Increasing growth and survival rate of tilapia larvae (Oreochromis niloticus) by adding polychaeta Nereis sp dry meal into feed formulation

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Abstract. Aquaculture has been known as one of solutions in dealing with food security problem through applying best practices of aquaculture and increasing fish production. In many countries, fish production is considered an essential activity contributing to household income and trade. However, fish feed has an expensive price, and it constitutes 60-70% total cost of production. The objective of this study is to reveal an alternative cheap source for additional ingredients to fish diet formulation of Tilapia larvae (Oreochromis niloticus). 240 fish larvae from local hatchery were used and cultured in 4 aquariums with different dose of protein diet (30%, 35%, 40%, and control). The source of protein diet is Polychaeta Nereis sp which was collected from surrounding Kastela Sea. Nereis sp was processed into dry meal and was added into the feed formulation. The result shows that the useful of 40% protein of Nereis sp gives the highest growth and survival rate of tilapia larvae, which is 44.99 g and 100%. It is followed by 35% and 30% Nereis protein, respectively. It is interesting that protein control which is feed commercial has almost similar growth level as the highest one. Specific growth rate (SGR) is 3.74%-3.79% per day for different protein dose of Polychaeta. Survival rate is 100% for all treatments which means all fish can survive and growth continuously from beginning to the end of the study.

1. Introduction
The fisheries and aquaculture sectors operate in an increasingly globalized environment. Aquaculture production, both in freshwater and marine, shifts the capture fisheries since capture production has slowed and decreased, and the fish stock proportion continuously exploited has declined to below 80% in 2018 [1]. It is also believed that aquaculture is one of solutions in dealing with food security problem worldwide by applying best practices of aquaculture and increasing fish production [2]. Nile Tilapia is generally known as an important freshwater fish species economically relating to its fast growth, breeding and low feed conversion, highly resistance to disease and harmful water condition, and the consumer acceptance [3]. Furthermore, Nile Tilapia can be reared through integrated fish farming systems such as polyculture and Integrated Multitrophic of Aquaculture (IMTA), the acceptance of artificial diet after yolk-sac absorption, and they are well-suited for culture in developing countries as in Indonesia [4], [5]. For aquaculture to play a major role in the food security as a source of high-quality protein, it is essential to produce the farmed species at low-cost. However, 40-60% operational expenditure in aquaculture is feed availability. One of the most important problems in aquaculture sector is the reduction in feed costs and production of high-quality feed.
The fact that fish meal production is limited, and its price is high leads the fish feed producers to use alternative raw materials. There are many raw materials sources from animals and vegetables. Seaworm or Polychaeta has important value as feed to shrimp culture and marine ornamental fish, particularly their ability as trigger the maturation of shrimp gamete cells up to 70% [6]. They also contain high level of amino acids and unsaturated fatty acids [7], [8]. Generally, the best results come from high lipid, Coldwater species such as Nereis spp. and Glyceria spp. [9].

The objectives of this study are to reveal an alternative cheap source for additional ingredients to fish diet formulation of Tilapia larvae (Oreochromis niloticus), to process raw materials (sea worm Nereis sp) into dry meal that will be added into diet formulation, and to measure the growth and survival rate of Tilapia larvae in fish tanks.

2. Materials and methods

Materials used were Tilapia larvae size 8 cm that collected from local hatchery (BBI Gambesi), 12 rectangular tanks, freshwater, analytical balance, and fish feed machine, pH meter, DO meter, and thermometer.

2.1 Experimental design and feed processing

A 3x4 randomized completely design was used to evaluate the effect of diet in four different level of Nereis protein (40%, 35%, 30%, and commercial diet as control) as shown at Table 1.

| Treatment  | Percentage of Nereis meal |
|------------|---------------------------|
| A          | 30%                       |
| B          | 35%                       |
| C          | 40%                       |
| D          | Commercial feed (control) |

For diet preparation, some raw materials were used to formulate larvae feed such as anchovy, tofu dregs, peanut, lamtoro leaves, sea worm Nereis sp, and vitamin premix. All raw sources were processed into dry meal under exact proportion. The proportion of those feedstuffs were calculated using Pearson square method and it is shown as Table 2.

| Food sources          | Protein ingredients in percentage (references) | Feed sources usage in gram | Protein usage in percentage |
|-----------------------|------------------------------------------------|---------------------------|-----------------------------|
| Anchovy meal          | 33.40                                          | 30%                       | 15.00                       |
|                       |                                                | 35%                       | 15.00                       |
|                       |                                                | 40%                       | 15.00                       |
| Tofu dregs meal       | 21.30                                          | 30%                       | 10.00                       |
|                       |                                                | 35%                       | 10.00                       |
|                       |                                                | 40%                       | 10.00                       |
| Peanut meal           | 8.80                                           | 30%                       | 6.00                        |
|                       |                                                | 35%                       | 6.00                        |
|                       |                                                | 40%                       | 6.00                        |
| Nereis meal           | 56.07                                          | 30%                       | 17.86                       |
|                       |                                                | 35%                       | 37.84                       |
|                       |                                                | 40%                       | 51.40                       |
| Lamtoro leaves meal   | 23.26                                          | 30%                       | 51.13                       |
|                       |                                                | 35%                       | 31.15                       |
|                       |                                                | 40%                       | 17.59                       |
| Vitamin premix        | 1.00                                           | 30%                       | 1.00                        |
|                       |                                                | 35%                       | 1.00                        |
|                       |                                                | 40%                       | 1.00                        |
| Total                 | 100                                            | 30%                       | 100                         |
|                       |                                                | 35%                       | 100                         |
|                       |                                                | 40%                       | 100                         |

240 fish larvae were used for the experiment. At the start, larvae were weighing to know the initial weigh, and they divided into 12 tanks that each tank contain 20 larvae. Larvae were fed at a rate of 5% of the body weight for 28 days. The quantities of formulated feeds required to feed fishes were determined based on the mean fish weight in each aquarium and appropriate feeding rate. The quantities of daily feed weights were adjusted during each sampling event (i.e. every week) to accommodate for fish weight changes. The weight data were collected from 60% of fish sampled every week to evaluate the fish’s growth performance and feed utilization under the different
experimental setups. Total weights were measured using analytical balance 0.01 g. The initial and final numbers of fish stocked were also taken. Water quality was checked in the beginning and at the end of experimental days. Orthophosphate (PO\textsubscript{4}-P) and Ammonia (NH\textsubscript{3}-N) were measured twice and it was measured in the laboratory of Balai Perikanan Budidaya Laut Ambon.

2.2 Parameter measurement

Data was analysed using one-way ANOVA. The various growth parameters, Feed conversion efficiency, survival rate, and production value were computed as follows [10]:

- Weight gain (WG, g) = Mean Wf – Mean Wi
  Where, Wf = final weight (g), Wi = initial weight (g)
- Daily Growth Rate (DGR, g day\textsuperscript{-1}) = WG/days
  Where, Days = Number of culture days
- Specific growth rate (SGR, % day\textsuperscript{-1}) = ((lnWf – lnWi)/Days)*100
- Survival rate (SR, %) = (Number of fish harvested/Number of fish stocked)*100
- Feed Conversion Efficiency (FCE, %) = WG (g)/Total weight of feed (g)
- Production value (PV) = Mean weight gain of fish cropped (kg or g)*Total number of survival (n)*Price per kg or g

3. Results and discussion

The present results indicate that treatment C has the highest body weight gained during experiment and followed by treatment D, A, and B, which is shown on the Figure 1. Based on statistical analysis, treatment C has significant different with treatment B (p<0.05), while there was no statistical difference with other treatments (A and D).

Diet formulation which contains 40% Nereis protein gives 44.99 g of Tilapia larvae weight gain. Though there is almost similar number with other treatments, the Polychaeta ingredients can be used as low-cost protein source for supporting growth of fish. It is interesting that the commercial feed obtains same growth level as the highest one. Otherwise, specific growth rate has the highest number in treatment D which is 4.21% day\textsuperscript{-1}, then followed by 3.79% day\textsuperscript{-1} for C, 3.78% day\textsuperscript{-1} for A, and 3.74% day\textsuperscript{-1} for B. The graph can be shown as Figure 2. Also, the result of daily growth reveals in Table 3.
Specific growth rate is defined as growth rate over time. To understand the relation between diet ingredient that given to fish’s SGR, Pearson’s correlation was applied. Based on result, correlation coefficient has 0.540 protein feed content and SGR of fish. The correlation is strong by pointed out the positive sign. Through statistical analysis on the average SGR among treatments, it demonstrates that there is no significant difference (p>0.05). 40% and 30% of Nereis protein in diet formulation give similar growth rate in body length and weight daily. Tilapia larvae gain their weight 4.00 g a day, while treatment D, A, B attain 3.99 g, 3.77 g, and 3.63 g, respectively. Growth is defined as weight gain or body length at a given time and it also called as the absolute growth. The daily growth rate of larvae shows the change of average weight for 28 days, means that average weight has been increased by increasing the maintenance time of cultured fish.

The utilize of Nereis as protein source in diet formulation that fed to Tilapia larvae obtains better performance to larval growth. Nutrition content of Nereis is a potential source to diet in fish and shrimp aquaculture [7]. Polychaeta worms have the potential to substitute fishmeal as raw material for shrimp feed [11]. Other studies revealed that Polychaeta have high level of PUFA (Polyunsaturated Fatty Acid) [12], and can control physical and chemical conditions in aquarium by reducing ammonia and eventually released as nitrogen gas [13].

All larvae survive 100% until the end of the experiment. The use of Nereis sp in feed formulation kept the fish in good condition.

![Figure 3](image_url)

**Figure 3.** Survival rate of Tilapia larvae for 28 days

### Table 3. The average of Weight gain, Specific growth rate, Daily growth rate, Survival rate, Feed conversion efficiency, and Production value of each treatment

|       | A   | B   | C   | D   |
|-------|-----|-----|-----|-----|
| WG    | 44.82 | 44.78 | 44.99 | 44.97 |
| DGR   | 3.77 | 3.63 | 4.00 | 3.99 |
| SGR   | 3.78 | 3.74 | 3.79 | 4.21 |
| SGR   | 100 | 100 | 100 | 100 |
| FCE   | 41.66 | 43.81 | 45.67 | 47.32 |
| PV    | 672.300 | 671.300 | 674.850 | 674.550 |

Animals including fish turn diet into meat or body weight are very efficiently and it varies between species and method of production. FCR or Feed conversion ratio is mentioned to calculate the ratio of feed intake to weight gain [15]. Nearly all aquaculture producers use feed conversion ratios (FCRs) as an index of feed use efficiency. FCR is determined by dividing the total feed use in a production unit or entire aquaculture facility by the net production of the culture species. The varieties of FCR depends on several factors, including species, feed type and quality, production system, feeding technique, and water quality conditions. Warmwater fish and shrimp farms typically achieve
FCR values of 1.5 to 2.5, and for general purposes, FCR often is assumed to be 2.0. Although FCRs are an excellent measure of feed use and economic efficiency, they can be misleading ecological indicators [16]. For example, attainment of a 1.0 FCR does not imply that no wastes resulted from feeding. Besides FCR, FCE (Feed conversion efficiency) is believed as precise calculation to identify the aquaculture’s efficiency production. In this study, the highest FCE is reached by treatments C, B, and A, respectively.

For economic analysis, Tilapia larvae that fed by commercial diet has similar cost and benefit with larvae which consume 40% Nereis protein in their feed. The total PV around Rp. 674.550-674.850. Treatment A gives Rp. 672.300 and Treatment B has Rp. 671.300. Generally, Nereis protein that mixed into feed formulation of larval rearing gives suitable profits economically.

Water quality parameters are measured every week during the experiment except the content of N and P that collected twice at the initial and the end of study. From table 4, it shows that temperature, pH, Dissolved oxygen, Ammonia (NH₃-N) and Phosphate (PO₄-P) in the tanks are within optimal levels for growth of Tilapia larvae.

| Table 4. Water quality parameters during experiment |
|---------------------------------------------------|
| Treatment | Water quality parameters |
| Temp (°C) | pH | DO (ppm) | Ammonia (mg/l) | Phosphate (mg/l) |
| A | 27.3-28.1 | 7.5-7.7 | 4.2-5.6 | 0.269-0.274 | 0.199-0.295 |
| B | 27.5-28.4 | 7.5-7.8 | 5.4-5.5 | 0.093-0.868 | 0.195-0.330 |
| C | 27.4-28.1 | 7.5-7.7 | 5.5-6.2 | 0.290-0.297 | 0.213-0.309 |
| D | 27.6-28.1 | 7.6-7.7 | 5.2-5.4 | 0.117-1.613 | 0.104-0.214 |

Water quality is of utmost importance in fish and shrimp farming. Regardless of the particular aquaculture system used, maintaining balanced levels of water quality parameters is fundamental for both the health and growth of farmed aquatic species. Water temperature can affect fish and shrimp metabolism, feeding rates and the degree of ammonia toxicity. Temperature also has a direct impact on biota respiration (oxygen consumption) rates and influences the solubility of oxygen (warmer water holds less oxygen than cooler water).

Toxic ammonia comes from major waste products of shrimp and fish and uneaten feed. High levels occur most likely in summer when feeding rates, water temperature and pH are high and when algae population is low. It causes stress, gill damage and poor growth when the concentration is > 2.0 mg/l. At more than 2.0 mg/l ammonia causes death of shrimp and fish. Keeping ammonia levels in ponds below 0.5 ppm is important. To avoid an accumulation of ammonia, preventive measures must be taken through optimum feeding rates, maintaining healthy algae blooms and water exchange.

4. Conclusion
The result of study demonstrated that affordable and feasible fish feed can be formulated from locally available low-cost ingredients for Tilapia larvae culture production. Though the Polychaeta *Nereis* sp can be applied as one of sources in feed formulation, on average the fish shows better growth performance, feed utilization, fish performance, and survival rate at 40% protein level which almost has similar pattern with fish that fed by commercial feed.

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