken to manage this waste is by composting them or give them directly as a feed source (Gunawan & Talib, 2017).

There are other ways to improve the value of cocoa pod husk (CPH) other than composting. One of them is by using bioconversion method of Black Solider Fly (BSF). BSF larvae are a voracious organic waste eater. They can consume a significant amount organic waste per day. By doing so, their adult stage does not feed since they have already stored the energy they need in adult stage during larval stage (Rana, 2014). Therefore, this fly does not become a vector and transmit any diseases. Furthermore, the larvae also can significantly reduce the population of *Salmonella* and *E. coli* in organic waste after they process the waste (Lalander *et al.*, 2013). Their instar 5 phase are a good source of protein since the protein content can reach 40% of their dry weight (Oonincx *et al.*, 2015) and, therefore, have a potential to be the protein source for feed. The residue from bioconversion process also can be used as fertilizer.

Feed price proportion towards total production costs is considered high. BSF can be an alternative feed protein source and it has been studied on poultry (Cockcroft, 2018, Cullere *et al.*, 2016) and fish (Kroeckel *et al.*, 2012, Magalhães *et al.*, 2017). However, study of BSF in small ruminants, especially sheep, is still very few.

The integrated system between cocoa, BSF, and sheep can promote sustainable farming for cocoa farmer. CPH as organic waste was bioconverted into fertilizer and larvae biomass which the latter was processed further into concentrated protein source for feed. This system may be better than the existing cocoa-sheep integration system stated by Gunawan dan Talib (2017) in terms of feed nutrition composition. When CPH is given directly or as a silage, the crude protein content is ranging from 4.86 – 7.92%. If CPH is bioconverted first, the crude protein content might be higher, as much as 40%, than give the husk directly to sheep. What we need to investigate is the biomass balance sheet of how many larvae was used to increase sheep's weight. Therefore, this project is limited to the measurement of the increase of sheep's weight, not until the manure from sheep and fertilizer from BSF are given back to the cocoa field. In the end, cocoa farmers were benefited more from this system because they will get more products (fertilizer from BSF's residue and concentrated protein source for feed) and the increase of their sheep's weight.

1.2 Objectives

1. To obtain data on waste reduction index, relative growth rate, and efficiency of conversion of ingested food from BSF fed with various CPH treatments and select the best treatment for CPH for further analysis
2. To do the proximate analysis of BSF larvae
3. To formulate the appropriate feed for sheep using various concentration of BSF and CPH residues as a substitute for soybean meal and coffee skin, respectively, in *business-as-usual* ration

1.3 Expected output and outcomes

1. To integrate CPH bioconversion system into existing system of integration cacao-sheep as a part of sustainable cocoa farming

2. Compare the feeding treatments and select the best treatment based on average daily weight gain, average daily feed consumption, and feed conversion ratio of studied sheeps.

2. BENEFIT AND IMPORTANCE OF THE RESEARCH

CPH is the largest part of cocoa fruit (74%), while the cocoa seed is just 24% (Gunawan & Talib, 2017). CPH is not needed by chocolate industry and become a waste that need to be processed. CPH was already used as sheep feed in several places in Indonesia. However, the use of CPH as a sheep feed was not widely implemented and has several problems. Cocoa peel contained an antinutrients such as lignin, tannin, and theobromine. Feeding a livestock with feed that contains theobromine will reduce the growth of livestock, while tannin and lignin will lower digestibility of livestock (Laconi & Jayanegara, 2015). CPH also contains a lot of water that caused a short period of storage.

Therefore, to solve that problem, we integrate the black soldier fly (BSF) (*Hermetia illucens*) as a bioconversion agent into the existing cacao-sheep system. The BSF larvae will consume the cocoa pod husk and convert it into their biomass. The BSF larvae were used as a high nutritious component for feed supplement to increase body weight of sheep livestock. BSF provide a bioconversion of CPH into high protein content biomass and they also generate residue that can be given back to the field as a compost.

Furthermore, the use of BSF larvae as a feed supplement for sheep has not been studied. Several studies on the use of BSF as a feed mainly focus on aquaculture (Rana, 2014, van Huis, 2013) and recently on poultry (Schiavone *et al.*, 2017a). All the studies showed good results and suggest BSF larvae as a promising new feed ingredient. Therefore, the suitability of BSF larvae to use as a feed in sheep need to be investigated.

3. METHODOLOGY

3.1 Rearing BSF

3.1.a Eggs Hatching

As much as 5 gr of BSF eggs was placed approx. 5 cm above container that filled by chicken feed for starter chicks that mixed with 70% of water. BSF larvae will hatch in several days. After hatching, larvae will fall into food source in the nursery container. First instar of BSF larvae remain in the same nursery container for five days after hatching.

3.1.b Larvae Development

After five days on the nursery container, BSF larvae should be moved to new container that divided based on the age of larvae. BSF larvae will reach full maturity and entering the pre-pupae stage after two weeks, the pre-pupae was seeking a dry place to pupate. As much as 10% of the pre-pupae population was transferred into transfer container that contain sand or soil for pupation and mating stage. Another 90% pre-pupae population was used as a protein source for feed formulation.

3.1.c Pupation and Mating

The transfer container should be placed inside a cage (insectarium) that made from muslin in the square shaped (3m x 3m x 3m). The BSF adults will emerged from pupae after ten days. This insectarium contained the plant for new-hatched BSF adults to perched. The new-hatched BSF adults mate immediately after emerged from pupae and was ovipositing in 3-4 days. To collecting the eggs, the stack of wooden sheets with small gaps in between or a cardboard was placed above the food source container that covered with muslin (for the attractant).

3.2 Cocoa pod husk bioconversion

Enlargement of the newly hatch larvae was carried out on baby chicken feed. After 5 days, the larvae are transferred into each container according to the treatment. The number of larvae is 100 larvae per container. The amount of daily feed given to the larvae referring to the result by Manurung *et al.* (2016) is 100 mg / larvae / day. Hence, the total amount of the treated CPH given per day is 10 g / container / day. To give the BSF larvae time to process the feed treatment given, the new feeding is done every three days so that the amount of new feed given to replace the old feed is 30 grams / 3 days. The CPH treatments for feeding the larvae are as follows:

1. Control: feeding the larvae with left-over food waste from the beginning until the trial period ends

2. Composted CPH (LK): feeding the larvae with composted CPH from the beginning until the trial period ends

3. Chopped CPH (LF): feeding the larvae with chopped-around-two centimetre-cube CPH from the beginning until the trial period ends

4. Composted CPH + left-over food: feeding the larvae using left-over food first for 6 days followed by composted CPH until the trial period ends

5. Chopped CPH + left-over food: feeding the larvae using left-over food first for 6 days followed by chopped-around-two centimetres-cube CPH until the trial period ends

6. Blended CPH (LFB): feeding the larvae using blended CPH from the beginning until the trial period ends.

The container used in this experiment is a jam bottle covered with gauze. Feed is treated according to the treatments and stored in the refrigerator to avoid decomposition (specifically for the treatment of chopped CPH feed). The treatments are replicated three times so that 18 jam bottles are used in the experiment. Feed is given every three days until the larvae develop into the pre-pupae phase.

The development time of BSF larvae was observed by calculating the wet weight of five larvae in each jam bottle sampled every three days until one of the larvae turned into fly. As soon as one of the larvae flies, observation is stopped.

For waste reduction index (WRI) calculation, the remaining feed is replaced every three days and BSF larvae are transferred to a new jam bottle containing new feed. The remaining feed was then weighed to calculate the weight of the wet residue. WRI, RGR, calculations obtained using Equation 1

$$WRI = (D/t) \times 100$$
$$D = (W-R)/W \quad (1)$$

Where:
D = Overall material degradation
W = Total amount of food provided during the experiment
t = Duration of the experiment (days)
R = Total amount of residue during the experiment

Relative growth rate (RGR) are calculated using Equation 2

$$RGR = [(w_2 - w_1)/w_1]/(t_2 - t_1) \quad (2)$$

Where:
w_2 = final total wet weight
w_1 = initial total wet weight
t_2 = last observation day
t_1 = initial observation day

Calculation of Efficiency of Conversion of Ingested Food (ECI) uses Equation 3

$$ECI = [(w_2 - w_1)/(W-R)] \times 100\% \quad (3)$$

3.3 Proximate analysis of BSF larvae

BSF larvae fed by the best CPH treartment was then analyzed for its proximate composition prior to feed formulation, i.e. water content, ether extract, crude protein and crude fibre. Water content was obtained by drying the sample in oven at 105 °C until it reaches constant weight. Ether extract was measured gravimetrically after diethyl ether extraction in a Soxhlet system. Prior to Soxhlet extraction, hydrolisis with 3 mol/L HCl at 100ºC for 60 minutes was performed. Crude protein of the larvae was measured using conversion factor of 5.71 multiplied by total nitrogen which were determined using Kjeldahl method (Caligiani *et al.*, 2018).

3.4 Propragation of large-scale BSF larvae

3.4.1 Large scale CPH waste composting

The best treatment of small scale BSF larvae enlargement is LKS treatment. Therefore, composting of 4 tons of CPH was immediately started after conclusions were obtained from the small experiment. Composting of CPH waste is carried out using Promi decomposers at a dose of 25 kg / ton in a cocoa plasma plantation located in Bobojong Village, Mande Sub-district, Cianjur District, West Java Province. CPH waste is collected near the harvesting route in the field closest to the main road and the collection schedule followed the harvest schedule of cocoa beans. The CPH waste is stacked as high as 50 cm in an area of 2 x 1 m (Figure 1a) as many as five spots along the main road which intersects with harvesting route. The Promi decomposer is spread evenly on the mound (Figure 1b). To speed up composting and prevent evaporation, the mound is covered with transparent plastic (Figure 1c). Mature compost is characterized by a change in color from brownish red to blackish gray (Figure 1d). The process takes approximately three weeks. The mature CPH compost is then transported to the BSF larvae enlargement facility in PT Biomagg, Depok

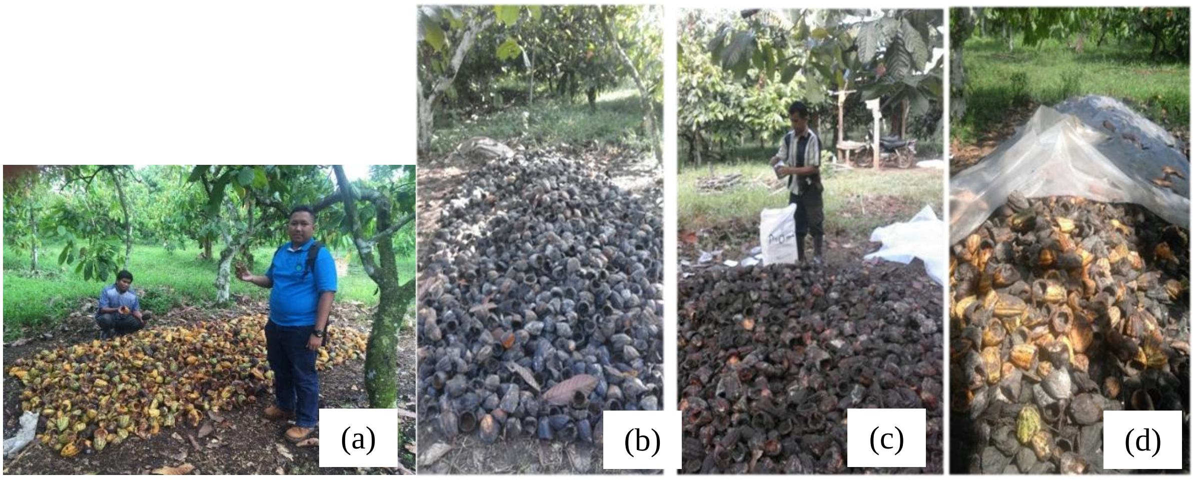

Figure 1 The composting process carried out in the farmer's cocoa field. (a) CPH waste is collected and square-formed, (b) Promi decomposer application, (c) CPH mound is covered with transparent plastic, (d) results of CPH composting after three weeks

3.4.2 Large-scale enlargement of BSF larvae with LKS treatment

Large scale BSF larvae enlargement activities are carried out at PT Biomagg, Depok. Three trays measuring 3 x 1 x 0.2 m were used in this experiment (Figure 2). This large-scale production system uses a batch system so that the larvae enlargement process cannot be completed in a single process (Figure 2a). There is a slight difference on feeding between large- and small-scale trials. In small scale experiment, left-over food waste is given separately with CPH compost, while on a large scale, left-over food waste is poured first as the first layer then the larvae as the second layer followed by CPH compost as the third layer (Figure 2b). This is done to prevent the composted CPH from drying out which can result in the loss of the eating preferences of BSF larvae on composted CPH. The number of BSF larvae eggs, left-over waste, and (a) compost in one tray are 10 grams, (b) ; and 50 kg, respectively. The production target of BSF larvae for experimental f2eed formulations in sheep is 210 kg

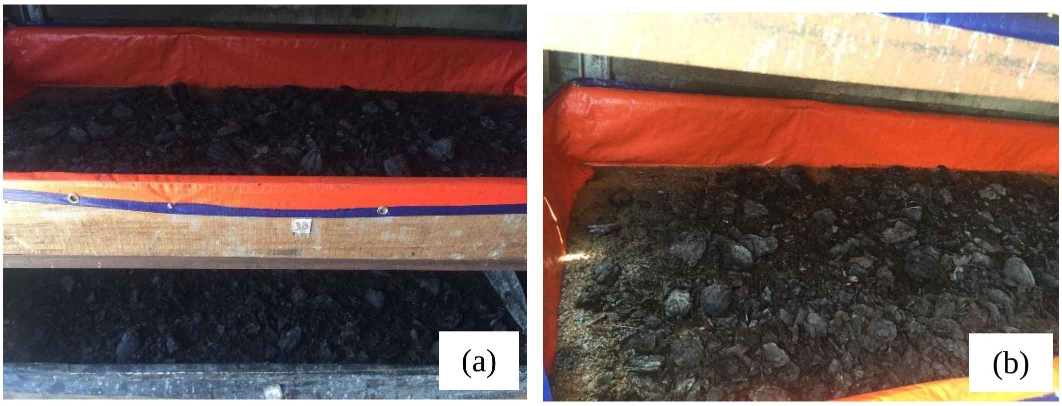

Figure 2 Large scale BSF larvae enlargement in Depok. (a) one batch consists of three trays, (b) the pouring stages are left-over food waste, BSF larvae, and composted CPH as the first, second, and third layers, respectively

3.5 Feed formulation

After determining its nutrient content, biomass of instar 5 BSF larvae was formulated into complete ration for sheep. Table 1 summarizes five formulas being used in the experiment. All the experimental rations was formulated according to energy and protein requirements of small ruminants in Indonesia as recommended by Jayanegara *et al.* (2017b).

Table 1 Five treated rations using BSF and CPH residue to substitute soybean meal and coffee skin, respectively, in business-as-usual ration

Treatments	**CPH Residue**(*	**BSF**	**Note**
R1	0%	0%	Control ration
R2	0%	5%	BSF replaced soybean meal 100%
R3	20%	0%	CPHR replaced coffee skin 100%
R4	20%	5%	CPHR replaced 100% coffee skin BSF replaced soybean meal 100%
R5	10%	2.5%	CPHR replaced coffee skin 50% BSF replaced soybean meal 50%

Note: (* CPH Residue (CPHR) is maggot's excreta resulted from bioconversion process

3.6 Sheep performances

Five experimental treatments above were fed to sheep. A total of 20 fat tail male sheep was used as the experimental unit. Sheep was divided into five groups according to experimental treatments so that each treatment is consisted of four sheep (served as replicates). Allocation of experimental rations to sheep was based on a randomized complete block design. Diet and water was provided *ad libitum*. Experimental period was performed for 10 weeks in which the first two weeks are adaptation period and the remaining weeks (week 3-10) are measurement period. Variables measured during *in vivo* feeding trial to sheep are feed and nutrient intake, rumen fermentation characteristics (pH, volatile fatty acid and ammonia concentration) and animal performance (initial body weight, final body weight and average daily gain)

3 RESULTS AND DISCUSSION

4.1 Cocoa pod husk bioconversion

Development time of BSF larvae fed on various treated CPH can be seen on Figure 3 The best response was found by control (fed with left-over food) which was characterized by the highest final wet weight and the fastest development time from larval stage to pre-pupae among all treatments, which are 77.33 grams per 100 larvae and 21 days, respectively. The treatment which was preceded by the administration of left-over food waste until the 6th day, both LKS and LFS, gave a better response compared to the treatment without left-over food waste at the beginning of the development phase. This can be seen from the final total wet weight of LKS and LFS which is above 40 grams with a development time of under 30 days, while the final total wet weight and the development time of LK, LF, and LFB treatments are below 40 grams and over 30 days, respectively. This is presumably because at the initial stage development of the larvae, the mandible is still small and cannot be used to chew hard foods such as CPH, even it has been composted, chopped or blended.

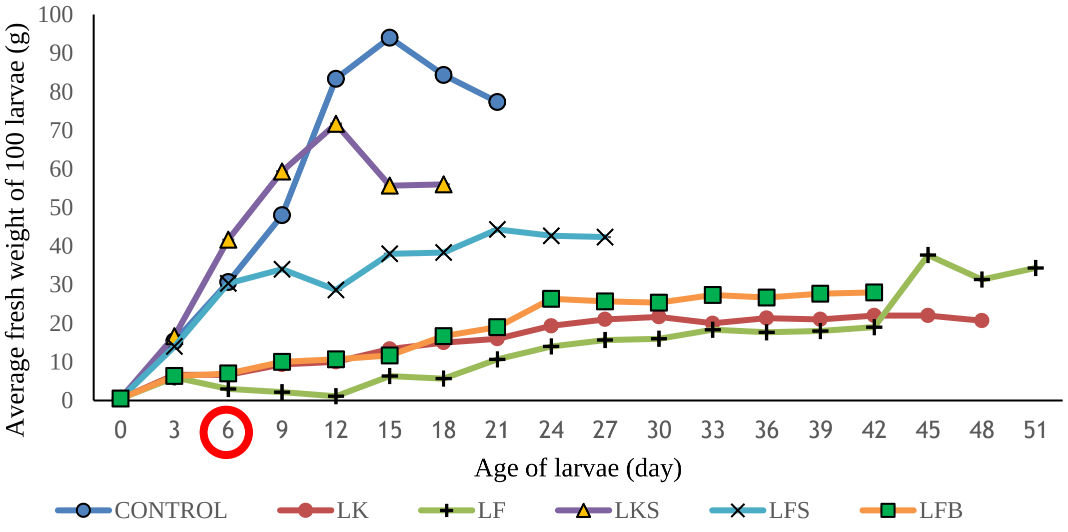

Figure 3 Development time of BSF larvae until pre-pupae stage fed by various CPH treatments. The treatments are left-over food (control), composted CPH (LK), chopped CPH (LF), composted CPH preceded by left-over food (LKS), chopped CPH preceded by left-over food (LFS), and blended fresh CPH (LFB). Left-over food in LKS and LFS treatment are given at the beginning of the experiment until day 6 (marked with a red circle). Standard Errors are not seen in the graph because all the values are below 0.1

Calculation of WRI, RGR, and ECI values can be seen in Table 2. Overall, the feed for larvae which has the highest score on the three parameters observed is left-over food waste (control). However, the WRI value in the control is the second best compared to the LKS treatment. The higher the WRI value index, the higher the reduced organic waste, so that it can be said that the LKS treatment can reduce more organic waste (in this case, cocoa pod waste) than control. In addition, the LKS treatment has the second best RGR and ECI values after that of the control. Therefore, the selected treatment to be characterized further was composted CPH mixed with left-over food waste.

Table 2 The average values of waste reduction index (WRI), relative growth rate (RGR), and Efficiency of conversion of ingested food (ECI). Numbers followed by different letters in the same column show significant differences at the 5% level according to the Duncan Multiple Range Test

Treatments	WRI	RGR	ECI
Control	1,87 ^{e}	7,69 ^{d}	81,29 ^{d}
LKS	2,33 ^{f}	5,72 ^{c}	63,00 ^{c}
LFS	0,97 ^{d}	3,14 ^{b}	54,03 ^{c}
LF	0,60 ^{c}	1,44 ^{a}	20,47 ^{a}
LFB	0,40 ^{b}	1,33 ^{a}	34,35 ^{b}
LK	0,30 ^{a}	0,86 ^{a}	28,14 ^{ab}

4.2 Proximate analysis of BSF larvae from the best treatment

Proximate analysis showed high protein levels (39.09% dry weight) but lower lipid levels (10.68% dry weight) when compared with control (Table 3). Higher protein values in the best CPH treatment (LKS) presumably due to protein intake by the larvae in the form of fungi mycelia contained in the composted CPH. Similar phenomenon was observed when cocoa pod husk was composted using *Phanerochaete chrysosporium* (Laconi & Jayanegara, 2015) The digestibility of feed mixtures supplemented with BSF larvae has been investigated through *in vitro* rumen fermentation study (Jayanegara *et al.*, 2017a). In this study, one of the factors that reduced digestibility was the lipid content measured using the ether extraction method. The higher the lipid content, the lower the *in vitro* digestibility due to the disruption of the growth of cellulolytic microflora in the rumen in the presence of lipids. It is expected that the low lipid content resulted from the best enlargement treatment in this experiment can overcome the feed digestibility containing BSF larvae, although the lipid concentration is not as low as the highly defatted larvae (4.6%) (Schiavone *et al.*, 2017b).

Table 3 Proximate analysis of BSF fed by left-over food (control) and composted CPH preceded by left-over food

Parameters	Control	LKS
Water content	33,76%	19,88%
Crude ash	3,88%	13,82%
Crude Protein	31,33%	39,09%
Crude Fat	30,73%	10,68%
Carbohydrate	0,30%	16,52%

4.3 Sheep performances

Sheep performances that can be measured up until now are average daily consumption, average weight gain, feed conversion ratio, and % dry matter over a month. Average daily consumption, feed conversion ratio, and % dry matter values are not significantly different among treatments (Table 4). Therefore, the treated rations could be substitutes for control ration. Further, treatment R2 (BSF replaced 100% soybean meal) was the second best in terms of average weight gain compared to control ration, although it did not significantly differ (Figure 4). However, longer observation periods (up to 3 months) are needed to make a valid statement along with the measurement of rumen fermentation characteristics.

Table 4 Treatments effect towards average daily consumption, feed conversion ratio, and dry matter digestibility of sheep fed with studied rations within one month. Numbers followed by same letters in the same column show no significant differences at the 5% level according to the Duncan Multiple Range Test

Treatments	**Average Daily Consumption (kg)**	**Feed Conversion Ratio**	**% Dry Matter**
Ration 1	1.01 [a]	15.8% [a]	3.2% [a]
Ration 2	0.94 [a]	15.7% [a]	3.0% [a]
Ration 3	0.92 [a]	13.6% [a]	3.4% [a]
Ration 4	1.01 [a]	12.9% [a]	3.3% [a]
Ration 5	1.01 [a]	12.4% [a]	3.5% [a]

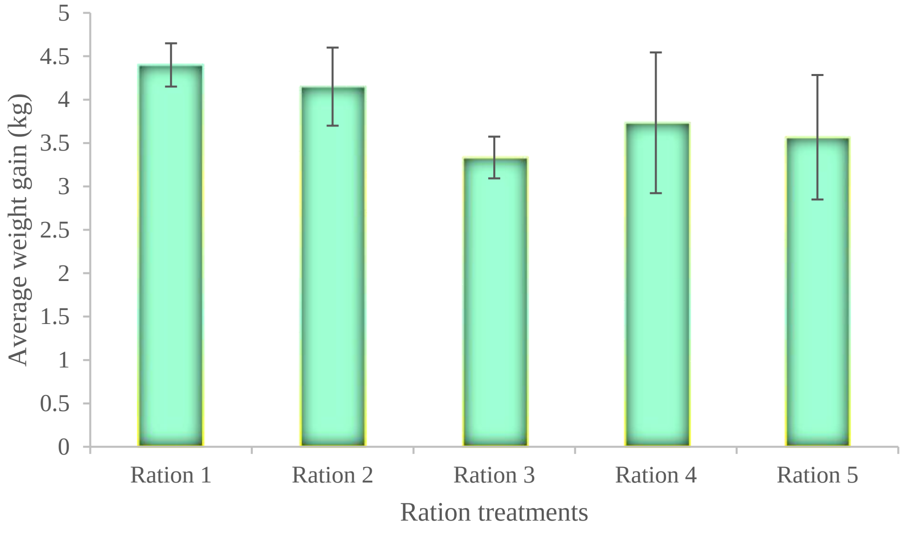

Figure 4 Average weight gain (kg) of sheep in a month fed with various concentration of BSF larvae and CPH residues mixed into standard ration

5. CONCLUSIONS

The best CPH treatment to reared BSF larvae is composted CPH preceded by the left-over food waste. BSF larvae fed on the best CPH treatment gave better results in terms of crude protein and crude fat when compared to that of control. BSF could be a substitute for soybean meal based on the performance of R2 ration on average daily consumption, feed conversion ratio, % dry matter, and average weight gain of sheep over a month. However, further studies are needed to confirm the statement.

6. PERSONAL INVESTIGATOR AND OTHER RESEARCHERS

No.	Name	Institution	Expertise	Job Desc.	Allocated time (hour/week)
1	Dr. Siswanto, DEA	Indonesian Research for Biotechnology and Bioindustry	Biochemistry	Coordinating the research and supervise crude protein measurement	6
2	Haryo Tejo Prakoso, M.Agr.Env	Indonesian Research for Biotechnology and Bioindustry	Biologist	crude protein measurement	2
3	Deden Dewantara Eris,	Indonesian Research for	Agronomist	Cocoa farmer coordinator and	2

	SP	Biotechnology and Bioindustry		cocoa pod husk collection and composting	
3	Ciptadi Achmad Yusup, M.Si.	Indonesian Research for Biotechnology and Bioindustry	Entomologist	BSF biomass production and bioconversion measurement	2
4	Aminudi, SP.	Biomagg Indonesia	Entomologist	BSF biomass production and bioconversion measurement	2
5	Dr. Anuraga Jayanegara	Bogor Agricultural University	Feed Nutritionist	Feed formulation and sheep weight measurement	4

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