The Use of Different Diets for Feeding Rate and Growth of Shortfin Eel (*Anguilla bicolor bicolor*)

N Taufiq-Spj[1,3], S Sunaryo[1], A Wirasatria[2,3] I Pratikto[1], D H Ismunarti[2] and M I Syaputra[1].

[1] Departement of Marine Science, Faculty of Fisheries & Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Indonesia.
[2] Departement of Oceanography, Faculty of Fisheries & Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Indonesia.
[3] Center for Coastal Disaster Mitigation and Rehabilitation Studies, Center of an Excellence Research University, Diponegoro Univ. Semarang, Cent. Java, Indonesia.

E-mail: taufiqspj_1999@yahoo.com

Abstract. Growth and feed rate accompanied with proximate, texture and condition of the organism. The objective of this study was to obtain the effect of different diets to the feed rate and growth of shortfin eel *Anguilla bicolor bicolor.* The study used three replications and 3 treatments of different sources of feed diet during 84 days culture. The fish were fed with proximation of 2.2% wet basis d^{-1} (ratio of dry feed and water = 4 : 3). Initial density of approx. 15 kg seed stock of eel fingerling size (early elver) per m^3 water in the recirculating water system with ranging of means$\pm$SD of temperatures, pH and DO were 27.14$\pm$0.11 to 27.66$\pm$0.05 °C, 7.44$\pm$0.09 to 7.66$\pm$0.05, and 3.54$\pm$0.19 to 4.66$\pm$0.09 ppm respectively. The result shows that feeding rate has relation to lipid content of feed but not significantly different among treatments ($\alpha > 0.05$), the highest *FR* shows by F_2 followed by F_3 and F_1, where: F_1= 2.04$\pm$0.07% d^{-1} and F_2= 2.12$\pm$0.05% d^{-1} and F_3= 2.10$\pm$0.06% d^{-1}). Absolute growth of individuals and biomass were related to the protein content; the highest was F_1 followed by F_2 and F_3. Growth margin F_1, F_2 and F_3 of individual were 25 g (46%), 22 g (44%), and 15 g (27%) respectively, whilst biomass margin were 3,855 g (26%), 3,629 g (24%), and 2,834 g (18%) respectively during 70 days culture. Marginal mean of Individual-specific growth rate (SGRi) shows no significantly different ($\alpha > 0.05$) between F_1 (0.53$\pm$0.05% d^{-1}) and F_2 (0.52$\pm$0.01% d^{-1}), but both of F_1 and F_2 have significantly different ($\alpha < 0.05$) to F_3 (0.35$\pm$0.07% d^{-1}). Biomass-specific growth rate (SGRb) also shows no significantly different ($\alpha > 0.05$) between F_1 (0.33$\pm$0.09% d^{-1}) and F_2 (0.31$\pm$0.02% d^{-1}), but both of F_1 and F_2 have significantly different ($\alpha < 0.05$) to F_3 (0.24$\pm$0.08% d^{-1}).

Keywords: Eel, *Anguilla bicolor bicolor*, Feeding Rate, Specific, Marginal growth.

1. Introduction

Anguilla sp. is mainly as carnivorous species [5] [12] [15] [16] with shorter gut line compare to either omni and herbivorous species. As nocturnal species, *Anguilla* preys mainly pelagic swimmer or drifting organism and some of the benthic organism. Atlantic menhaden *Brevoortia tyrannus* had been

used for baiting American eel *A. rostrata* at Hudson River [15]. Early glass eel of *A. bicolor bicolor* and *A marmorata* pray crustacean and zooplankton in Segara Anakan (southern seawater of Cilacap) Central Java [16]. As predators, eels become increasingly piscivorous by increasing body length. [12] conclude that most research on the diet of eels has been in nutrient-rich basic habitats and the eels which less than 300 mm in length fed mostly on Salmonid ova and parr, *Trichoptera* sp. larvae, *Simulium* sp. larvae, Asellus sp., Nymphs *Ephemeroptera, Gammarus pulex,* and *Diptera larvae.*While eels more than 300 mm in length fed on *Gammarus, Asellus,* crayfish, and fish of Sculpin and Stickleback. Due to this feeding habit, the sources of feed are important in racing this species.

Study on feed intake mainly related to feeding efficiency and economic sustainability should take into consideration. The relationship between feed intake and growth in finfishes has been conducted extensively using bioenergetic approaches [6] [7] [1]. However, Heinsbroek et al [8] stated that as bioenergetic models are focussed on the energy they do not take into account the differences in attributes and functions of the different energy-containing nutrients as well as the differences in transformation efficiencies, including a deposition. Due to these energy-containing nutrients it has been indicated that the non-protein macronutrients, lipids, and carbohydrates, are to some extent interchangeable in their effect on feed intake and growth and can be grouped as nonprotein energy (NPE), provided that the level of carbohydrates does not exceed the limit of the fish species investigated [14].

The shortage of eel in Asia (Japan), Europe, and America made Indonesian government enforcing regulation for eel seed export [17]. Nevertheless, eel aquaculture in Indonesia nowadays is just starting to develop compared to other species, i.e., prawn (*Penaeus monodon*), shrimp (*P. merguensis*), milkfish (*Chanos canos* F), or Nile (*Oreochromis niloticus*). Hence, food industry of those species has already been found in the right formula with reasonable price. However pasted feed for eel has still being imported or produced with relatively high price and low supply assurance. By using the pelleted feed of shrimp, hopefully, can minimize the production cost with consistent assurance in supply. The previous study using pasted feed of 2.2 % in dry basis [17] gave the best absolute growth slightly more than 100 % during 100 days culture. Trial on wet basis needs to be conducted to get another figure of feed ratio and growth. For this reasons, the study on different diets in wet basis will hopefully get the figure of feeding rate and weight gain of eel. Hereafter, this study aimed to evaluate different diets for feeding rate and growth performance.

2. Methodology

Fingerling elver (approx. 50 gr) of eel *A. bicolor bicolor* were obtained from seed collector farm in Purwokerto (Central Java). This study used a biomass of fish seeds (approx. 15 kg) which was distributed to 1 m^3 of 9 tanks with basic recirculating system conducted by [17]. The fish were maintained at 26.86 ± 0.58°C water temperatures, 7.39 ± 0.18 pH, 6.48 ± 0.11 ppm of dissolved oxygen and normal photoperiod in the semi-enclosed farm (HDPE roof and aluminum fence). Two kind of commercial pasted feeds F_1 (Sakae), and F_3 (UNS) and commercial shrimp feed (Feng Li) F_2 (Table 1) was given by 2.2 % d^{-1} in wet basis and give twice a day. The best-pasted feed performance using water weighed 75 % of dry food given. Hence the actual feed powder (dry) given was 1.26 % and mixed with water of 0.94 % (with ratio feed water of 4 : 3). CMC, cellulose gum (5% of food weight) was added for F_2 as a stabilizer and food binder. Uneaten food was collected and weighted after two hours feeding. Feed given assembled with biomass of eel weight and weight sampling of eel were taken in every fortnight. Feeding Rate (*FR*) were observed daily during 84 days and calculated by using the formula, i.e., amount of food given (*fg*) per day minus uneaten food (*uf*) divided by fish biomass (*b*) in every tank (*Form.1.*). To reduce mistaken calculation, the feed was put on floating net, and weighted if any remainder and the residue collected by flushing and netting the water.

$$FR = \frac{fg - uf}{b} \times 100\% \qquad (1)$$

Table 1. Feeds proximate analysis given to fish

Feeds	Water (%)	Ash (%)	Protein (%N)	Lipid (%)
F_1	10,51	13,18	39,67	12,17
F_2	10,18	8,65	34,11	4,84
F_3	9,25	10,98	27,71	12,04

Specific growth rate (*SGR*) of individual and biomass of fish per tank in group of treatments (F_1, F_2, and F_3) was calculated during 70 days observation by the difference of normal logarithmic of weight at "*t*" time (W_t) and initial weight (W_0) divided by time (*t*) in percent d^{-1} (*Form.2.*) [17]. Statistical analysis uses a completely random design and analysis co-variance with three treatments and three replications. The estimated marginal mean was used for feeding rate (*FR*). Individual and biomass-specific growth were analyzed by using generalized linear model repeated measurement ANOVA and post hoc test using multiple comparisons based on observed means.

$$SGR = \frac{Ln(Wt) - Ln(W0)}{t} \times 100\%$$
(2)

Water was a pump with a maximum capacity of approx. 5,000 l h^{-1} by using Ø ¾ inc inlet hose, recirculated through biological filter. Medium aeration was administered in every tank. For growing nitro bacteria (*Nitrosomonas* and *Nitrobacter*) and reduce metabolite excess, filter bucket filled with solid phase media of coral, zeolite stone, and charcoal. To prevent accumulated uneaten food and feces, bioplastic filter (dacron) were placed on the surface of the solid phase. The dirt produced were separated between uneaten food and feces. Medial aeration was also administered in every tank during the culture.

3. Result and Discussion
As a carnivorous species, i.e. *Anguilla bicolor bicolor* grow related to the protein content in feed given, the growth apparently increases by increasing protein. Feed rate fluctuates every fortnight accompanied by a condition of an organism, the texture of feed but seems to not correlate with the degree of the lipid content in the feed. As nocturnal species, eel tends to be active in the night time, but during feeding time especially in the morning, the fish tend to be active to prey while the food is moving onto floating net due to dynamic schooling of fish.

3.1. Feeding Rate
Feed rate observation conducted every day which assembled with fish biomass [17] weighted fortnightly. By using one way ANOVA shows that the feeding rate seems to be stable or no significantly different during 84 days observation (α > 0.05). The *FR* value in between 1.98 to 2.16 % (Table 2), fluctuation condition apparently depend on the condition of the organism. The wet basis of feed treated mainly to compare the previous study which used dry basis with *FR* between 1.30 – 2.07% [17]. Hence present study has a higher value of *FR* and more efficient in term percent of feed swollen by fish compare to the previous study of dry basis, and will probably give economic advantages. Due to a smaller quantity of feed given with smaller uneaten and debris of feed, will automatically give a better water quality.

Table 2. Mean of Feeding Rate (% d^{-1}) of eel during 84 days observation

Treat-ments	Days of observations						Mean	±SD
	01-14	15-28	29-42	43-56	57-70	71-84		
F_1	1.98	2.05	2.09	1.99	1.99	2.16	2.04	0.07
F_2	2.05	2.08	2.12	2.15	2.16	2.15	2.12	0.05
F_3	1.99	2.10	2.15	2.13	2.08	2.16	2.10	0.06

Nevertheless, treatment F_2 had a highest mean value with lowest deviation (2.12±0.05% d^{-1}) compare to F_1 (2.04±0.07% d^{-1}) and F_3 (2.10±0.06% d^{-1}) (Table 2). In fact, this F_2 of commercial shrimp feed which was added with cellulose gum (5%) have slightly more compact than original pasted feed with a similar water content of 75%. The influencing factors for feeding rate are food and feeding habit [17], once type of food introduced, the fish will take the feed to go along with starving conditions. Even rancidity rate was not measured, but the trend of these *FR* apparently related to lipid content where the feed rate increased by decreasing lipid content (Table 1). In correlation of this rancidity condition, all of the treatment replications of treatment F_1 have lower DO than others (Table 3).

Observation on temperatures, salinities, hydrogen ion concentrations (pH) and dissolved oxygen (DO) conducted in every second day remain stable (Table 3). Hydrogen ion concentration (pH) was between 7.44 and 7.66. This pH will be related to Alkalinity, and the pH will affect to NH_3 and NO_2^- concentration. This corresponds with stating *Nitrosomonas sp* which can grow better in pH between 7 to 8 [9], this bacteria will be responsible for converting NH_4 to NO_3.

The highest *FR* in this study was 2.16% with a natural temperature of 27.66±0.05°C, while [11] by using water heater of 33°C have the best *FR* for *A. bicolor pacifica* was 2.41±0.04% day^{-1} and *A. marmorata* (2.23±0.10% day^{-1}). Meanwhile, by using temperature of 28°C [11] just can attain of *FR* only 1.82±0.01% day^{-1} for *A. marmorata* and 2.13±0.03% day^{-1} for *A. bicolor pacifica*. Natural environment conditions of this study seem to have a better *FR* compare to what which [11] have conducted. Hereafter, in regulating feeding of aquatic organisms, natural environment, and adjacent conditions may as an important role to provoke a feeding rate.

Table 3. Temperatures, salinity, pH and dissolved oxygen measured every second day in every replication of treatments.

| Treatment	t (°C)	S (‰)	pH	DO (ppm)
$F_{1.1}$	27.14±0.11	1.22±0.04	7.44±0.09	3.78±0.11
$F_{1.2}$	27.24±0.05	1.28±0.08	7.44±0.05	3.54±0.19
$F_{1.3}$	27.26±0.05	1.36±0.05	7.50±0.00	3.64±0.15
$F_{2.1}$	27.50±0.07	1.22±0.04	7.44±0.05	4.28±0.11
$F_{2.2}$	27.54±0.05	1.28±0.04	7.56±0.05	4.42±0.18
$F_{2.3}$	27.66±0.05	1.30±0.00	7.52±0.05	4.36±0.09
$F_{3.1}$	27.56±0.09	1.16±0.09	7.62±0.08	4.58±0.11
$F_{3.2}$	27.66±0.05	1.20±0.10	7.63±0.04	4.56±0.22
$F_{3.3}$	27.64±0.05	1.18±0.04	7.66±0.05	4.66±0.09

3.2. Growth performance

3.2.1. Specific growth rate

Specific growth rate gives a figure or performance in certain technique, and methods had been used in giving percent daily weight attainment during the culture of organisms. Base on ANOVA analysis of individual fish growth repeated measurement during five times observation the grown shows significantly different among treatment ($\alpha < 0.05$), in continues test, indicated that F_1 similar to (=) F_2, but F_1 and F_2 different to ($\neq$) F_3. Table 2. shows that individual specific growth fluctuates fortnightly, the lowest *SGRi* occurred in the local pasted feed (UNS) of $F_{3.1.}$ during second measurement (0.05% d^{-1}), mean while the highest *SGRi* on Korean formula pasted feed (Sakae) of $F_{1.3.}$ during third measurement (0.80% d^{-1}). Base on standard deviation repeated measurement of *SGRi* during 70 days culture, F_1 shows lowest followed by F_2 and F_3 (Table 2). This conditions indicate that treatment F_1 gave a stable impact of growth in every replication of tanks compared to F_2 and F_3, meanwhile, F_2 more stable than F_3.

When the feeding rate is over maintenance requirement since the energy consumed equals to the energy required by aquatic organisms to maintain them self without mobilizing endogenous energy reserves. Hence the growth rate will rapidly increase [3]. Furthermore, the growth will increases at a decreasing rate to a maximum point of feeding rate. For this conditions, this study shows that fish *A. bicolor bicolor* have a fluctuation of growth, but remain increase above zero line (Figure 1 and 2) even the *FR* remain stable (Table 2).
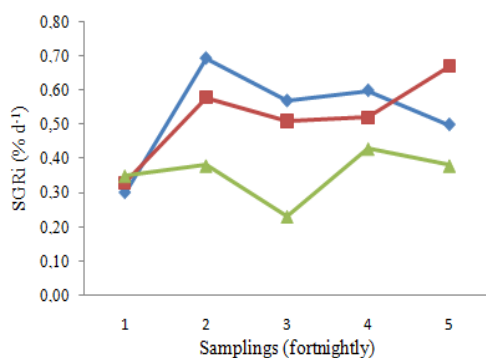
Figure 1. Individual-specific growth rate (%) of fish (*A. bicolor bicolor)* measured in every fortnight during 70 days culture fed with different feed.
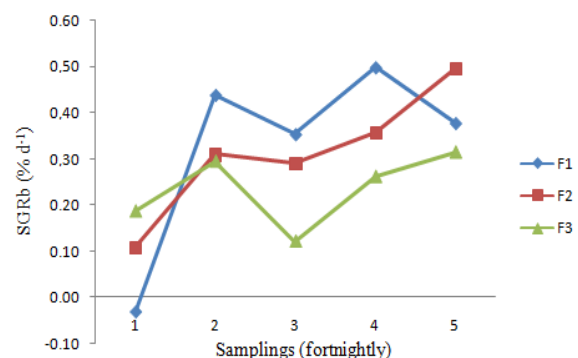
Figure 2. Biomass-specific growth rate (%) of fish (*A. bicolor bicolor)* measured in every fortnight during 70 days culture fed with different feed.

Figure 1 and 2 shows that the treatment 1 (F_1) gave the best growth either in individual and biomass of fish followed by F_2 and F_3 base on time series of samplings. The growth pattern of both individual and biomass seems to be similar. During previous study growth performance seems related to the feeding rate, the highest feeding rate gave the best growth, while, the lowest feeding rate followed by low growth of *A. bicolor bicolor* [17].

Nevertheless, unknown cause made quite high death rate of fish in one of the replications of F_1 ($F_{1.1}$, 7.4%) compare to other replications during the first period of sampling (1st-fortnight culture). Hence, total biomass gone drop under zero line after two weeks innoculation (Figure 2). Adaptation processes may affect in certain individual of fish, [17] stated that adaptation of captive fish requires high energy. They also stated that protein is sparing effect of lipid if the energy in the feed for an organism is inadequate to be used for environmental adaptation. Hence protein energy will be used for pertaining life, not for cell development. In this case, even though feed in F_1 treatment have significantly higher protein content (Table 1), but during first-week innoculation, in replication 1 of

F_1, some of the fish bundle may face longer handling during transportation. Moreover, during second samplings of treatments, first treatment of F_2 also have high (6.9%) death rate. Hence, this death rate has no correlation with food given. Holmgren et al [10] have experience in an initial decrease in weight of *A. anguilla* , this decrease can be regarded as a common phenomenon for the eels to complete their metamorphosis and to adapt to their new environment. Tesch [18] has also described an initial reduction in length and weight of European glass eels in aquaria, either fed or unfed, while pigmentation developed.

Possibility causes of unhealthy seed in a certain bundle during transportation were temperatures, feeding before transportation, extruded undigested feces, and finally low dissolved oxygen. This temperature fluctuation during transportation will lead to unstable metabolic rate hence feed given before transported will digest then protruded as undigested feces. Hereafter, The organism will take more energy and oxygen consumption as the water temperature increases [2]. Furthermore [11] stated that as tropical and subtropical species *A. marmorata* and *A. bicolor pacifica* could tolerate higher water temperatures, also [4] mention that some of the Indonesian shortfin eels can tolerate to 41°C temperature. As a proof [11] tried the experiment on *A. bicolour pacifica* and *A. marmorata* exposed at different temperatures, i.e., 8, 13, 18, 23, 28, and 33°C, both species most died within 10 days at both 8 and 13°C and both of them can survive at ≥18°C.

The graphs either individual and biomass-specific growth rate have a similar pattern (Figure 1 and 2), after second-fortnight samplings (28 d) the growth increase steeply either F_1 and F_2 treatments. Third samplings show a decrease in the percent of growth to all of the treatments. During this fortnight of culture (days of 29th to 42nd) there were some obstructions of noises and tremble waves from building construction. Even though the obstacles still happen during next fourth samplings, the growth of fish can still increase may due to the ability of environmental adaptation. Correlation of feed rate in this case of obstruction seems to be slightly increase to go along with increasing feces during 29 to 42 days of culture. However, the next samplings (4th, 43 – 56 d) the *FR* slightly decrease assembled with continues obstruction of noises and tremble wave, but the number of feces was lower. Hence F_1 and F_3 individual *SGR* remain increased. Moreover the 5th samplings (57 – 70 d) the *FR* remain similar to 4th samplings and both *SGR* of F_1 (pasted feed of Sakae) and F3 (pasted feed of UNS) tend to be decreased. On the other hand, *FR* of F_2 remains consistently increased (slightly) during obstructions (days 29th to 70th), hereafter, both individual and biomass growth also consistently increased after first decreased in the 3rd samplings (Table 2, Figure 1 and 2).

From this study seems to have no correlation between growth performance and feeding rate (Figure 1 and 2, Table 2), but apparently related to protein available in the feed (Table 1). The *FR* seems to have a correlation with the lipid content in the food. Taufiq-Spj et al [17] stated that protein is sparing effect of lipid, if the energy in the feed for an organism is inadequate to be used for environmental adaptation, protein energy will be used for adaptation, not for growth. Hereafter, the growth itself as one of captive fish adaptations and this process require a high energy. Moreover, even the figure of feeding rate remains stable and tend to be increased at the end of sampling in F_1 and F_3 treatments (Table 2), but specific growth rate figures show a decrease for F_1 and F_3 but increase for F_2 (Figure 1 and 2).

3.2.2. Growth margin

Absolute growth of individual eel *A. bicolor bicolor* was bigger than biomass absolute growth. This calculation of the margins from the initial individual weight of fish and at the end of the measurement. The calculation shows that F_1 gave highest individual absolute growth (25 g, 46%) followed F_2 (22 g, 44%) then F_3 (15 g, 27%) during 70 days of culture. Biomass absolute growth gave values of F_1, F_2, F_3 were 3,855 g (26%), 3,629 g (24%), and 2,834 g (18%) respectively during 70 days culture (Calculated from Table 4). No different between F_1 and F_2 (α > 0.05) either in individual and biomass, but F_1 and F_2 have different to F_3 (α < 0.05) also in individual and biomass. F_2 of shrimp feed gave more consistent in weight among tanks replication where the deviation was smaller than F_1 and F_3.

Table 4. Initial and t time weight of Individual (I_0, I_t), Biomass (B_0, B_t), Specific Growth Rate (%) of Individual (SGR_i) and Biomass (SGR_b) of *A. bicolor bicolor* fed with different diets during 70 days culture.

Treatments	Observed mean of growth margin					
	I_0 (kg)	I_t (kg)	% SGR_i	B_0 (kg)	B_t (kg)	% SGR_b
$F_{1.1.}$	0,056	0,078	0,48	15,025	17,481	0,22
$F_{1.2.}$	0,054	0,080	0,55	15,007	19,463	0,37
$F_{1.3.}$	0,053	0,079	0,57	14,988	19,641	0,39
25Mean	0,054	0,079	0,53	15,007	18,862	0,33
±SD	0,001	0,001	0,05	0,018	1,199	0,09
$F_{2.1.}$	0,049	0,072	0,53	14,843	18,490	0,31
$F_{2.2.}$	0,050	0,071	0,52	14,910	18,751	0,33
$F_{2.3.}$	0,051	0,074	0,52	14,833	18,233	0,29
22Mean	0,050	0,072	0,52	14,862	18,491	0,31
±SD	0,001	0,001	0,01	0,042	0,259	0,02
$F_{3.1.}$	0,055	0,068	0,30	16,595	18,908	0,19
$F_{3.2.}$	0,055	0,069	0,32	15,947	18,256	0,19
$F_{3.3.}$	0,054	0,073	0,43	14,915	18,796	0,33
15Mean	0,055	0,070	0,35	15,819	18,653	0,24
±SD	0,001	0,003	0,07	0,847	0,349	0,08

Total margin of individual-specific growth ($SGRi$) during 70 days culture F_1 shows the best mean value of 0.53±0.05% followed by F_2 (0.52±0.01%) and F_3 (0.35±0.07%) (Table 3). Moreover, the margin in biomass specific growth (SGR_b) tend to be smaller than $SGRi$. The trend of the growth pattern of SGR_b (total margin of biomass) was similar to $SGRi$ either in statistical analysis. Table 3 shows that percent of SGR_b on F_1 show the best growth (0.33±0.09%) followed by F_2 (0.31±0.02%) and F_3 (0.24±0.08%).

The previous study by using the bigger size of *A, bicolor bicolor* (±70 g tail[-1]) [17] shows the absolute biomass growth 52.94±6.17% with an initial density of ±20 kg m^{-3} and the *FR* of 1.89±0.06% d[-1] during 100 days culture. While, present study using a smaller size of eel (±50 g tail[-1]) absolute growth attained 24.42% (calculated from Table 4) with initial density of ±15 kg m^{-3} and the *FR* of 2.12±0.05% d[-1] during 70 days culture. If the T_2 in a previous study [17] equal to F_2 in the present study in term of water volume and protein content of the feed, hence during 100 days culture, F2 should attain approximately 34.88% absolute growth (calculated from Table 4). For this reasons, bigger sizes of eel will grow faster than smaller. According to [3] stated that the FCR decreases as the feeding rate increases towards to the maximum rate, and the level of *FR, FCR* as well as growth rate varies according to especially the size of cultured organisms, species, and water temperature.

4. Conclusion

From 3 different feed treatments, the feeding rate of *A. bicolor bicolor* do not correlate with protein content but apparently, follows the degree of lipid in the foodstuff. Higher lipid content gave slightly lower of feeding rate, on the other hand, a higher protein on feed gave higher growth performance.

The initial weight of eel seeds gave a figure of survival rate followed by biomass growth; smaller seed inoculated will gave smaller survival rate and smaller biomass growth. The calculation of efficiency value of this study represents by biomass density per liter of water volume, have a value of 3.8 g l^{-1} during 70 days culture.

Acknowledgements

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