Polyculture Engineering technology of *larasati* red tilapia (*Oreochromis niloticus*) and white shrimp (*Litopenaeus vannamei*) based for protease enzyme

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**Abstract.** The objective is polyculture technology of red tilapia larasati fish and white shrimp with different combinations density. The material is saline red tilapia larasati 3.29 ± 0.018 g and white shrimp with initial weight 1.39 ± 0.025 g. Seeds are density of red tilapia larasati larvae 5 and 10 larvae / m² fish. And white shrimp 5 larvae / m² and 10 larvae / m². An artificial feed used enzyme dose of 2.25 g / kg. The experimental using complete randomized design 4 treatments and 3 replications that is given seeds 5 larvae / m² larva red tilapia larasati and given 5 larvae / m² white shrimp (A), 5 larvae / m² red tilapia (B), 10 larvae and 5 m² white shrimp (C), 10 m² larvae and 10 m² white shrimp (D). The data were growth of absolute weight, survival rate, FCR, and water quality data (temperature, salinity, pH, O₂, NO₂, NH₃). Data were analyzed of variance (F test). The results showed significantly effect (P <0.01) on the growth. The highest absolute growth in D treatment were red tilapia larasati (185.75 ± 0.50g) and white shrimp (25.25 ± 0.95 g).

1. Introduction

Red tilapia and white shrimp are important protein sources worldwide, especially in Indonesia. In Indonesia the majority of fish consumption is caught in the ocean, with amounts around 90 million tons in recent years [1,2,15,45]). Meanwhile production of inland fisheries in 2003 in Central Java reached 339,000 tons (Marine and Fisheries Agency of Central Java Province, 2004) [37]. Fishery production was dominated by marine fisheries for about 236,240 tons (approximately 74 % of total fishery production) with a value of IDR 0.77 trillion. In 2003, aquaculture and fisheries businesses in public waters of Central Java, both in terms of production and value, were higher than the previous year. The production of aquaculture and fisheries businesses in public waters reached 75 to 88 thousand tons and 14.33 thousand tons with production value reached IDR 0.88 billion and IDR 91.90 billion, respectively. cur rently, the fish farmers on the northern coast of Central Java, particularly in Semarang, still use the conventional monoculture fisheries system, thus, their production value is low [3,4,5,6].

The problems that often arise in fisheries include high mortality (60-90 %) of *larasati* red tilapia and white shrimp in fisheries that is caused by lack of nutrient intake and poor water quality [5,7].

*larasati* Red tilapia and white shrimp are commodities that have important economic value and they can be cultured together, which bring benefits of increased growth of red tilapia in 3-5 months to
reach the weight of 200 -300 g of and the white shrimp to reach the size of 25-30. Hassan et al. [11] reported that the most important polyculture [20, 21, 22, 23]. Hassan et al.[11] stated that one solution of monoculture fish farming problems is to replace it by polyculture fish and shrimp simultaneously farming where there is no competition so both species grow simultaneously. Bardach et al. [5] Bautista [7] described polyculture fisheries in pond supplied with manure is a widespread practice in many countries. explained this system usually use a combination of planktopagic silver carp, bighed carp, macrophytphagic grass carp, benthphagic common carp, and omnivorous tilapia sp. To take advantage of the various feeding pond riches [9, 10, 11].

Other advantages of combined farming of larasati red tilapia and white shrimp are their synergism, and they can be cultivated in ponds together using seaweed as biofilter system that is able to absorb organic material to improve water quality. In aquaculture environment, red tilapia and white shrimp with seaweed biofilter system can quickly adapt, can be fed with various types of food from artificial feed, clashing, plankton to disposal in the form of organic materials, have a fast growth, are easy to multiply, and relatively resistant against disease. The studies suggested that to improve polyculture by using enriched artificial feed with the protease papain could enhance feed utilization and digestibility in most polyculture of larasati red tilapia and white shrimp.

The importance of papain in artificial feed to improve the quality of red larvae and vannamei prawns in ponds is affected by abrasion or the highest tides of seawater. Papain is enzyme found naturally in unripe papayas with is usually used as meat tenderizer, which helps to break down the tough band between fibers in muscle tissues. Papain is extracted from carica papaya with an activity of approximately 100.000 mU/mg. it is used to catalyze proteolysis and speed up the digestive process in fish [45] [57] [58][59] [60] [61].

The study was mainly aimed to investigate the role of polyculture technology of larasati red tilapia and white shrimp with different ratio of larasati red tilapia and white shrimp on their growth and survival rates.

2. Methodology
2.1. Experimental fish
The test feed used in this study were made in the form of pellet with 35 % protein content and energy of 300.36 kcal (1 kcal = 4 186.8 J) [22]. The diet contained fish meal, soybean meal, corn meal, rice bran, wheat flour, fish oil, corn oil, vitamin mix, and mineral mix modification [28].

The material in this study were seed of marine larasati red tilapia (average initial weight 3.29 ± 0.018 g) and Litopenaeus vannamei white shrimp (average initial weight 1.25 ± 0.025 g). The artificial feed contained 35 % protein and enriched with a protease (100 mg / kg of feed) with the amount of feed given at 3 % biomass/day based the method modified from Mo et al (2016) [45], who reported the use of the enzyme papain at a dose of 170 mg / kg of feed that produced the best growth in goldlined seabream (Rhapdosargus sarba), mud grouper (Ephinephelus Sp Bleeker), and Trachinotus blochii. Feed ingredients and proximate analysis can be seen in Table 1.

2.2. Diet preparation
Ingredients and proximate composition of experimental diets are presented in Table.1.

| Ingredient (g per 100 g diet) | Analysis of materials |
|------------------------------|-----------------------|
|                              | Moisture | Ash  | Crude lipid | Coarse Fiber | Crude Protein | BETN  |
| Fish meal                    | 10,89    | 22,75| 7,98        | 9,25         | 45,40         | 3,73  |
| Soybean meal                 | 11,06    | 5,65 | 9,23        | 5,46         | 38,71         | 29,88 |
| Corn meal                    | 13,71    | 1,77 | 2,03        | 0,01         | 9,38          | 73,09 |
### Table 2. Composition of experimental diets

| Ingredient (g per 100 g diet) | (g per 100 g diet) |
|------------------------------|-------------------|
| Enzim protease               | 2.25              |
| Fish meal                    | 33                |
| Soybean meal                 | 33                |
| Corn meal                    | 8.25              |
| Rice meal                    | 8.1               |
| Dextrin                      | 10.2              |
| Fish oil                     | 1.5               |
| Corn oil                     | 1.5               |
| Vitamin & mineral            | 1.1               |
| CMC                          | 1.1               |
| **Total**                    | **100**           |

### Procedure for making artificial feed for the experiment:
An appropriate dose of papain was dissolved first into warm water (45 °C), and then mixed evenly with artificial feed. The mixture was stored in an airtight container for 24 h [16] [17][18] [24]. The artificial feed was prepared by mixing the least amount of the ingredients first; then the more voluminous ingredients were gradually added and mixed. The corn oil and fish oil were added last to the mixture. After that, granules with diameter of 1-2 mm were formed from the mixture. Lastly, the artificial feed was dried in the oven at 40 °C [28].

Material (g) Composition protease papain enzyme (g/kg feed) 0.08 Fish meal 33 Soybean 33 Corn meal 8.25 Rice meal 8.1, Dextrin 10.2, Corn oil 1.5 Fish oil 1.5, Vitamin & mineral 1.1 , CMC 1.1 Total 100 Energy (kcal) 300.02 Ratio E/P 8.7.

The material used in this research was _larasati_ marine tilapia (3.29 ± 0.018 g) and Vannamei shrimp with initial weight of 1.39 ± 0.025 g. Seeds were stocked with the density of red larvae at 5 and 10 tail/m² fish and vannamei shrimp at 5 tail/m² and 10 tail/m². An artificial feed with protein content of 35% was enriched with protease at 22.5 g/kg of feed was given at a dose of 3% per biomass per day. The dose of papain used in this study was modified from the study by Mo et al (2016) [45]. The results suggested that the protease papain enzyme as much as 100 mg kg⁻1 diet was the optimum level for the growth of red tilapia _larasati_ and white shrimp with the average weight of 3.29 ± 0.018 g and Vannamei shrimp with initial weight 1.39 ± 0.025 g - fingerlings-1. The experimental was conducted in the brackish water pond using Randomized Complete Design (RCD) with four treatments and three replications namely, that is given seeds 5 tails / m² _larasati_ red tilapia fish and given 5 tails / m² white shrimp (A), 5 tails / m² red tilapia Larvae) and 10 m² / m² of white shrimp (B), 10 m² larvae and 5 m² white shrimp (C), 10 m² larvae and 10 m² white shrimp (D)). The data obtained were data of growth of absolute weight, life, FCR, and water quality data (temperature, salinity, pH, O₂, NO₂, NH₃). Data were analyzed by various analysis (F test) and descriptive. The study was conducted in media maintenance of polyculture technology of ± 1200 m², with each research plot area of 100 m².
2.3. Absolute Growth
The absolute growth formula is calculated by the formula Steffens (1989)[13][53][55], with the following formula:

\[ W \text{ (Absolute growth weight)} = \text{Weight of end test animal research} - \text{Weight of animal initial test of} \]

\[ \text{FCR} : \frac{\text{The amount of feed consumed}}{(\text{Final weight} + \text{Total weight of dead fish}) - \text{Initial weight}} \times 100\% \]

\[ \text{SR (Survival)}: \frac{(\text{Final count})}{\text{Initial count}} \times 100\% \]

2.4. Statistical Analysis
The experimental design used Completely Randomized Design (CRD) four treatments and three replications that is given seeds 5 larvae / m² red tilapia larasati fish and given 5 larvae / m² white shrimp (A), 5 larvae / m² red tilapia larasati Larvae) and 10 m² / m² of vannamei shrimp (B), 10 m² larave and 5 m² vannamei shrimp (C), 10 m² larvae and 10 m² vannamei shrimp (D). The data obtained were weight growth, survival rate, FCR, and water quality data (temperature, salinity, pH, O₂, NO₂, and NH₃). Data were analyzed by analysis of variance (F test) and descriptive analysis. The study was conducted in ponds (± 1200 m²) with polyculture technology, with each research plot area sized 100 m².

The effect of the treatments was tested using an analysis of variance (ANOVA) [56]. Before analysis, the normality, additivity, and homogeneity of the data were first tested. If the analysis of variance was significant (p<0.05) or highly significant (p<0.01), Tukey test was conducted to find out the difference among treatments [56]. To determine optimal dose of papain, analysis was conducted using Minitab 16. Water quality data were descriptively analyzed.

3. Results and Discussion
The results showed that there was a highly significant effect artificial feeding enriched with papain (P <0.01) on growth, food conversion ratio (FCR), survival rate, and feed conversion ratio (FCR).

3.1. The absolute growth of Larasati Red Tilapia.
The results showed that treatment D (10 m² larvae of larasati red tilapia and 10 m² / m² white shrimp) gave the highest weight growth in Larasati Red Tilapia with 185.75±0.50 g and in vannamei shrimp with 19.97 ± 1.75 g (Table.3). This result showed that Treatment D gave better weight growth due to higher protein content in the feed that affected the absolute weight of larasati red tilapia . Protein is useful for growth and it highly affected the growth of red tilapia and white shrimp protein quality energy content of feed and balance can increase growth rate d the nutrient and feeding rate [17]. Protein deficiency could decrease the weight of red tilapia and white shrimp due to muscle loss in the muscles and vital organs [2]. Similar with previous researches, the current study has shown that artificial feeding with protein content of 35% enriched with papain enzyme was able to increase the weight growth of larasati tilapia red from 179.5 g to 185.25 g [13][51][55].

The use of papain at 2.25 g/kg feed to enrich the artificial feed could increase the absolute growth of tilapia [13] [51] [52] [55]. Addition of exogenous protease such as papain could mitigate the adverse effect of plant protein. In our previous experiment, common carp Cyprinus carpio (freshwater fish) fed with papain-digested soybean residues mixed with beef liver showed significantly higher weight gain (12.43%) after 93 days, (Mo et al., 2016). In addition, Mo et al. (20016)[45] reported using papain at a dose of 17 g/kg pellets in artificial feed that could increase relative weight gain (%) of gold lined sea bream from 157.1±20.3 % to 320.9±67 % with the final weight between 150.8 ± 20.8 to 189.3±29.6 g. Mo et al (2016)[45] also reported an increase in final weight of brown spotted grouper from...
100.0±27.4 g to 163.0±47.8 g and relative weight gain from 305.6 % to 599.4±49.8 by using similar dose of papain. But application of technology polyculture red Tilapia *larasati* and white shrimp different growth of absolute was better good that the fish fed with digested soybean residues mixed with effective digestion was useful to maintain body condition, hence, contributes also in the growth. Papain is needed by fish even in small amounts because the body cannot synthesize it; thus, fish should get extra papain from artificial feeds [45]. Therefore, complete and balanced nutrient feed formula is necessary for *larasati* red tilapia and white shrimp fish. Moreover, polyculture system is one of the important systems that can increase growth and survival rate of red tilapia and white shrimp [6][49][50][51][52][53][54][55].

### Table 3. Absolute growth based on weight (g), survival rate, food conversion ratio on a variety of treatments and replications on the polyculture of red tilapia Larasati and white shrimp.

| Treatment | A (5N+5V) | B (5N+10V) | C (10N+5V) | D (10N+10V) |
|-----------|-----------|------------|------------|-------------|
| 1. Absolute growth of red tilapia of larasati (g) | 174.52±2.45<sup>b</sup> | 176.19±4.46<sup>ab</sup> | 178.97±5.87<sup>ab</sup> | 185.75±0.50<sup>a</sup> |
| 2. Absolute growth of white shrimp (g) | 21.72±0.54<sup>b</sup> | 21.90±0.53<sup>b</sup> | 23.29±0.94<sup>ab</sup> | 25.25±0.95<sup>a</sup> |
| 3. Survival rate of red tilapia of larasati (%) | 75.48±0.47<sup>c</sup> | 88.55±1.24<sup>b</sup> | 91.49±1.66<sup>b</sup> | 97.25±0.33<sup>a</sup> |
| 4. Survival rate of white shrimp (%) | 75.99±0.55<sup>c</sup> | 89.58±1.23<sup>b</sup> | 91.88±1.72<sup>b</sup> | 95.75±0.52<sup>a</sup> |
| 5. FCR of red tilapia larasati and white shrimp. | 3.16±0.11<sup>a</sup> | 2.32±0.07<sup>b</sup> | 1.95±0.05<sup>c</sup> | 1.22±0.13<sup>d</sup> |

Note: N = *larasati* red tilapia, V= white shrimp

Different superscript letters within the same column indicate significant differences between samples at the level of (P < 0.01)

3.2. Absolute growth of White Shrimp

The highest rate of absolute weight growth was shown by Treatment D (10 m<sup>2</sup> larvae of *larasati* red tilapia and 10 m<sup>2</sup>/ m<sup>2</sup> white shrimp) in ponds culture in Kandang Panjang, Pekalongan. Effect of the difference in the density of *Larasati* red tilapia of and white shrimp on the absolute growth of white shrimp (g) in polyculture system was highly significant (P < 0.01) (Table 3).

The highest value was from Treatment D (10 individuals per m<sup>2</sup> for red tilapia of *Larasati* and 10 individuals per m<sup>2</sup> for white shrimp) with the value of 25.25±0.95. g. *Larasati* red tilapia could use the feed well, and the addition of protease in the artificial feed helped to break down protein into amino acids that were easy to be breed by *larasati* red tilapia properly According to [23][24][25][26][27], fish growth is influenced by two main factors: internal factors related to the fish itself including genetic and physiological characteristics, and external factors related to the environment, such as chemical composition of water, temperature, metabolic rate, oxygen availability, and feed.

Growth occurs when there is excess energy after it is used for body maintenance, basal metabolism, and activities. Growth is supported by feeding that is tailored to the nutritional needs of the fish and it should have a high digestibility value. In the current study, artificial feed was given with a protein content of 35 % to 40 %. polyculture system that simultaneously maintaining white shrimp and milkfish (30 individuals per m<sup>2</sup> of white shrimp and 30 individuals per m<sup>2</sup> of milkfish) was able to
provide the highest absolute weight growth, because the amount of feed and the pond’s density were appropriate. Indicators of The growth was change in length and weight in a given period. Individual growth is due to the addition of tissue mitotic cell division that causes changes in size [29][30][31][32][33][34]. Marine and fisheries agency of central java province (2004) [35][36][37][38][39][40] reported that growth factors that affect the feed ratio and the weight of the fish are external and internal factors. External factors include water and environmental conditions, while the internal factors are the species, sex, genetic, and physiological status of fish. Indicator of physical growth includes number or size of cells that build up the body tissue and morphologically-visible growth of body. Growth occurs when the energy needed for metabolism and tissue maintenance fulfill the needs of the fish [23][24][41], or when the amount of consumed feed was greater than the amount needed for body maintenance, and when feed is used as an energy source for the fish [42][43][44][45][46].the highest value was obtained from polyculture technology with 30 individuals of white shrimp and 30 individuals of milkfish per m² in a polyculture system.

3.3. Survival rate of Larasati Red Tilapia.
The results showed that the highest survival rate in larasati red tilapia was obtained from Treatment D (10 individuals per m² for red tilapia of Larasati and 10 individuals per m² for white shrimp) with 97.25±1.15 % (Table.3). Analysis of variance showed a highly significant(P < 0.01) survival of larasati red tilapia. This is likely because of the innovation in polyculture system that used recirculation and biofilter system from seaweed. Other factors also contributed such as administration of artificial feed containing 35 % of protein enriched with papain at a dose of 2.25 g/kg feed to improve survival rate and growth rate, good environmental media, and proper number of stocking density, where all of them helped to increase survival rate, i.e. 97.25±1.15. Result of the current study was better by that of [51], which showed polyculture with white shrimp per m² and 30 individuals of milkfish per m² that had survival rate of 75±0.13 %. This shows the Effect of the difference in density of white shrimp L. vannamei and milkfish on the survival rate of milkfish in polyculture system.

Survival is the ratio between the number of fish or individuals living at the end of maintenance period with the number of fish or individuals living at the start. According to [3][13][45][56][63], fish survival is influenced by biotic and abiotic factors. Biotic factors include parasites, density, population, competitors, while abiotic factors include physical and chemical properties of aquatic environment. [2][25][56] explained that good growth of fish will increase the production volume that depends on the rate of growth and survival of the cultivated fish.

3.4. Survival of White Shrimp
The results showed that the highest survival rate in white shrimp reared in polyculture system was obtained from Treatment D (10 individuals per m² of Larasati red tilapia of and 10 individuals per m² of white shrimp) with the value of 95.76±1.02 %. (Table.3).

Table 3 shows the effect of density difference between larasati red tilapia and white shrimp on the survival rate of white shrimp in polyculture system. Analysis of variance showed a significant influence of density (P < 0.01) on survival of white shrimp. The survival rate was relatively high at Treatment D (95.76±1.02a %). This is more likely due to several factors: e application of biofilter with a high protein content that was enriched by 35 % with papain at a dose of 2.25 g/kg feed, which increased growth and survival rates. Artificial feed that was added with papain also contributed to proper tissue maintenance. The stocking density of larasati red tilapia and white shrimp was relatively sufficient, thus, contributing to high survival rate (95.76±1.02a %), better than the result of [51] who reported 95 ± 0.33 % of survival of white shrimp and milkfish in polyculture system. Result of the current study is consistent with previous researches by [11][14][20][21][22][45][63] who reported good water quality is essential in fish cultivation. Water quality affects survival of white shrimp and milkfish, which led to better growth rate [30][31][32][42][56]. Furthermore, maintaining water quality using biofilter and application of seaweed could increase the survival rate of white shrimp and
milkfish polyculture up to 80 to 90 % [18][19][20][21][22][45][63]. Eventually it could improve production and economic returns of white shrimp and milkfish reared in polyculture system higher than those in the crop rotation polyculture system. Furthermore, previous research on polyculture of tilapia shrimp showed an improvement on water quality in white shrimp and milkfish cultivation ponds by reducing diseases and chemicals that could increase survival rate.

3.5. Food conversion Ratio (FCR) of Larasati Red Tilapia and White Shrimp.

Feed conversion ratio was a very important parameter to examine whether the feed was able to increase the growth of larasati red tilapia and white shrimp. Feed conversion values are also useful to see how far the feed was converted further into meat of larasati red tilapia or white shrimp. Better FCR could be obtained from treatment D feed conversion values are also useful to see how far the feed was converted further into meat of larasati and white shrimp (Table.3). In the current study, the FCR value was of 1.22 ± 0.13 was better compared to the research of [51] who reported FCR of 1.25 ± 0.02, since larasati tilapia fish and vannamei shrimp can utilize feed well. Amal et al (2008) [3][11][47][60] also reported Food Conversion Ratio (FCR) of white shrimp and milkfish reared in polyculture system. it can be concluded that the feed formulated was suitable for polyculture system; thus, it increases growth rate better. Moreover, lower FCR value means more efficient feed administration [15] [25] [39] [40].

3.6. Water quality

The water quality was suitable for larasati red tilapia and vannamei shrimp. The existence of application technology of red tilapia culture cultivation as a tourism object of maritime education in Pekalongan city.

Table 3 shows that all parameters, i.e. the dissolved oxygen content (4.75 mg L−1 to −6.75 mg L−1), temperature (26.5 − 29.5 °C), salinity (20.5 L−1 to 29.5 mg L−1), pH (7.5 to 8.5), and ammonia (0.01 mg L−1 to 0.14 mg L−1), were still in the range of viable and capable for the life of larasati red tilapia and white shrimp, which were reared in polyculture system [3][8][15][25][39][40][52]. Several other researchers reported the application of closed polyculture system of tilapia shrimp combined with tagelus could increase productivity up to 28 %, which was higher than polyculture system for shrimp and tagelus.

Table 4. Water quality maintenance media polyculture system red tilapia larasati and white shrimp used biofilter system

| Water quality variable   | Range       | Worthiness (literature) |
|--------------------------|-------------|-------------------------|
| Oksigen terlarut (mg/l)  | 4.75 − 6.75 | >4 mg/l [a,b]           |
| Temperature (°C)         | 26.5 − 29.5 | 25.5 − 35 °C [c,d,f]    |
| Salinity (ppt)           | 20.5 − 29.5 | 15 − 30 [c,d]           |
| pH                       | 7.5 − 8.5   | 7.5 − 8.7 [c,d,e]       |
| Ammonia (mg/l)           | 0.01−0.14   | <1 mg/l [c,d,e]         |

Note: [40]a, [39]b, [52]c,[3]d, [8]e, [25]f.

Based on Table 4, it is shown that biofilter helped to produce decent water quality for polyculture system maintenance and the source should be environmentally friendly, because it uses seaweed as biofilter placed in inlet and outlet at 7.25 - 5.85 mg / L. The temperature of 25.5 mm was found to increase the survival rate of fish (93.73 ± 0.39c%) and vannamei shrimp (96.71 ± 0.85c%). The water quality shows the feasibility of the system for maintenance of milkfish and vannamei shrimp [15][25][39][40][52].
4. Conclusion
The results showed that the density difference of *larasati* red tilapia and white shrimp gave a highly significant effect (P <0.01) on growth, survival rate, and feed conversion ratio (FCR) of *larasati* red tilapia and white shrimp. The highest absolute weight growth was from treatment D (10 m² larvae and 10 m² / m² white shrimp) with 185.75 ± 0.50g *larasati* red tilapia () and 25.25 ± 0.95 g of white shrimp. The survival rate of *larasati* red tilapia was 97.25 ±0.33%, white shrimp 95.75 ± 0.52%, FCR 1.22 ± 0.13

The quality of the water was still feasible for the life of *larasati* red tilapia and white shrimp The existence of application technology of red tilapia *larasati* culture cultivation.

5. Reference
[1] Akegbejo and Y Samsons 1999 J Appl Trop Agric 49 (1): 37–41
[2] Akiyama D M, W G Dominy, and A L Lawrence 1991 Penaid Shrimp Nutrition for The Commercial Feed Industry. In Akiyama, D M and R K H Tan (eds.). [Proceedings of the Aquaculture Feed Processing and Nutrition Workshop, Thailand and Indonesia Singapore 19– 25 September 1991 American Soybean Association Singapore] 80–89.
[3] Amal S H, S H Sayed and E M Ibrahim 2008 Effect of Stoking Rates and Supplementary Feed on The Performance of Blue Tilapia (Oreochromis aureus) and Grass Carp (Ctenopharyngodon idella) Reared in Earthen Pond. [In Elghobashy, H., K].
[4] Fitzsimmons and A S. Diab (eds) 2008. Proceedings of 8th International Symposium on Tilapia in [Aquaculture] 12–24 October 2008 949–964.
[5] Aslam A, G S Hossain, M R Biswas, S K Barman and K Anisulhuq 2009 Int J Sustain Crop Prod 4(4): 23–27
[6] Badan Pengkajian dan Penerapan Teknologi 2007 Tiger Shrimp Cultivation Traditionally. [Research Report. Jakarta: BPPT].
[7] Bautista M N 1986 J.Aquaculture 3(3–4): 229–242
[8] Boyd H E, Burgess, Pronek and Walls 1982 Water Quality in Warm Water Fish Pond [Auburn: Auburn University, Aquaculture Experiment Station].
[9] Davis J 2011 Polyculture Opportunities in The Mid-hills of Nepal for Resource Poor Farmers. Ecological Aquaculture Studies & Reviews. [Kingston: University of Rhode Island].
[10] De Silva S S and F Y Anderson 1995 Fish Nutrition in Aquaculture [New York: Chapman and Hall].
[11] Dirisu S O, B Muinat and D M Yakubu 2007 J Animal Research International 4(3): 737–740
[12] Edwards) in Koilsagar Reservoir of Mahabubnagar District (TS), India International Journal of Fisheries and Aquatic Studies 2(4): 147–152.
[13] Effendie M I 1979 Methods of Fisheries Biology. [Bogor: Yayasan Dewi Sri]. [Bahasa Indonesia].
[14] Endrawati, H., S. I istiyanto, and A. Indarjo 2001 Application and Cultivation Technology Community Business Group Polyculture Nila Gift and Tiger Prawn in Ponds In An Effort to Empower Coastal Communities] Journal Info. 4(1): 6–18
[15] FAO (Food and Agriculture Organization) 2016 FishStat: a tool for Fishery statistics analysis version 2.12.2 http://.fao.org/fishery/statistics/software/fishstat/en(accessed 14.07.16.
[16] Fox J M, Addison LL, Anthony J S, Allen D, Denis RM, Elizabeth C D,and Tzachi MS 2006
[17] Furnichii, M 1988 Dietary Requirement in Fish Nutrition in Farmulture. [Japan: Japan International Coroporation Agency].
[18] Halver J E 1980 Fish Nutrition. [New York: Academic Press Inc].
[19] Halver J E and T Lovell 1989 Nutrition and Feeding of Fish. New York: [Van Nostrand Reinhold].
[20] Hassan A S, S H Sayed and 2008 Effect of stocking rate and supplementary feed on the growth performance of blue tilapia (Oreochromis aureus) and grass carp (Ctenopharyngodon idella) reared in earthen pods.[International symposium on tilapia in aquaculture] P 949-964
[21] HASSAN M S, SOLTAN M A, AGOUZ H M, and BADR A M 2013 Egyptian Journal of Aquatic Research 39(3):205–213.
[22] Hassan S, Altaff K, Satyanarayana T 2009 Pakistan Journal of Nutrition 8(4):341–344.
[23] Herpher B 1988 Nutrition on pond fisheries Cambridge, USA: Cambridge University Press. 388p.
[24] Herpher B 1988 Nutrition of Pond Fishes, Formerly of Fish and Aquaculture Research Station. [Cambridge: Cambridge University Press].
[25] Herpher B and Y Praginin 1981 Comercial Fish Farming. New York: [John Wiley Sons].
[26] He H L and R Liv 1992 J. Aquacultur 103: 177–185.
[27] Huet M 1971 Fish Culture, Breeding and Cultivation of Fish. [London: Fishing New (Books)] Ltd.
[28] [NRC] National Research Council 1993 Nutrient requirements of fish. [Washington DC: National Academy Press]. 124p.
[29] Nurjana M 2007 Proceedings of the National Seminar of, Aquaculture Society (MAI) Indonesia. Surabaya, Indonesia. 5–7 June 2007].
[30] Samidjan I 2001 Effect of Various Combinations of Natural Feed (Tetraselmis chui, Chlorella sp, Brachionus plicatilis Muller, Nauplius Artemia salina Leach) on Growth and Survival Rate Mangrove Crab (Scylla paramamosain). [Paper Presented at the National Seminar of Crustacean 2001. Institut Pertanian Bogor, Bogor. 20 – 21 July 2001].
[31] Samidjan I 2001 Enlargement of Mude Crabs (Scylla paramamosain) in Pond with Feed Trash in Different Dose. [Paper Presented at the National Seminar of Crustacean 2001. Institut Pertanian Bogor, Bogor. 20–21 July 2001].
[32] Samidjan I 2008 Engineering of Monoculture Technology for Superintensive System on Mudcrab (Scylla paramamosain) Using Different Feeds on The Growth and Survival Rate. In Hartoko, A. (ed.). [Proceedings of International Conference, Geomatic, Fisheries and Marine Science for a Better Future and Prosperity Marine Geomatic Centre (MGC). Semarang, Indonesia. 21– 22 October 2008].
[33] Samidjan I, E Arini, and D Rachmawati 2012 Applicaton of Technology and Science in (IbM) Business Group Polyculture of Shrimp, Fish and Seaweed (Gracyllaria sp.) Based on The Biological Filter Mangkang Wetan Village, District Tugu, City Semarang]. [Research Report. Semarang: Universitas Diponegoro].
[34] Samidjan I 2009Use of Various Types of Probiotic Bacteria (Bacillus, Alcaligenes, Flavobacterium, and Lactobacillus) as The Commercial Feed in Crumble from Vannmei]. [Research report] Semarang: Universitas Diponegoro].
[35] Jaspe J C, C M A Caipang, and B J G Elle 2011 J. ABAH Bioflux. 3(2): 96–104.
[36] Kanazawa A 1985 Nutrition of Penaeid and Shrimp. In: Taki Y, J H Primavera and J A. Liobrera (eds). [Proceedings of the First International Conference on Culture of Penaeid / Shrimp. Aquaculture Dept. SEAFDEC. Iloilo, Philippines]. 4–7 December 1984. 123–130.
[37] Kementerian Kelautan dan Perikanan 2004 Production of Shrimp and Milkfish in Fisheries Central Java, Indonesia]. [Jakarta: KKP].
[38] Kurmaly K 1995 Shrimp Nutrition and Disease: Role of Vitamins and Astaxanthin. [Bangkok: Roch Aquaculture Centre].
[39] Laxmappa B, S M Khrisna 2015 Polyculture of The Freshwater Prawn Macrobrachium malcolmsonii in (H.M. 98 Istiyanto Samidjan & Diana Rachmawati / Jurnal Teknologi (Sciences & Engineering) 78:4–2 ;91–98
[40] Li D S and S L Dong 2000 J Chinese Journal of Oceanology and Limnology 8: 61–66.
[41] Makwinja R and F Kapute 2015 Net Journal of Agricultural Science 3(3): 62–67.
[42] Mangampa M and Burhanuddin 2014 Jurnal Saintek Perikanan 10 (1): 30–36
[43] Marine and Fisheries Agency of Central Java Province 2004. Basic Data Production Potential and Fisheries Central Java [in The Figures. Semarang: Marine and Fisheries Agency].
[44] Miroslav C, T Dejana I, Dragana D and Vesna 2011 J. Tehnologija Mesa 52(1): 106–121.
[45] Mo W, WY, R S S.Lau, A C Kwok, M H Wong 2016 J. Fish growth and water quality
environmental pollution :1-6.

[46] Murachman, N Hanani, Soemarno, S Muhammad 2010 Journal of Sustainable Development and Nature 1(1): 2087–3522

[47] Nikolova L 2013 J. Bulgarian Journal of Agricultural Science 19(6): 1391–1395

[48] Phytase supplementation in aquaculture diets improves fish, shrimp growth performance. Retrieved from http://www.ag.auburn.edu/~davisda/publications/ publication_files/ca30_gaa-fox-feb06.pdf on 10 Jun 2016.

[49] Porchas M M, L. R M, Cordova M A P, Cornejo and J A L Elias 2010 J Reviews in Aquaculture 2: 73–85.

[50] Reksono B H, Hamdani and Yuniarti 2012 Journal of Fisheries and Marine 3(3): 41– 49

[51] Samidjan I and D Rachmwat 2016 Jurnal Teknologi (Sciences & Engineering) 78:4–2 ;91–98.

[52] Solomon J R and M N Ezigbo 2010 Polyculture of Heteroclarias / Tilapia Under Different Feeding Regimes New York Science Journal 3(10): 42– 57

[53] Steffens 1989 Principles of Nutrition England: Ellis Horwood Limited

[54] Stickney R R 1979 Principle of Warm Water Aquaculture New York: John Weley and Sons Inc

[55] Stell R G D, Torrie J H and Dickey D A 1996 Principles and procedures of statistics, [3rd ed. New York: McGraw Hill International Book Company, Inc] 672p.

[56] Suyono, I Samidjan, D Rachmwat, and T Yasman 2010 Application Science and Technology in (IbM) Groups of Fish Farming Milkfish and Seaweed (Gracilaria sp.) in The Village of Muara Church, West Tegal Tegal]. In Suyono, N. Isdarmawan, and N. Zuhri (eds.). Proceeding of National Seminar on Development Strategy for Environmentally-Based Fisheries and Marine. [Pancasakti University, Tegal, Indonesia. 9 December 2011. 123– 46].

[57] Tacon 1987 Nutrition and Farmed Fish and Shrimp Training Manual Brazil: The Essential Nutrients Food and Agricultural Organization of the United Nations

[58] Watanabe 1988 Fish Nutrition and Marineculture. [Tokyo: Department of Aquatic Biosciences].

[59] Xie B, W Jiang and H Yang 2011 J. Bulgarian Journal of Agricultural Science 17(6): 851–858

[60] Yang Y and K Fitzsimmons 2002 Tilapia Shrimp Polyculture in Thailand Research Report. Thailand: Asian Institute of Technology

[61] Yasin M 2013 Scientific Journal Edition March Agriba 1: 86–99

[62] Yuvaraj D ,R Karthik and R Muthezhilan 2015 Asian Journal of Crop Science 7(3):219-232.

[63] Rachmwat D and I Samidjan 2017 Philippine J. of Science 146 (3):237-245

Acknowledgements
This work was supported by grants from Marine and Fisheries of Government. The authors are indebted to the Mr.T.Miftahuddin heads of POKDAN fish farmers Muara Rejeki in Pekalongan, who has lent his polyculture in pond culture to research.