Bioedukasi
Jurnal Biologi dan Pembelajarannya

Vol. 21 No 3, October 2023, page 299-308
e-ISSN: 2580-0094; p-ISSN:1693-3931
DOI: 10.19184/bioedu.v21i3.45120
jurnal.unej.ac.id/index.php/BIOED

The Effect of Pellet Made from Mixture of Maggot Flour, Mealworm Flour, And Golden Snail Flour on Growth of *Cherax quadricarinatus*

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Article Info

Article history:
Received August 4, 2023
Revised September 22, 2022
Accepted September 29, 2023

Keywords:
Alternative feed;
Cherax quadricarinatus;
growth performance.

ABSTRACT

Feed is one of the main points that determine crayfish successful cultivation. The feed commonly use fish flour tends to be expensive; therefore, it becomes an obstacle for crayfish cultivators. This study aimed to determine the effect of pellet feed made from maggot flour, mealworm flour, and golden snail flour on crayfish growth as an alternative feed that are more affordable. This research was conducted using laboratory experiment design consisted of three treatments and three replications of each. The research design used fish flour-based pellet for control (P0), maggot flour, mealworm flour, and golden snail flour-based pellet for treatment 1 (P1), maggot flour, mealworm flour, golden snail flour, and fish flour-based pellet for treatment 2 (P2). The experiment conducted during one month, using 90 individuals' crayfish. The effect of treatments on the growth measured by three main parameters such as: dry and wet biomass, carapace length, and supported by feed analysis using feed conversion ratio (FCR) and feed utilization efficiency (FUE). The results showed that P1 has significant effect on the dry biomass growth (p=0.001), wet biomass growth (p=0.000), carapace length growth (p=0.049). Feed analysis showed that P1 has significant effect on FCR (p=0.004) and FUE (p=0.010).

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1. INTRODUCTION

Freshwater crayfish (Cherax quadricarinatus von Martens) has the potential to be developed as a cultivation commodity. Cherax quadricarinatus has advantages over other types of crayfish, including easy cultivation, fast growth rate, superior fecundity, and environmental tolerance (Mamuaya et al., 2019). The composition and content of feed plays crucial role on the growth of crayfish until they can be marketed. Main formulation of feed in aquaculture is fish flour (Hakim et al., 2019). However, production of fish flour-based feed poses a challenge because fish flour is an imported commodity that fluctuates in price and availability (Miranti et al., 2019). One of the solutions to overcome this problem is use different feed ingredients. Alternative feed ingredients must fulfill numerous criterias such as nontoxic to farmed animals, containing adequate nutrients, and easy to get or always available (Fauzi and Sari, 2018).

Insect larvae is considered as promising, good quality, efficient and sustainable alternative protein material that has not been utilized optimally. Insect larvae contain high levels of protein, fat and minerals, making them a potential substitute for fish-based feed substitution (Hender et al., 2021). Black soldier fly (Hermetia illucens) larvae that is commonly known as maggot and mealworm (Tenebrio molitor) larvae are species that can be used as alternative feed ingredients. BSF (Hermetia illucens) larvae contains 32-53% protein and high fat content 18-33% (Lu et al., 2022). Furthermore, BSF flies are non-disease-carrying flies and their larvae can be cultured easily in a short time. Mealworn contains nutrients in the form of 48% crude protein, 40% crude fat, 3% ash content, 8% non-nitrogen extract content, and chitin (Fuah et al., 2021). Fibre content in mealworm ranges between 1.97 and 18.84%. Mealworm also contains varies minerals and vitamins. There are high amounts of potassium, calcium, iron and magnesium. It also provides sources of vitamins such as riboflavin, panthothenic acid, and biotin (Gkinali et al, 2022). The selection of insects as alternative feed ingredients has promising prospects because they are easy to breed at relatively affordable prices (Rumokoy et al., 2019). Besides insect larvae, the golden apple snail

(Pomacea canaliculata) is an alternative component for feed. Golden snail can be found in nature, particularly in rice fields, due to their powerful reproductive capability and ability to adapt well to dry circumstances. The golden snail is a pest that destroys rice plants, posing a risk to farmers. The golden snail, on the other hand, has high protein content and can be utilized as an alternate feed component. The protein content in the golden apple snail which has been processed into flour reaches 54.17%, besides, there are other nutrient contents including 4.83% crude fat, 2.37% crude fiber, and 20.13% ash content (Prabewi et al., 2019).

The quality of feed is a factor that influences the growth of freshwater crayfish. Freshwater crayfish can grow optimally when their feed needs are met in terms of quantity and quality. Research is needed to determine the impact of the pellet feed combination of the three alternative components on the growth of freshwater crayfish since the combination of the three types of prospective feed ingredients has not yet been studied. Research on effect of feed pellet made from mixing of maggot flour, mealworm flour, and golden snail flour based on the growth of freshwater crayfish can be used as material for consideration and breakthroughs in freshwater crayfish feed innovations.

2. RESEARCH METHOD

Materials used in this research include freshwater crayfish, fish flour, maggot flour, mealworm flour, golden snail flour, vitamin min mix, soybean flour, corn flour, wheat flour, bran flour, water, and CMC (Carboxymethyl Cellulose).

This study used laboratory experiment method with a completely randomized design. Treatments consisted of control (P0) with the basic ingredients of fish flour, treatment 1 (P1) with maggot flour, golden snail flour, and mealworm flour, treatment 2 (P2) with a maggot flour, mealworm flour, golden snail flour, and fish meal. The study was conducted for 4 weeks with growth parameters including dry biomass, wet biomass and carapace length. Feed analysis is done by calculating FCR (feed conversion ratio) and FUE (feed utilization efficiency).

Feed test

The pellet that is used in this study were modified from the proportion of feed raw materials research according to Hutabarat et al (2015). The following Table 1 presents the proportion of raw materials for combined pellets.

Table 1. Proportion of feed pellets for each treatment

Ingredients	**Proportion for each treatment**		
	P0 (%)	**P1 (%)**	**P2 (%)**
Fish flour	29.275	0	7.32
Golden snail flour	0	9.8	7.32
Maggot flour	0	9.8	7.32
Mealworm flour	0	9.8	7.32
Soybean flour	27.1	27.1	27.1
Corn flour	16.1	16.1	16.1
Bran flour	14.9	14.9	14.9
Wheat flour	9.42	9.42	9.42
Vit Min Mix	2.1	2.1	2.1
CMC	1.1	1.1	1.1
Total	100	100	100

Nutritional values of the feed is assessed using proximate test on pellets. Proximate test was carried out at Jember State Polytechnic Food Analysis Laboratory in March 2023. The proximate test results are listed in Table 2.

Table 2. Proximate test results

Feed test parameters	Results of each treatment		
	P0 (%)	P1(%)	P2(%)
Water	5.96	9.56	9.55
Ash	13.81	8.37	8.55
Fat	6.52	7.94	8.69
Protein	15.99	20.56	21.64
Carbohydrate	57.69	53.43	52.08

Notification:

P0: Fish flour-based feed pellet
P1: Maggot flour, golden snail flour, and mealworm flour-based feed pellet
P2: Maggot flour, golden snail flour, mealworm flour, and fish flour-based feed pellet

Data retrieval

a. Data on the growth of freshwater crayfish were obtained every 7 days for 4 weeks through several measurements consisted of wet biomass, carapace length, dry biomass, feed conversion ratio (FCR), feed utilization efficiency (FUE), as well as measurements of abiotic factors in the freshwater crayfish rearing environment. The following formulas are used for collecting data of the crayfish growth: Wet biomass, carapace length, and dry biomass growth

Wet biomass growth was calculated using the equation (Yusapri et al., 2022):

$W= W_4-W_0$

Notification:

W = Wet biomass growth (g)
W_4 = Mean of wet biomass on the week 4 (g)
W_0 = Mean of wet biomass on the week 0 (g)

Carapace length growth was calculated using the equation (Nightingale et al., 2021):

$L= L_4– L_0$

Notification:

L = Carapace length growth (mm)
L_4 = Mean of carapace length on the week 4 (mm)
L_0 = Mean of carapace length on the week 0 (mm)

Dry biomass was calculated using allometric equation formula (Daba and Soromessa, 2019):

$Y=aB+X$

Notification:

Y= Dry biomass (g)
X= Wet biomass (g)

Growth rate of crayfish is calculated using the equation formula (Stumpf et al., 2011):

Growth rate (%) = $100 \times (\ln W_f - \ln W_i)/t$

Notification:

W_f = Final weight (g)
W_i = Initial weight (g)
t = Number of weeks

b. Feed conversion ratio (FCR) an Feed utilization efficiency (FUE)

Analysis of the efficiency of feed pellet for the growth of freshwater crayfish is measured based on the calculation results of FCR (Feed Conversion Ratio) and FUE (Feed Utilization Efficiency). FCR is obtained from the comparison between the weight of the feed that has been consumed in a certain period of time to the biomass of freshwater crayfish at the end of the study (Maftuch et al., 2021).

FCR is calculated using the equation (Li et al., 2022)

FCR=F/(Wt-W0)

FUE is calculated using the equation (Indriastuti et al., 2022):

EPP=(Wt-W0)/F x 100%

Notification:

FCR : Feed conversion ratio
EPP : Feed utilization efficiency (%)
F : Average feed consumed during the study (g)
W_t : Average freshwater crayfish biomass obtained at the end of study (g)
W_0 : Average freshwater crayfish biomass obtained at the beginning of study (g)

Abiotic factors measurement

Abiotic factors measurement was carried out every day during the study at 10.00 WIB. The abiotic parameters measured in this study included water temperature was measured using a digital thermometer, water pH was measured using a digital pH meter, dissolved oxygen was measured using a digital DO meter, and light intensity was measured using a lux meter.

Data Analysis

ANOVA test (Analysis of Variances) was used to analyze the data. The results obtained from the ANOVA test was continued with the Post Hoc test if the results are significant. The Post Hoc test aims to determine the treatment that has the most significant effect. The Post Hoc test conducted is Duncan's test.

3. RESULT AND DISCUSSION

As displayed in Table 3, treatment 1 (P1) showed significant effect to dry biomass, wet biomass, and carapace length growth during 4 weeks of study (P<0.05). Table 4 showed significant effect occurs on feed analysis including FCR and FUE.

Table 3. Effect of alternative feed pellet on crayfish growth performance

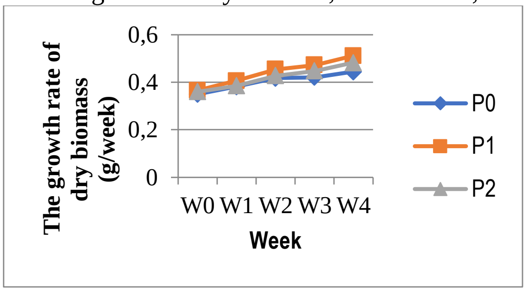
Items	Mean square	F	P-value
Dry biomass	0.002	24.728	0.001
Wet biomass	0.051	46.694	0.000
Carapace length	1.160	5.178	0.049

Table 4. Effect of alternative feed pellet on FCR and FUE

Items	Mean square	F	P-value
FCR	0.273	15.303	0.004
FUE	311.899	10.799	0.010

Wet biomass, dry biomass, and carapace length growth

The growth rate performance of crayfish during 4 weeks of study is represented as figure in Figure 1. The slope indicates that feed pellet increases growth of dry biomass, wet biomass, and carapace length of crayfish.



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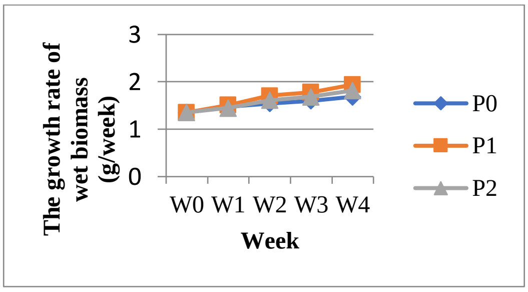

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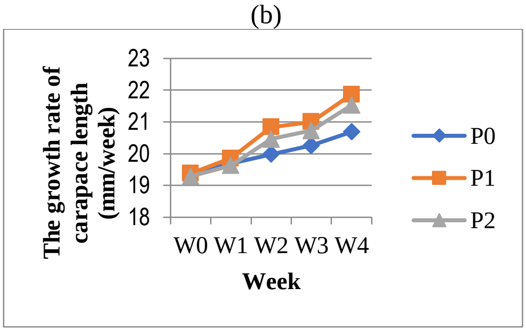

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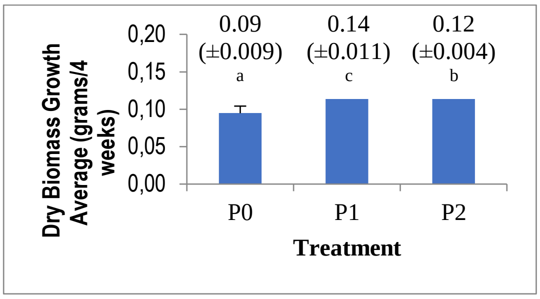
Figure 1. Effect of the treatment on the growth rate of dry biomass (a), wet biomass (b), and carapace length (c) per week of the crayfish during 4 weeks.



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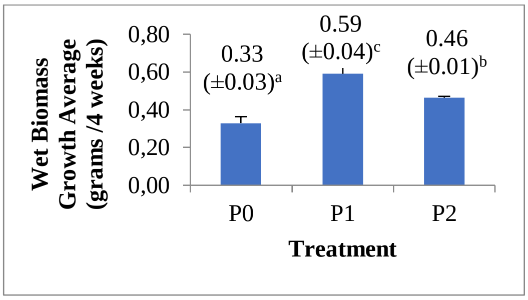

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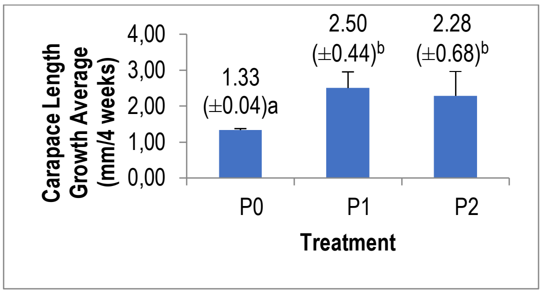


(c)

Figure 2. Effect of the treatment on the growth of dry biomass (a), wet biomass (b), and carapace length (c) during 4 weeks.

Figure 2 showed growth average of crayfish including dry biomass, wet biomass, and carapace length. Each treatments gave different results on crayfish growth. Based on the materials that used for making pellets, P1 which contain mixing of maggot flour, mealworm flour, and golden snail flour gave the best growth average on dry biomass, wet biomass, and carapace length.

Research on the effect of pellets made from maggot flour, mealworm flour, and golden snail flour gave significant results on the growth of wet biomass (p=0.000) with the highest average growth in treatment P1 with an average of 0.59 (±0.04) gram/4 week, followed by P2 which produced an average wet biomass growth of 0.46 (±0.01) gram/4 weeks, and P0 with average wet biomass growth of 0.33 (±0.03) gram/4 weeks. The pellets made from maggot flour, mealworm flour, and golden snail flour also gave significant results on the growth of dry biomass (p= 0.001) and carapace length (p=0.049). The highest dry biomass average was obtained from P1 with an average of 0.14 (±0.011) gram/4 weeks, followed by treatment P2 with an average of 0.12 (±0.004) gram/4 weeks, and P0 with an average of 0.09 (± 0.009) gram/4 weeks. The highest carapace length growth average was obtained from P1 with an average of 2.50 (±0.44) mm/4 weeks, then P2 with an average of 2.28 (±0.68) mm/4 weeks, and P0 with an average of 1.33 (±0.04) mm/4 weeks.

The differences in growth that occured are mainly influenced by the nutrients contained in the pellets. In pellet P1, protein was obtained from a combination of using three different types of flour consisted of maggot flour, mealworm flour, and golden snail flour. The proximate test results showed that the protein content in the P1 pellet was 20.56% and the carbohydrate content was 53.43%. As for the P2 pellet, it has a protein content of 21.64% and carbohydrates of 52.08%. Then for the treatment with the lowest growth, P0, the proximate test results showed a protein content of 15.99% and carbohydrates of 57.69%. Protein in feed has several functions needed to support the growth of freshwater crayfish (Greco, et al., 2022; Jones et al., 2022). Some of the functions of protein include as a building substance that forms new tissues, replaces damaged tissue, forms enzymes and hormones, and regulates various metabolic processes (Manik and Arleston, 2021). *Cherax quadricarinatus* requires about 13-43% protein to achieve optimal growth (Saoud et al., 2012). The lower protein content at P0, which was 15.99%, resulted in lower growth compared to the P1 and P2 treatments. Previous research by Subchan et al. (2023), providing feed made from a combination of mealworm and fish flour gave better results because of a nutrient balance between protein and carbohydrates contained in the feed.

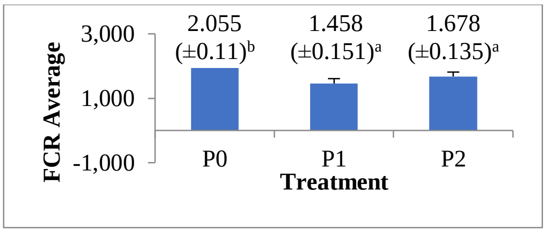
Carbohydrate is another type of nutrient that crucial for the growth of freshwater crayfish seeds. The proximate test revealed that the pellet feed's carbs content was quite high, at 57.69% in the P0 treatment, 53.43% in P1, and 52.08% in P2. Carbohydrate acts as the main source of energy for crayfish. In freshwater crayfish, the need for carbohydrate increases when molting occurs. Carbohydrates are converted in cellular respiration to produce energy in the form of ATP. This energy is used to carry out several physiological processes, such as active transport of ions or minerals needed for the formation of a new exoskeleton. Energy is also used to maintain the osmotic pressure of freshwater crayfish so that the osmoregulation remains balanced (Cheng et al., 2002). According to Raharjo et al. (2020), if the energy needs at the time of molting are not enough, crayfish can running out of energy to release their shells.

In addition to the nutrient of the feed, palatability also affects growth differences between each treatment. The desire degree of freshwater crayfish for meal is known as palatability. Even though the feed formulation has been developed as well as it can, if the palatability of the feed is low, the feed is not being consumed or being less liked, hence the feed has a low benefit value or nutrient utilization (Widodo, 2017).

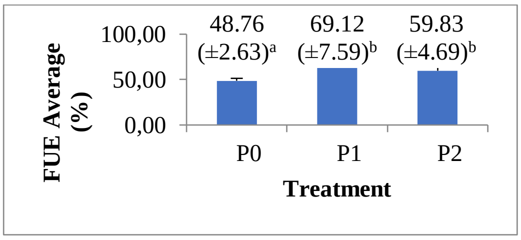
The amount of feed that can be transformed to energy and growth by freshwater crayfish decreases as fewer feed is consumed. In addition, a number of other parameters that affect feed consumption are also influenced by the nutritional values of the feed. Feed consumption is also impacted by the high fat content. Feed that contains too high fat-levels can lead to low feed consumption (Pamungkas, 2013). The difference in fat content in P1 and P2 is the cause of the difference in the amount of feed consumed which results in lower P2 growth.

Feed Conversion Ratio (FCR) and Feed Utilization Efficiency (FUE)

The effect of alternative feed pellet on crayfish during 4 weeks of study is represented as figure in Figure 3. These FCR and FUE values indicate feed efficiency of each treatment.



(a)



(b)

Figure 3. Effect of the treatment on the FCR (a) and FUE (b) during 4 weeks.

Notification:

P0: Fish flour-based feed pellet
P1: Maggot flour, golden snail flour, and mealworm flour-based feed pellet
P2: Maggot flour, golden snail flour, mealworm flour, and fish flour-based feed pellet

Feed analysis was obtained from the calculation of feed conversion ratio (FCR) and feed utilization efficiency (FUE). Treatment of different types of feed P0, P1, and P2 had a significant effect ($p=0.004$) on the FCR of freshwater crayfish. Feed conversion ratio (FCR) represents the efficiency level of the feed given. The conversion referred to the amount of feed weight needed for growth or to increases biomass (Fran and Akbar, 2013). The results showed that the lowest feed conversion value was found in treatment P1, which had an FCR value of 1.458 (±0.151). According to Hartono and Barades (2022), FCR determines the level of efficiency of crayfish in converting feed into body tissue that is resulting in biomass growth. As for the P0 and P2 treatments, the FCR values were 2.055 (±0.11) and 1.678 (±0.135) respectively.

A low FCR value indicates better feed quality. The lower feed conversion value means the higher the feed efficiency and the higher the feed conversion value means lower on the efficiency (Saputra et al., 2018).

Maggot flour, mealworm flour, and golden snail flour based feed pellet had significant effect (p=0.01) on the feed utilization efficiency (FUE) of freshwater crayfish. The treatment with the highest FUE value was P1 which was 69.12%, then P2 with EPP value 59.83% and the lowest was P0 with an EPP value 48.76%. The percentage of feed utilization shown in the EPP is defined as feed that can be utilized for growth which is represented by increased biomass compared to the amount of feed consumed (Prariska et al., 2020).

The results of abiotic factors measurement during the 4 weeks of the study is presented in Table 5 below. Abiotic factors measured including pH, DO, water temperature, and light intensity are in the suitable ranges for crayfish cultivation.

Table 5. Abiotic factors

Week	pH	DO (mg/L)	Water temperature (°C)	Light intensity (lux)
W1	7.29 ±0.03	4.55 ±0,16	25.34±0.14	198,180 ±6.84
W2	7.47 ±0.05	4.77 ±0,18	25.12±0.23	194,300 ±9.93
W3	7.60 ±0.04	4.76 ±0,12	25.15±0.10	191,600 ±4.14
W4	7.69 ±0.04	4.79 ±0,07	25.03±0.10	191,800 ±5.35

The abiotic factors observed in this study included the water acidity (water pH), water temperature, dissolved oxygen levels, and light intensity. The first abiotic factor is the degree of acidity (pH) of the media water. The appropriate pH range for crayfish is in the range of 6.5 to 9. If the pH is less than 5 or too acidic it can cause death whereas if the pH is above 9 it can reduce appetite so that growth becomes slow (Rihardi et al., 2013). The degree of acidity (pH) of water in this study ranged from 7.29 -7.69 so that it is included in a good category to support the growth of freshwater crayfish. Water temperature is one of the abiotic factors that affect the ongoing metabolism of crayfish during the rearing period. The optimal temperature range for the growth of crayfish is 25-29°C (Lukito and Prayugo, 2007), 23.6 (±3)°C (Westhoff and Rosenberger. 2016). In this study, the water temperature is in the optimal range between 25.03 -25.34°C.

The third abiotic factor is dissolved oxygen (DO). Dissolved oxygen (DO) plays important role in the process of converting feed into energy. The energy is then used for activity, movement, and growth. Therefore, if the dissolved oxygen (DO) level is low, this condition can disrupt the life of freshwater crayfish seeds (Taufiq et al., 2016). According to Hutabarat et al. (2015), suitable oxygen levels for crayfish range from 4-6 mg/L. Dissolved oxygen levels in this study were ranges about 4.55 to 4.79 mg/L so that it fits the crayfish dissolved oxygen needs. The range of light intensity in this study is 191,600-198,180 lux. Freshwater crayfish are nocturnal animals that are sensitive to light. When crayfish are cultivated in the environment with low light intensity, it will make them actively move and allow them to interact more with others than staying in the shade or shelter. However, being in low light intensity conditions for a long time can lead to increased cannibalism due to competition between individuals (Satyantini et al., 2009).

Economic analysis

Pellet price was analyzed based on material composition that is used for pellet production. Price comparison between research pellets and commercial fabric pellets is presented in Table 4.

Table 4. Pellets Price Analysis Comparison

Kind of Pellets	Price (Rp)	Sources
P0	6,863,-	Research product
P1	6,170,-	Research product

Kind of Pellets	Price (Rp)	Sources
P2	6,344,-	Research product
M1	14,000,-	CP Prima, Mitra Hewan Gresik
M2	21,799,-	Feng Li, Wu Aqua Expert Surabaya
M3	12,000,-	Comfeed, Poultry Shop, Deli Serdang

Notification:

P0: Fish flour based feed pellet
P1: Maggot flour, golden snail flour, and mealworm flour based feed pellet
P2: Maggot flour, golden snail flour, mealworm flour, and fish flour based feed pellet
M1: Fabric pellets brand 1
M2: Fabric pellets brand 2
M3: Fabric pellets brand 3

The three kinds of pellets product that is resulted from the research is cheaper than fabric pellets. The cheapest pellet is P1 with price Rp. 6,170,- which is cheaper compared than P0 (fish flour based pellet) which is costs Rp. 6,863,- and P2 (maggot flour, golden snail flour, and fish flour based feed pellet) which is costs 6,344,- . Based on the comparative analysis of pellet prices, research product pellets can be used as alternative feed for freshwater crayfish cultivators because it has competitive prices.

4. CONCLUSION

Maggot flour, mealworm flour, and golden snail flour based pellet had significant effect on growth of freshwater crayfish ($p<0.05$). The best growth occurs on treatment 1 (P1) by pellets made from maggot flour, mealworm flour, and golden snail flour with average growth of dry biomass 0.14 (±0.011) grams/4 weeks, wet biomass 0.59 (±0.04) gram/4 weeks, and carapace length 2.5 (±0.44) mm/4 weeks. Thus, pellets made from mixing of maggot flour, mealworm flour, and golden snail flour can be used as replacement for fish-flour based pellets from commercial fabric that tends to be expensive and fluctuating in price.

5. ACKNOWLEDGEMENT

We would like to express our gratitude to freshwater crayfish research group and Zoology Laboratory of Biology Education Program, University of Jember, for facilitating and providing support in this research.

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