Fermented Black soldier fly (*Hermetia illucens*) meal utilization in artificial feed for carp (*Cyprinus carpio*)

[1]Vivi E. Herawati, [1]Pinandoyo, [2]YS Darmanto, [1]Johannes Hutabarat, [1]Seto Windarto, [3]Nurmanita Rismaningsih, [4]Ocky K. Radjasa

[1] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia; [2] Department of Fishery Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia; [3]Department of Materials Chemistry, Graduate School of Engineering, Nagoya University, Nagoya, Japan; [4] Department of Marine Science, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia. Corresponding author: V. E. Herawati, viviendar23@gmail.com

Abstract. Black soldier fly (*Hermetia illucens*) meal has a high protein content, and it is used as an alternative replacement of fish meal in artificial feeds. It is easily available and affordable compared to fish meal. This study aimed to determine and analyze fish meal substitution with fermented Black soldier fly meal in an artificial feed, in order to increase the production and nutritional quality of carp (*Cyprinus carpio*), especially improvements of the fatty acid profile. A completely randomized design with four treatments and three replications was used. The treatments applied consisted in different dosages of fish meal substitution with fermented Black soldier fly meal. They were: A - 0% substitution; B - 12.5% substitution; C - 25% substitution; D - 37.5% substitution. A fix feeding rate of 5% of the biomass was applied. The fish used in this study were common carp with approximately 3.5 cm length and an average weight of 0.62 g/fish, with a stocking density of 1 fish L^{-1}. The primary data observed were total feed consumption (TFC), feed utilization efficiency (FUE), protein efficiency ratio (PER), specific growth rate (SGR), survival rate (SR), and fatty acid profile. Treatment D presented the best results, with a significant effect ($P<0.05$) on FUE, PER, and SGR, but no significant effect on TFC and SR. Treatment D produced a FUE of 75.66%, PER of 2.13%, SGR of 2.83% and SR of 91.11%. The best nutritional quality was also observed in fish from treatment D, with linolenic acid having the highest content in the identified fatty acids, of 9.59%.

Key Words: common carp, fermentation, maggot meal, nutritional quality, production.

Introduction. Black soldier flies (*Hermetia illucens*) grow widely in animal wastes such as cow manure, goat manure, poultry manure, and organic wastes under good conditions. Black soldier flies can be processed into meal that can substitute fishmeal. The best life stages to produce meals are the prepupa and larval phases. St-Hilaire et al (2007) reported that larvae have a crude protein and fat content of 36-48% and 31-33%, respectively, and could be an alternative source of protein to fishmeal, as a raw material for fish feed (Bondari & Sheppard 1981). Common carp is a freshwater fish with high economic value and has a high demand in markets compared to Nile tilapia (*Oreochromis niloticus*), gourami (*Osphronemus goramy*), and African catfish (*Clarias gariepinus*) (Atanasoff 2014).

The increase in demand for common carp has not been utilized properly by farmers, because feed administered does not usually meet the needs of carp. One of the causes is the decreasing supply of fishmeal, which makes it more expensive. Some agricultural ingredients and livestock industry wastes such as soybean meal, corn gluten, and meat and bone meals are now widely used, but the amount of those ingredients is not that high. These conditions lead to the constant search of replacements for fishmeal (Kroeckel et al 2012).

Black soldier fly meal used to substitute fishmeal is fermented first to improve the nutritional quality of the feed. The fermentation process aims to increase the nutrient content in the feed, so that it can increase fish production and quality (Herawati et al 2017). Probiotic bacteria are bacteria that support the health of other organisms. Probiotic bacteria are a source of nutrition that contribute to enzymes in the digestion system of fish and help the absorption of organic material mediated by probiotics. In aquaculture, probiotics increase fish immunity to pathogens and greatly contribute to fish digestive enzymes (Nwachi 2013).

Research on substitution of fishmeal with Black soldier fly meal has been carried out for Bala shark (*Balantiocheilos melanopterus*) (Priyadi et al 2009), and African catfish catfish (*Clarias gariepinus)* (Kurniasih et al 2014) with satisfactory results. Therefore, further research is needed to determine the effect of fishmeal substitution with Black soldier fly meal on the efficiency of feed utilization and growth of common carp. Based on the foregoing description, this study examined and discovered larvae meal nutrients, and experienced in substituting fishmeal with *H. illucens* larvae meal to improve the nutritional quality and production of carp hatcheries. This study aimed to analyze the substitution of fishmeal with fermented Black soldier fly meal in artificial feed to increase the production and improve the fatty acid profile of common carp.

Material and Method. This research was conducted from March 2019 to May 2019 at the Aquaculture Laboratory of Diponegoro University, Indonesia. The fish used in this study were common carp, 3 to 4 cm in length and 0.62±0.14 g mean weight. The stocking density was 1 fish L^{-1}. Carp was selected this study based on a previous study where Jian carp presented good results when 25% of fishmeal was substituted with Black soldier fly meal (Li et al 2017). The 25% substitution ratio acts as a median in this experiment. Acclimatization was carried out for 1 hour to prevent fish from becoming stressed. The tested fish were maintained in the same conditions for 14 days, to adapt to their new environment.

The tested feed used in this study was pellet-shaped artificial feed, with 30% crude protein. It is in line with a previous study conducted by Takeuchi et al (2002), who stated that carp needs 30-35% crude protein in its feed.

This study was conducted using a completely randomized design (CRD) with four treatments and three replications. The meal was made from dried prepupae. They were grinded and blended. The meal was left to ferment for 7 days. Different dosages of fishmeal substitution with fermented Black soldier fly meal were used in the following treatments: A (0%), B (12.5%), C (25%), and D (37.5%). The tested fish were administered feed by a fix feeding rate of 5% of the total biomass. Feeding was carried out 3 times a day, in the morning (08:00), noon, and afternoon (16:00), in the 45 days of experiment.

The proximate chemical composition of the feed and samples was determined using a standard procedure (AOAC 2005; Herawati et al 2018). The fatty acid profile of the samples was determined using a gas chromatograph (Shimadzu) (AOAC 2005; Herawati et al 2015; Herawati et al 2018).

The measured variables of the study were the total feed consumption (TFC), feed utilization efficiency (FUE), protein efficiency ratio (PER), specific growth rate (SGR), and survival rate (SR).

The TFC was calculated using the following formula (Pereira 2007):

$$TFC = F1 - F2$$

Where: F1 - initial amount of feed (g); F2 - amount of leftover feed (g).

The FUE was calculated using the following formula (Watanabe 1988):

$$FUE = \frac{W_t + D - W_0}{F} \times 100\%$$

Where: W_t - tested fish biomass weight at the end of the observation (g); W_0 - tested fish biomass weight at the beginning of the observation (g); D - dead fish weight (g); F - the amount of feed administered during observation (g).
The PER was calculated using the following formula (Tacon & Metian 2008):

$$PER = \frac{W_t - W_0}{Pi} \times 100\%$$

Where: W_t - tested fish weight at the end of the observation (g); W_0 - tested fish weight at the beginning of the observation (g); Pi - weight of feed consumed × % of feed protein.

The SGR is calculated using the following formula (Abdel-Tawwab et al 2010):

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\%$$

Where: W_t - average fish weight at the end of the study (g); W_0 - average fish weight at the beginning of the study (g); t - time of the study (day).

The SR of fish was calculated using the following formula (Effendi 1997):

$$SR = \frac{N_t}{N_0} \times 100\%$$

Where: N_t - number of fish at the end of the study; N_0 - number of fish at the beginning of the study (fish).

The water quality parameters monitored were temperature, dissolved oxygen (DO), pH and ammonia content (NH_3). The ammonia content was determined at the beginning and at the end of the study. Temperature, DO, and pH measurements were carried out every morning, afternoon, and evening. Ammonia content was analyzed at the Laboratory of the Department of Environmental Engineering, Diponegoro University. The temperature was measured using a thermometer, and pH measurements were carried out using a pH meter. DO levels were determined using a DO meter.

Data was analyzed using the variance analysis (ANOVA). There were several normality tests, homogeneity tests and additivity tests conducted prior to ANOVA. Where significant differences were observed ($P<0.01$), Duncan's multiple range tests were conducted. All tests were carried out using SPSS version 23.0 software, except for the additivity testing, which was carried out using Microsoft Excel.

Results and Discussion. The proximate composition of the experimental feeds and the ingredients used are presented in Table 1. Since the amount of fermented Black soldier fly meal varied between treatments, there were different levels of wheat flour and rice bran flour to adjust the composition in order to meet the carbohydrate content needed in the preparation of feed formulations. The preparation of feed formulations was based on crude protein and carbohydrate requirements of carp.

The fatty acid contents of the feeds from each treatment are presented in Table 2. The fatty acid with the highest content out of essential fatty acids was linolenic acid with 7.85% of total FAME.

The values of the TFC, FUE, PER, SGR, and SR of carp in the 3 treatments and control are presented in Table 3. The substitution of fishmeal with fermented Black soldier fly meal proved to have a significant effect ($P<0.05$) on FUE, PER and growth of carp, but did not have significant effects ($P>0.05$) on the SR of carp. All treatments in this study (with fishmeal substituted by *H. illucens* meal) have a high SR value, results confirmed by other authors, but for tilapia (*Oreochromis niloticus*) (Ogunji et al 2008).

The highest value of the final weight, TFC, FUE, PER, SGR, and SR were in treatment D, with fishmeal substituted by 37.5% with fermented Black soldier fly meal, while the lowest values were observed in the control treatment, without fishmeal substitution.

Table 1

Composition of feed ingredients and proximate analysis of feed (% dry weight)

Feed ingredients	*Feed composition (%)*			
	A	*B*	*C*	*D*
Fishmeal	25	21.87	18.75	15.625
Fermented Black soldier fly meal	0	4.04	8.08	12
Soybean meal	33	33	33	33
Corn starch	3	3	3	3
Rice bran flour	15.5	13.84	14.67	13.76
Wheat flour	14	14.25	13.5	14
Fish oil	3	3	2	2
Palm oil	3	3.5	3.5	3
Vitamins-minerals mix	2.5	2.5	2.5	2.5
Carboxymethylcellulose	1	1	1	1
Total	100	100	100	100
Protein	30.08	30.06	30.02	30.03
Nitrogen-free extract (NFE)	32.92	31.38	32.56	32.50
(Crude fat) (%)	13.22	13.09	12.18	11.77
Energy (kcal)	294.66	289.64	285.13	281.72
E/P Ratio	9.79	9.64	9.49	9.38

Note: E/P - the energy to protein ratio for optimal growth of fish ranges between 8-12 kcal g^{-1} (De Silva 1987).

Table 2

Fatty acid content of feeds administered in each treatment

Fatty acid methyl esters (FAME)	*Samples*			
	A	*B*	*C*	*D*
Saturated Fatty Acids % of total saturated fatty acids				
Butyrate	0.73±0.09	0.33±0.06	0.88±0.03	1.95±0.09
Hexanoate	1.85±0.09	2.16±0.02	2.09±0.04	2.75±0.03
Undecanoate	1.90±0.08	2.82±0.05	2.75±0.03	3.09±0.02
Laurate	2.95±0.08	3.86±0.07	3.09±0.02	3.83±0.02
Tridecanoate	3.75±0.09	6.15±0.02	2.83±0.02	5.23±0.06
Pentadecanoate	5.83±0.06	2.28±0.07	4.19±0.06	6.95±0.08
Palmitate	2.19±0.09	5.37±0.03	4.95±0.08	8.95±0.02
Heptadecanoate	4.15±0.03	2.16±0.02	3.95±0.02	4.19±0.07
Arachidate	1.85±0.09	3.72±0.05	3.19±0.07	6.64±0.07
Tricosanoate	1.66±0.02	1.93±0.06	1.85±0.02	2.55±0.02
Unsaturated Fatty Acids % of total unsaturated fatty acids				
Linolenic	2.35±0.02	2.97±0.06	4.85±0.04	7.85±0.04
Linoleic	2.97±0.06	3.75±0.07	4.90±0.06	6.99±0.06
Erucate	3.08±0.09	4.56±0.05	5.95±0.01	6.95±0.01
Eicosapentaenoate	2.62±0.05	3.05±0.02	3.17±0.01	4.17±0.01
Docosahexaenoate	1.07±0.03	2.75±0.03	3.59±0.07	4.59±0.07

Treatment D produced the highest FUE value of 75.66% and PER value of 2.13%. These results indicate that the protein quality is not optimal, because out of the utilization of feed for growth (75.66±0.91%), protein only accounts for 2.13%. PER was calculated in this study to evaluate the quality of protein in feed absorbed and utilized by carp, based on weight gain of tested fish divided by the amount of protein in feed consumed in one study cycle (Bhilave et al 2012). There was a higher PER value than the PER value from a study where Black soldier fly larvae meal substituted fishmeal in a diet for juvenile *O. niloticus* (1.2-1.3%) (Devic et al 2018).

Table 3

The values of growth and feeding parameters

Treatment	*TFC (g)*	*FUE (%)*	*PER (%)*	*SGR (%)*	*SR (%)*	W_t *(g)*
A	16.85±1.64[a]	42.05±1.82[b]	1.23±0.07[bc]	1.76±0.24[bc]	87.00±2.85[a]	16.98±0.81[a]
B	17.76±2.86[a]	44.54±1.95[b]	1.17±0.41[c]	1.98±0.18[c]	86.67±2.67[a]	17.16±0.81[a]
C	18.66±1.84[a]	55.57±1.57[b]	1.65±0.13[b]	2.07±0.12[b]	88.33±2.55[a]	18.68±0.18[a]
D	19.46±0.23[a]	75.66±0.91[a]	2.13±0.08[a]	2.83±0.07[a]	91.11±2.85[a]	25.23±0.81[a]

Note: TFC - total feed consumption; FUE - feed utilization efficiency; PER - protein efficiency ratio; SGR - specific growth rate; SR - survival rate; W_t - final weight.

Based on the FUE values of the carp, the orthogonal polynomial tests were carried out (Figure 1). The FUE value in treatment D was significantly different from the FUE value in treatment A, while the values in treatments A and B were not different from that of treatment A.

The results of the calculation of the orthogonal polynomial test show a quadratic pattern relationship: $y = 5.123x^2 - 15.671x + 52.582$, with $R^2=0.9377$. There is no optimum point reached. The substitution ratio of fishmeal with Black soldier fly meal, by the value of R^2, shows that the linearity of the graph is 93.77%.

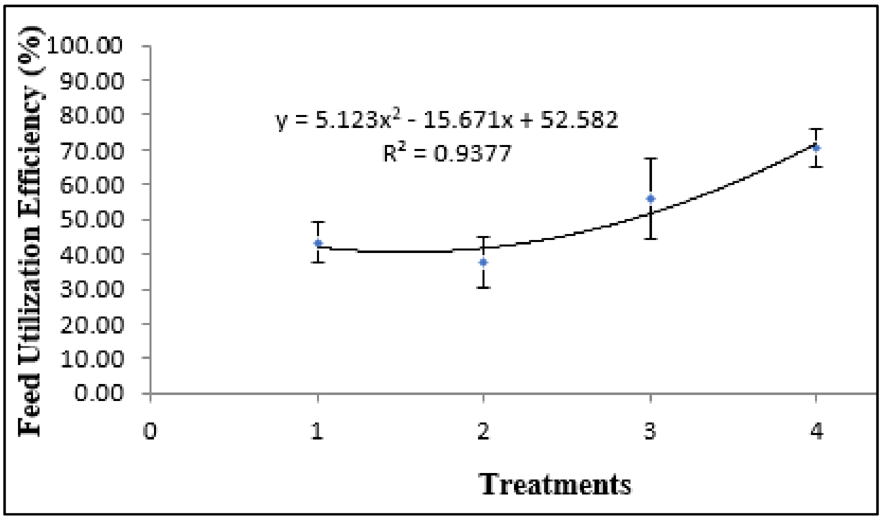

Figure 1. Orthogonal polynomial histogram of feed utilization efficiency (%) of carp (*Cyprinus carpio*).

Based on the PER values determined for carp, the orthogonal polynomial tests were carried out (Figure 2). Based on the orthogonal polynomial test, the following quadratic pattern relationship was obtained: $y = 0.188x^2 - 0.6444x + 1.5703$, and $R^2=0.8993$. There is no optimum point reached. The substitution ratio of fishmeal with Black soldier fly meal, by the value of R^2, showed that the linearity of the graph is 89.93%.

The increase in the value of PER is followed by an increase in the value of the SGR. The highest PER and SGR values were 2.13% and 2.83% resulting in treatment D, while the lowest were observed in the control lot. The results showed that the high PER value was directly proportional with the SGR value. This is because the protein in the feed can be easily broken down, so it can be maximally absorbed and utilized for supporting growth. This is reinforced by other studies as well (Madeira et al 2013). Good protein absorption has a positive impact on biomass weight growth. A higher protein conversion value of a feed indicates the higher efficiency of a feed, because the existing protein can be used optimally (Xiao et al 2018).

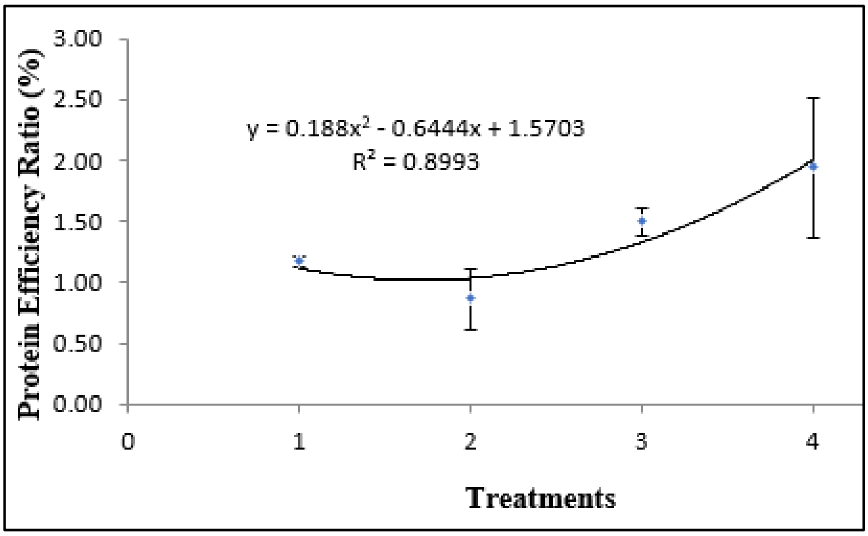

Figure 2. Orthogonal polynomial histogram of the protein efficiency ratio (%) of carp (*Cyprinus carpio*).

Based on the SGR results, the orthogonal polynomial tests were carried out during the study (Figure 3). The best SGR value was in treatment D, 2.83%, while the lowest value was in treatment A, with 1.76%. This result was lower than that of Zhou et al (2018) who replaced dietary fish meal with Black soldier fly larvae meal for Jian carp (*C. carpio var.* Jian), and obtained a SGR of 3.7-3.8%. Based on the orthogonal polynomial test, a quadratic pattern relationship was obtained, with $y = 0.2027x^2 - 0.7809x + 2.6073$, and R^2=0.9453. There is no optimum point reached. The substitution ratio of fishmeal with fermented Black soldier fly meal, by the value of R^2, showed that the linearity of the graph is 94.53%.

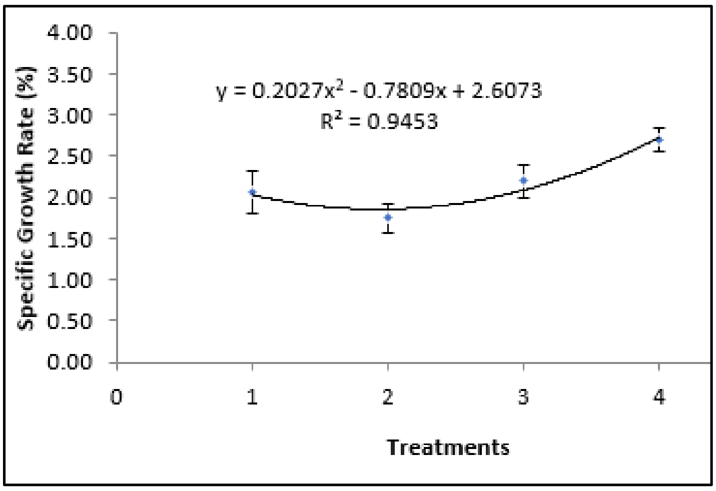

Figure 3. The orthogonal polynomial histogram of the specific growth rate (%) of carp (*Cyprinus carpio*).

The protein to energy ratio (E/P) of feed ranges from 9.38 to 9.78 kcal g^{-1} protein, which is within the range of appropriate energy ratio, of 8.9 to 12.3 kcal g^{-1} protein (NRC 1993).

The fatty acid profile of carp. The fatty acid profile of common carp fed by artificial feed with fermented Black soldier fly meal is presented in Table 4. The fatty acid profile of carp in treatment D presents the highest levels of linoleic acid out of essential fatty acids, with was 9.59% of total FAME. The highest level for an essential fatty acid was observed in treatment D, where linolenic acid represented 9.59% of total identified fatty acids. This result confirms the results of Zarantoniello (2019), where linoleic acid (18:2n6) was the most abundant PUFA in all the dietary treatments where Black soldier Fly (*Hermetia illucens*) based diets were administered to zebrafish (*Danio rerio*). Linolenic acid functions as a base in the formation of linoleic acid and long-chain PUFA (Zengin et al 2013; Herawati et al 2015).

Table 4

Fatty acid profile of common carp (*Cyprinus carpio*) fed artificial diets with fermented Black soldier fly (*Hermetia illucens*) meal

Fatty acid methyl esters (FAME)	*Treatments*			
	A	*B*	*C*	*D*
Saturated Fatty Acids				
% of total saturated fatty acids				
Butyrate	0.88±0.09	0.92±0.03	0.99±0.07	1.93±0.09
Hexanoate	1.89±0.03	1.97±0.05	2.01±0.05	2.59±0.04
Undecanoate	3.09±0.02	3.47±0.04	3.63±0.05	5.98±0.05
Laurate	1.83±0.02	1.95±0.01	1.98±0.04	2.79±0.06
Tridecanoate	3.82±0.06	3.97±0.06	4.02±0.05	5.78±0.08
Pentadecanoate	3.86±0.08	3.94±0.01	4.32±0.06	8.20±0.08
Palmitate	3.13±0.02	3.33±0.02	4.68±0.04	6.96±0.04
Heptadecanoate	1.28±0.07	2.43±0.07	3.79±0.08	4.83±0.04
Arachidate	3.37±0.07	3.40±0.07	3.59±0.09	4.19±0.07
Tricosanoate	1.07±0.01	2.15±0.07	2.38±0.07	3.59±0.08
Unsaturated Fatty Acids				
% of total Unsaturated Fatty Acids				
Linolenic	6.07±0.01	6.15±0.07	7.38±0.07	9.59±0.08
Linoleic	1.08±0.04	1.19±0.08	1.27±0.09	1.57±0.08
Erucate	1.72±0.02	1.67±0.02	1.75±0.02	1.98±0.07
Eicosapentaenoate	0.81±0.05	1.02±0.04	1.36±0.05	1.89±0.05
Docosahexaenoate	2.07±0.01	2.15±0.07	4.38±0.07	6.19±0.08

The water quality parameter values during the study are presented in Table 5. Water quality during the study is appropriate for the growth and development of carp. Water quality is very influential on the SR of fish. The temperature in the cultivation medium fluctuated from 23 to 29^{o}C. Unstable temperatures can cause stress and lead to death. Oxidative stress can occur due to an increase in temperature and there seems to be a relationship between thermal stress response and oxidative stress response (Madeira et al 2013). Fish mortality can occur at the time of water change. Water changes can cause fish to experience stress. The inability of weak fish to adapt can result in death. Some mortalities were observed in this study, but the differences were not significant.

Table 5

Water quality parameter values

Treatments	*Temperature (^{o}C)*	*pH*	*DO (mg L^{-1})*	*NH_3 (mg L^{-1})*
A	23-29	7.3-7.8	3.4-4.8	0.02-0.19
B	23-29	7.3-7.9	3.4-4.8	0.02-0.19
C	23-29	7.4-7.8	3.4-4.8	0.02-0.19
D	23-29	7.3-7.8	3.4-4.8	0.02-0.19
Appropriate range (a)	23-30	6.5-9	>3	<0.2

Note: DO - dissolved oxygen; a - Zonneveld et al (1991).

Conclusions. Fermented Black soldier fly meal could be applied as an alternative replacement of fishmeal in artificial feeds for common carp. The meal is easily available and affordable compared to fishmeal, it has a good nutritional quality, meeting the needs of carp. However, finding the best feed formulation to produce high quality and quantity in carp farming is important and sometimes difficult to reach. Based on the results, the highest values of final weight, total feed consumption, feed utilization efficiency, protein efficiency ratio, specific growth rate, and survival rate were found in the treatment with carp fed using a 37.5% substitution of fishmeal with fermented Black soldier fly meal.

Acknowledgements. This research was funded by funding sources of The Non-National Budget of LPPM RKAT Diponegoro University in the 2019 fiscal year with a letter assigning implementation of Research, Development, and Implementation activities (RPP) with number of 385-29 / UN7.P4.3 / PP / 2019.

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Received: 18 November 2019. Accepted: 22 November 2019. Published online: 26 April 2020.
Authors:
Vivi Endar Herawati, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: viviendar23@gmail.com
Pinandoyo, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: pinandjaya5758@gmail.com
YS Darmanto, Department of Fishery Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: ysdarmantofpik@gmail.com
Johannes Hutabarat, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: johannesfpik@gmail.com
Seto Windarto, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: seto.sidhartawan@gmail.com
Nurmanita Rismaningsih, Department of Materials Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, 464-8603 Nagoya, Japan, e-mail: nurmanitar@gmail.com
Ocky Karna Radjasa, Marine Science Department, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: ocky_radjasa@yahoo.com

How to cite this article:
Herawati V. E., Pinandoyo, Darmanto Y., Hutabarat J., Windarto S., Rismaningsih N., Radjasa O. K., 2020 Fermented Black soldier fly (*Hermetia illucens*) meal utilization in artificial feed for carp (*Cyprinus carpio*). AACL Bioflux 13(2):1038-1047.