

THE EFFECT OF SUBSTITUTION OF FISH MEAL WITH FRESH BLACK SOLDIER FLY LARVAE IN FEED OF TILAPIA (*Oreochromis* sp.)

Pengaruh Substitusi Tepung Ikan Dengan Larva *Black Soldier Fly* Segar Dalam Pakan Ikan Nila (*Oreochromis* sp.)

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ABSTRACT

The high price of feed is caused by the high price of fish meal as a raw material for protein-source of feed. *Black Soldier Fly* (BSF) larvae have good nutrient content, so they can be used as an alternative raw material for feed. This research aims to determine the effect of fish meal substitution with fresh BSF larvae on the growth performance of tilapia (*Oreochromis* sp.). The research was designed using a completely randomized design with four treatments in the form of fishmeal substitution with BSF larvae and triplicates, including P1 (0%), P2 (25%), P3 (50%), and P4 (75%). The average weight of individual fish used was $17,98 \pm 1,49$ g with a stocking density of 20 fish tank⁻¹ and kept for 60 days in fiber tanks measuring 50x50x60 cm³. Feed is given as much as 3% of the fish biomass with a frequency of giving twice per day at 08.00 am and 15.00 pm. The data were analyzed using analysis of variance with a significance level of 95%. If there is a significant difference, further testing is carried out using Duncan's Multiple Range Test. Water quality data were analyzed descriptively. The research results proved that fresh BSF larvae could replace fish meal by 75% ($P < 0,05$). Substitution of fish meal with fresh BSF larvae as much as 75% resulted in absolute growth rate based on length of $6,16 \pm 0,38$ cm, absolute growth based on weight of $53,41 \pm 10,40$ g, specific growth rate based on length of $0,10 \pm 0,01$ % day⁻¹, weight-based specific growth rate of $0,89 \pm 0,17$ % day⁻¹, feed conversion ratio of $1,13 \pm 0,14$ and feed efficiency of $89,71 \pm 10,81$ %.

Key words: fresh BSF larvae, tilapia, fish meal, growth performance, feed conversion ratio

ABSTRAK

Mahalnya harga pakan disebabkan oleh tingginya harga tepung ikan sebagai bahan baku pakan sumber protein. Larva *Black Soldier Fly* (BSF) mempunyai kandungan nutrisi yang baik sehingga dapat digunakan sebagai alternatif bahan baku pakan. Penelitian ini bertujuan untuk mengetahui pengaruh substitusi tepung ikan dengan larva BSF segar terhadap performa pertumbuhan ikan nila (*Oreochromis* sp.). Penelitian dilakukan secara eksperimen menggunakan rancangan acak lengkap dengan empat perlakuan dan tiga ulangan, meliputi P1

(substitusi tepung ikan dengan larva BSF segar sebanyak 0%), P2 (substitusi tepung ikan dengan larva BSF segar sebanyak 25%), P3 (substitusi tepung ikan dengan larva BSF segar sebanyak 50%), dan P4 (substitusi tepung ikan dengan larva BSF segar sebanyak 75%). Ikan yang digunakan mempunyai berat antara 17.98 ± 1.49 g, padat tebar sebanyak 20 ekor bak⁻¹, dan dipelihara selama 60 hari dalam bak fiber berukuran 50x50x60 cm³. Pakan diberikan sebanyak 3% dari biomassa ikan dengan frekuensi pemberian sebanyak dua kali per hari. Data dianalisis menggunakan analisis ragam dengan tingkat signifikansi 95%. Apabila ada beda nyata, maka dilakukan uji lanjut menggunakan *Duncan's Multiple Range Test*. Data kualitas air dianalisis secara deskriptif dengan membandingkan sumber pustaka. Hasil penelitian menunjukkan bahwa larva BSF segar dapat substitusi tepung ikan sebanyak 75% ($P < 0,05$) yang menghasilkan pertumbuhan mutlak berbasis panjang dan berat masing-masing sebesar 6.16 ± 0.38 cm dan 53.41 ± 10.40 g, laju pertumbuhan spesifik berbasis panjang dan berat masing-masing sebesar 0.10 ± 0.01 % hari⁻¹ dan 0.89 ± 0.17 % hari⁻¹, rasio konversi pakan sebesar 1.13 ± 0.14 dan efisiensi pakan sebesar 89.71 ± 10.81 % pada ikan nila.

Kata Kunci: larva BSF, ikan nila, tepung ikan, pertumbuhan, rasio konversi pakan

INTRODUCTION

In 2018, tilapia production reached 1.12 million tons or around 31.94% of Indonesia's total fisheries production and increased to 1.23 million tons in 2020 (FAO (2020)). According to the Decree of the Director General of Aquaculture (2020), in 2024 Indonesia is targeting tilapia production to be 2.24 million tons. This makes tilapia cultivation opportunities in Indonesia have bright prospects so that they can support food security, the national economy, and improve people's welfare. Tilapia (*Oreochromis* sp.) has advantages, namely relatively high resistance to disease, wide tolerance to the environment, and is able to grow well (Iskandar & Elrifadah, 2015).

Fish meal is the main source of protein in feed formulation, but because it is an imported commodity whose price continues to increase, alternative raw materials are needed to replace fish meal (Fauzi & Sari, 2018). The price of imported fish meal is IDR15,000 kg⁻¹ with a protein content of 74.6%, while local fishmeal is IDR 12,000/kg with a protein content of 55.4% (Marno *et al.*, 2016).

Black Soldier Fly (BSF) larvae contain crude protein ranging from 41-42%, ether extract ranging from 31-35%, ash content ranging from 14-15%, calcium ranging from 4.8-5.1%, and phosphorus ranging from 0.6-0.63% (Fauzi & Sari, 2018). Substitution of fishmeal with maggot flour as much as 75% resulted in a specific growth rate of 1.99 ± 0.10 % day⁻¹ and a feed consumption of 1125.51 ± 1.88 in tilapia (*O. niloticus*) (Arifin *et al.*, 2020). Utilization of maggot flour up to 270 g kg⁻¹ did not have a negative impact on growth performance and nutrient utilization in tilapia (Wang *et al.*, 2017). According to Murni (2013), giving a combination of 50% artificial feed with 50% fresh maggots to tilapia (*O. niloticus*) resulted in 100% survival, 44.21% relative growth, 1.63 feed conversion ratio, and 42.85% feed efficiency. This study aims to determine the effect of fish meal substitution with fresh BSF larvae on the growth performance of tilapia (*Oreochromis* sp.).

RESEARCH METHODS

Time and Place

The research was conducted from March to May 2023 at the Fish Food Science Sub-Laboratory, Aquaculture Laboratory, Department of Fisheries, Faculty of Agriculture, Gadjah Mada University.

Tools and Materials

The tools used are 50x50x60 cm³ fiber tub, aerator, water quality checker, net, digital scale, analytical scale, desiccator, oven, digestion unit, distillation unit, scrubber unit, muffle furnace, fat extractor, hematocrit centrifuge, spectrophotometer. The materials used include tilapia measuring 9 - 12 cm, H₂SO₄ solution, BCG-MR indicator, boric acid, HCL solution, aquadest, NaOH solution, N-Hexane, HNO₃ solution, fresh BSF larvae, fish meal, soybean meal, bran, corn meal, starch, mineral mix, vitamin C, fish oil.

Research Design

The study was conducted experimentally using a Completely Randomized Design consisting of 4 treatments with 3 replications. The treatments were based on the research of Arifin *et al.*, (2020) with modifications. The research treatments included:

P1 = Substitution of fish meal with fresh BSF larvae as much as 0%

P2 = Substitution of fish meal with fresh BSF larvae as much as 25%

P3 = Substitution of fish meal with fresh BSF larvae as much as 50%

P4 = Substitution of fish meal with fresh BSF larvae as much as 75%

Research Implementation Procedures

Preparation of feed formulation

The feed formulation was prepared using the Pearson square method, which is a simple method used to calculate fish feed rations with several feed ingredients. The feed formulation is shown in Table 1 below.

Table 1. Feed formulation and proximate analysis results

Feed Ingredients	Amount of feed ingredients			
	P1	P2	P3	P4
Fish meal	42.50	31.88	21.25	10.63
Fresh BSF larvae	0.00	10.63	21.25	31.88
Soybean meal	21.25	21.25	21.25	21.25
Corn meal	10.63	10.63	10.63	10.63
Bran	10.63	10.63	10.63	10.63
Starch meal	10.00	10.00	10.00	10.00
Mineral mix	2.50	2.50	2.50	2.50
Vitamin C	0.50	0.50	0.50	0.50
Fish oil	2.00	2.00	2.00	2.00
Total (%)	100	100	100	100

Feed making

The making of feed begins with weighing the feed ingredients according to the calculations in the feed formulation. All feed ingredients are mixed, hot water is added at a temperature of 85°C and stirred until homogeneous. After all feed ingredients are mixed, the dough is then molded and dried at a temperature of 40°C for 6-8 hours.

Proximate analysis of feed

Proximate analysis of feed includes testing the levels of crude protein (micro Kjeldahl method including the processes of destruction, distillation, and titration), fat (Soxlet method, which is extracting fat repeatedly using organic solvents), water (thermogravimetric method), ash (ashing method), crude fiber (qualitative method), carbohydrates (carbohydrate by different method), and feed energy.

Preparation of cultivation containers and distribution of tilapia seeds

Fish maintenance tanks measuring 50x50x60 cm³ as many as 12 units. The tanks are cleaned by brushing the walls to avoid germs. The density of tilapia in each tank is 20 fish. Before being stocked, the fish are acclimatized first to avoid stress and can adapt to their environment.

Tilapia fish seed maintenance

Fish maintenance was carried out for 60 days in the Aquaculture Laboratory, Department of Fisheries, Faculty of Agriculture, Gadjah Mada University. Feed was given as much as 3% based on the percentage of biomass with a feeding frequency of twice a day, namely at 08.00 and 15.00 WIB. During maintenance, observations were made on fish growth, water quality, and hematocrit and leukocrit.

Parameter Pengamatan

Length and weight gain

Growth observation was conducted by measuring length and weight, conducted on days 0, 15, 30, 45, and 60. Length and weight gain were observed from the average increase in length and weight each time sampling was conducted. Sampling was conducted by calculating the length and weight of fish amounting to 3% of the total number of fish per tank.

Survival

According to Rahayu *et al.*, (2013), survival is calculated using the following formula:

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

Description:

SR : Survival (%)

N_t : Number of fish alive at the end of maintenance (tail)

N_o : Number of fish alive at the beginning of maintenance (tail)

Absolute growth

According to Muchlisin *et al.*, (2016), absolute growth based on weight and length can be calculated using the formula:

Absolute growth based on weight

$$W = W_t - W_o$$

Description:

W : Absolute growth based on weight (g)

W_t : Fish weight at the end of maintenance (g)

W_o : Fish weight at the beginning of maintenance (g)

Absolute growth based on length

$$L = L_t - L_o$$

Description:

L : Absolute growth based on length (cm)

L_t : Fish length at the end of maintenance (cm)

L_o : Fish length at the beginning of maintenance (cm)

Specific growth rate

According to Muchlisin *et al.*, (2016), the value of the *specific growth rate* (SGR) can be calculated using the following formula:

Specific growth rate based on weight

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

Description:

SGR : *Specific growth rate* (% day⁻¹)

W_t : Fish weight at the end of maintenance (g)

W_o : Fish weight at the beginning of maintenance (g)

t : Maintenance time (days)

Specific growth rate based on length

$$SGR = \frac{\ln L_t - \ln L_o}{t} \times 100\%$$

Description:

SGR : *Specific growth rate* (% day⁻¹)

L_t : Fish length at the end of maintenance (cm)

L_o : Fish length at the beginning of maintenance (cm)

t : Maintenance time (days)

Feed conversion ratio and feed efficiency

The *feed conversion ratio* (FCR) can be calculated using the following formula:

$$FCR = \frac{(W_t - D) - W_o}{F}$$

Description:

FCR : *Feed conversion ratio*

F : Amount of feed given (g)

W_t : Fish weight at the end of maintenance (g)

D : Total weight of dead fish (g)

W_o : Fish weight at the beginning of maintenance (g)

Feed efficiency can be calculated using the formula based on Muchlisin *et al.*, (2016):

$$EP = \frac{(W_t - D) - W_o}{F}$$

Description:

EP : *Feed efficiency*

F : Amount of feed given (g)

W_t : Fish weight at the end of maintenance (g)

D : Total weight of dead fish (g)

W_o : Fish weight at the beginning of maintenance (g)

Hematocrit and leukocrit

Hematocrit and leukocrit levels were measured by inserting blood samples into capillary microhematocrit tubes, then centrifuged at a speed of 1500 g for 5 minutes to separate red blood cells, white blood cells, and plasma. Hematocrit and leukocrit were observed by measuring the length of red blood cells and white blood cells in the capillary tube, then the percentage was calculated based on research by Isnansetyo *et al.*, (2016). Observations of fish hematocrit and leukocrit were carried out on days 0, 30, and 60.

Water quality monitoring

Water quality observations were conducted on days 0, 15, 30, 45, and 60. Water quality analysis measured included dissolved oxygen content, water temperature, and pH using a water quality checker, free CO₂ and alkalinity using the titration method, and ammonia levels were observed from test reagents using a spectrophotometer.

Data Analysis

Analisis data yang digunakan adalah analisis ragam dengan tingkat signifikansi 95%. Hasil yang menunjukkan beda nyata diuji lanjut dengan menggunakan *Duncan's Multiple Range Test*. Data kualitas air dianalisis secara deskriptif.

RESULT

The results of the proximate analysis of the test feed in Table 1 show that the levels of protein, fat, and water in the feed are in accordance with the provisions of SNI (2006), namely the optimal range at protein levels $\geq 25\%$, fat $\geq 5\%$, and water $\leq 12\%$.

Table 1. Nutrient content of test feed

Nutrient	Treatment			
	P1	P2	P3	P4
Protein (%)	29.03±0.08	30.56±0.31	30.75±0.18	29.48±0.38
Fat (%)	11.88±0.70	13.05±0.35	13.55±0.35	13.75±0.35
Water (%)	7.31±0.30	7.51±0.36	7.83±0.51	8.21±0.27
Ash (%)	16.61±0.06	17.24±0.07	17.60±0.07	17.75±0.01
Crude fiber (%)	3.84±0.78	7.72±0.22	7.07±0.01	6.55±0.37
Carbohydrate (%)	35.46±0.04	31.51±0.57	30.14±0.09	31.01±0.59
Energy (cal 100 g-1)	3639.14±39.57	3922.17±83.78	4143.64±67.26	4210.93±6.08

Fish survival during maintenance ranged from 78.33±5.77 – 90.00±5.00%. This value is in accordance with SNI (2009), where good survival in tilapia cultivation is at least 75%. The results of the analysis of variance showed that feed treatment did not affect tilapia survival.

Table 2. Fish survival and growth

Treatment	Survival (%)	Absolute growth based on weight (g)	Specific growth rate based on weight (% day ⁻¹)	Absolute growth based on length (cm)	Specific growth rate based on length (% day ⁻¹)
P1	83.33±2.89 ^a	29.07±3.94 ^b	0.48±0.07 ^b	4.25±0.36 ^b	0.07±0.01 ^b

P2	78.33±5.77 ^a	28.55±5.01 ^b	0.48±0.08 ^b	4.15±0.47 ^b	0.07±0.01 ^b
P3	86.67±7.64 ^a	30.74±6.42 ^b	0.51±0.11 ^b	4.67±0.51 ^b	0.08±0.01 ^b
P4	90.00±5.00 ^a	53.41±10.40 ^a	0.89±0.17 ^a	6.16±0.38 ^a	0.10±0.01 ^a

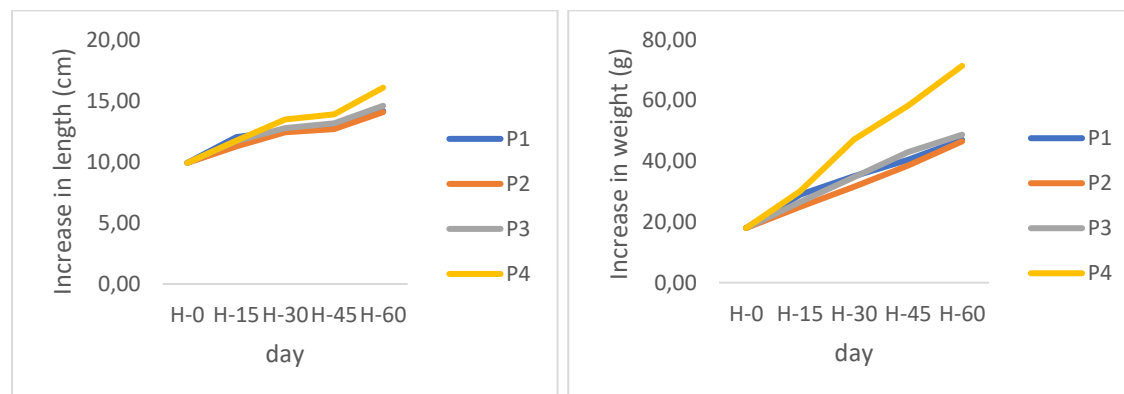


Figure 1. Increase in length and weight of tilapia during rearing

The average length and weight of tilapia at the time of stocking were 9.92 ± 0.24 cm and 17.98 ± 1.49 g, respectively. Figure 1 shows that the use of fresh BSF larvae up to 75% resulted in a continuous increase in length and weight. The highest increase was obtained in the P4 treatment and the lowest in the P2 treatment.

Table 3. Feed conversion ratio and feed efficiency

Treatment	Feed Conversion Ratio	Feed Efficiency (%)
P1	1.64 ± 0.19^a	61.60 ± 6.76^a
P2	1.55 ± 0.14^a	65.06 ± 6.08^a
P3	1.57 ± 0.24^a	64.71 ± 10.25^a
P4	1.13 ± 0.14^b	89.71 ± 10.81^b

Table 3 shows the feed conversion ratio of tilapia ranging from 1.13 ± 0.14 – 1.64 ± 0.19 . The best results were obtained in the P4 treatment, which was 1.13 ± 0.14 . The efficiency of tilapia feed showed results ranging from 61.60 ± 6.76 – $89.71 \pm 10.81\%$. The best results were obtained in the P4 treatment, which was $89.71 \pm 10.81\%$. The results of the analysis of variance showed that substitution of fish meal with fresh BSF larvae of 75% affected the feed conversion ratio and feed efficiency.

Substitution of fish meal with fresh BSF larvae of 75% produced hematocrit levels ranging from 22.00 ± 0.00 – $27.74 \pm 2.02\%$, while leukocrit ranged from 1.42 ± 0.01 – $1.47 \pm 0.00\%$. The results of the analysis of variance showed that feed treatment did not have a significant effect on the hematocrit and leukocrit levels of tilapia.

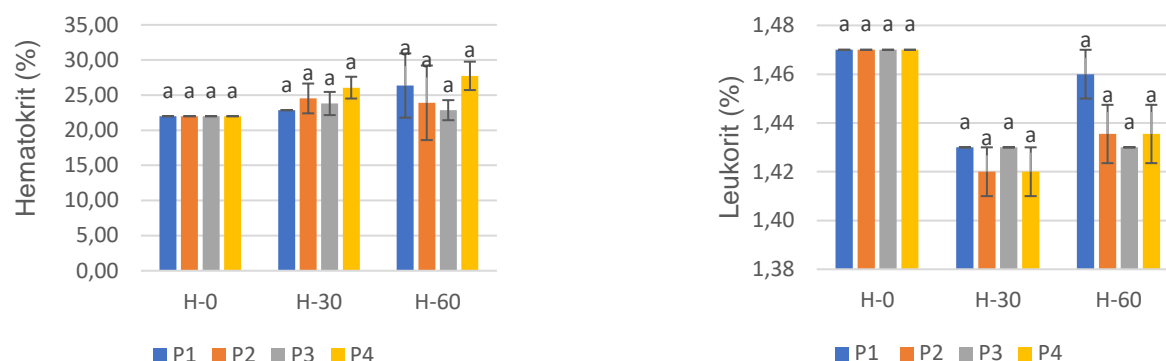


Figure 2. Hematocrit and leukocrit levels

Water Quality

Based on Table 4., the water quality parameters during maintenance produced values that were still feasible and optimal.

Table 4. Water quality

Water quality	Treatment				Optimal
	P1	P2	P3	P4	
Temperature (°C)	27.62±0.60	27.56±0.69	27.33±0.59	27.56±0.73	25–32 ^a
pH	7.64±0.18	7.59±0.14	7.61±0.13	7.72±0.23	6.5–8.5 ^a
Dissolved oxygen (ppm)	5.37±1.31	5.47±1.32	5.52±1.29	5.33±1.30	≥3 ^a
Alkalinity (mg l ⁻¹)	174.53±4.58	170.27±10.68	180.40±19.88	173.20±6.61	30-500 ^b
CO ₂ (mg l ⁻¹)	28.33±3.40	29.07±5.45	28.73±5.96	28.13±5.72	min 4 ^c
Ammonia (ppm)	0.003±0.002	0.001±0.001	0.006±0.004	0.005±0.004	< 0.02 ^a

Information :

a : SNI 7550:2009 Production of grow-out class tilapia (*Oreochromis niloticus* Bleeker) in still water ponds

b : Bintoro and Abidin (2013)

c : Pramleonita *et al.* (2018)

DISCUSSION

The test feed produced protein, fat, and water levels according to SNI (2006), while the ash, crude fiber, and carbohydrate levels showed values greater than SNI (2006). According to SNI (2006), the content of artificial tilapia feed in intensive cultivation has a protein content of ≥25%, fat ≥5%, water ≤12%, ash ≤15%, crude fiber ≤6%, and carbohydrates ranging from 20-30%. The high levels of fat and carbohydrates affect the high energy of fish feed (Fahmi *et al.*, 2009), so that fish can use this energy to maximize the function of protein for their growth (energy sparing effect).

Survival during maintenance ranged from 78.33±5.77 – 90.00±5.00%. According to SNI (2009), good *survival* in tilapia cultivation is at least 75%, so that survival is still categorized as good. Fawole *et al.* (2020), stated that substitution of fish meal with BSF larvae

in catfish up to 75% did not affect its survival. This is because there is no accumulation of abnormal fat due to the provision of BSF larvae and liver function and intestinal physiology are not inhibited.

Growth is a change in size, length, or weight over time. Factors that influence growth include genetic factors, hormones, and the environment. The growth process is caused by the process of adding tissue from cell division through mitosis due to the input of energy and protein from feed. This energy is then used by the fish's body as a process of metabolism, reproduction, movement, and replacement of damaged cells (Prajayati *et al.*, 2020). Substitution of fish meal with fresh BSF larvae as much as 75% gave the best results in all growth parameters. Substitution of fish meal with fresh BSF larvae significantly affected the absolute growth parameters based on length and weight, *specific growth rate* based on length and weight, which were 6.16 ± 0.38 cm and 53.41 ± 10.40 g and $0.10 \pm 0.01\%$ day⁻¹ and $0.89 \pm 0.17\%$ day⁻¹, respectively. The higher the substitution of fish meal with fresh BSF larvae, the better the growth of length and weight of tilapia. This shows a good response in fish in utilizing feed in the metabolism and growth process, as well as the balance of nutrients in the feed. Growth is influenced by nutrient balance because growth occurs when there is remaining energy from metabolism that has been used for body activities and maintenance (Prajayanti *et al.*, 2020).

The best *feed conversion ratio* (FCR) value during 60 days of tilapia rearing was obtained in the P4 treatment (substitution of fish meal with fresh BSF larvae as much as 75%). This proves that the utilization of feed for growth is quite efficient, so that it can create a good fish appetite pattern, while in P1 (substitution of fish meal with fresh BSF larvae as much as 0%) it produces a high FCR value of 1.64 ± 0.19 . Ihsanudin *et al.*, (2014) stated that fish appetite patterns can make the FCR value smaller. The smaller the feed ratio value, the better the quality of the feed. A good *feed conversion ratio* value ranges from 0.8 - 1.6. This value proves that all treatments in the observations that have been carried out obtained optimal FCR. Low FCR values are related to good weight growth. The higher the fish weight value, the higher the *feed conversion* value utilized, so the FCR value is lower, while the higher the FCR value, the more feed is needed and causes inefficient use of feed because it is not comparable to the increase in fish weight (Zahra *et al.*, 2019).

The best tilapia feed efficiency value for 60 days of maintenance was obtained in the P4 treatment (substitution of fish meal with 75% fresh BSF larvae) which was $89.71 \pm 10.81\%$, while the lowest feed efficiency was obtained in the P1 treatment (substitution of fish meal with 0% fresh BSF larvae) which was $61.60 \pm 6.76\%$. The feed efficiency value in the P4 treatment shows that the use of fresh BSF larvae up to 75% can improve feed quality. Increasing the feed efficiency value indicates better feed quality, where feed efficiency shows the proportional value of fish weight growth with the amount of feed consumed by the fish (Giri *et al.*, 2007). High feed efficiency and low *feed conversion ratio* indicate that the feed has been digested and absorbed optimally by the fish for their growth, so that the use of fresh BSF larvae in feed indicates better feed utilization in tilapia (Muchlisin *et al.*, 2016).

Hematocrit levels during maintenance ranged from $22.00 \pm 0.00\%$ - $27.74 \pm 2.02\%$. Royan *et al.*, (2014) stated that tilapia are in normal condition if the hematocrit levels range from 21.00 - 22.76%. Hematocrit levels of tilapia in normal conditions range from 27.3 - 37.8%, where hematocrit levels can be an indication of stress in fish due to environmental factors, handling, or pathogen infection.

Leukocrit levels of tilapia during 60 days of maintenance ranged from $1.42 \pm 0.01\%$ to $1.47 \pm 0.00\%$. Isnansetyo *et al.*, (2016), showed that leukocrit levels of tilapia in normal conditions ranged from 1.22 - 1.27%. Aisiah (2012), stated that the number of leukocrit of tilapia fish in the normal range is between 1.75 - 2.91. An increase in leukocrit levels can

describe the response of white blood cells to immunostimulants or their derivatives in the form of antigens or pathogenic microorganisms (Purbomartono *et al.*, 2019). Based on the results of the analysis of variance, the substitution of fish meal with fresh BSF larvae did not affect the hematocrit and leukocrit levels of tilapia fish, this is indicated by the condition of the fish being healthy and having good immunity.

Water quality is one of the determining factors for the success of fish farming. Water quality that is in accordance with the needs of fish can provide an influence on good survival and growth, so water quality management is very important in the living medium for aquaculture organisms (Panggabean *et al.*, 2016).

CONCLUSION

The results showed that substitution of fish meal with fresh BSF larvae can be given up to a dose of 75%. Substitution of fish meal with fresh BSF larvae as much as 75% in tilapia feed resulted in absolute growth based on length of 6.16 ± 0.38 cm, *specific growth rate* based on length of $0.10 \pm 0.01\%$ day⁻¹, absolute growth based on weight of 53.41 ± 10.40 g, specific growth rate based on weight of $0.89 \pm 0.17\%$ day⁻¹, feed conversion ratio of 1.13 ± 0.14 , and feed efficiency of $89.71 \pm 10.81\%$. The use of fresh BSF larvae up to 75% in feed formulation did not affect the *survival*, *hematocrit*, and *leukocrit* of tilapia.

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