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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 / m^2 fish. And white shrimp 5 larvae / m^2 and 10 larvae / m^2. 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 / m^2 larvae red tilapia larasati and given 5 larvae / m^2 white shrimp (A), 5 larvae / m^2 red tilapia) and 10 m^2 / m^2 of white shrimp (B), 10 m^2 larvae and 5 m^2 white shrimp (C), 10 m^2 larvae and 10 m^2 white shrimp (D)). The data were growth of absolute weight, survival rate, FCR, and water quality data (temperature, salinity, pH, O_2, NO_2, NH_3). 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. currently, 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

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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 simultaneous 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, bighead carp, macrophytophagic 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 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 sarb*a), 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.

Table 1. Proximate analysis of compositiont of Feed Composers Materials

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

Rice meal	12,43	9,25	10,97	18,94	13,62	34,79
Dextrin	10,60	0,20	0,59	0,00	0,10	88,51

Source: Laboratory of Animal Nutrition and Feed Science, Faculty of Animal Husbandry, Diponegoro University 2017

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
Vitamine& mineral	1.1
CMC	1.1
Total	100
Results of Proximate Analyses	
Protein (%)	35.01
Fat (%)	11.50
BETN (%)	33.72
Energy (kcal)	300.36
Ratio E/P(cal · g–1)	8.57

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 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 (kkal) 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/m^2 fish And vannamei shrimp at 5 tail/m^2 and 10 tail/m^2. An artificial feed with protein content of 35% enriched with protease at 22.5g / kg of feed was given at a dose of 3 % per biomass per day. The dose of papain used in this study were 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 / m^2 *larasati* red tilapia fish and given 5 tails / m^2 white shrimp (A), 5 tails / m^2 red tilapia Larvae) and 10 m^2 / m^2 of white shrimp (B), 10 m^2 larvae and 5 m^2 white shrimp (C), 10 m^2 larvae and 10 m^2 white shrimp (D)). The data obtained were data of growth of absolute weight, life, FCR, and water quality data (temperature, salinity, pH, O_2, NO_2, NH_3). Data were analyzed by various analysis (F test) and descriptive. The study was conducted in media maintenance of polyculture technology of ± 1200 m^2, with each research plot area of 100 m^2,

2.3. Absolute Growth

The absolute growth formula is calculated by the formula Steffens (1989)[13][53][55], with the following formula:

W (Absolute growth weight) = Weight of end test animal research – 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 / m^2 red tilapia *larasati* fish and given 5 larvae / m^2 white shrimp (A), 5 larvae / m^2 red tilapia *larasati* Larvae) and 10 m^2 / m^2 of vannamei shrimp (B), 10 m^2 larvae and 5 m^2 vannamei shrimp (C), 10 m2 larvae and 10 m^2 vannamei shrimp (D). The data obtained were weight growth, survival rate, FCR, and water quality data (temperature, salinity, pH, O_2, NO_2, and NH_3). Data were analyzed by analysis of variance (F test) and descriptive analysis. The study was conducted in ponds (± 1200 m^2) with polyculture technology , with each research plot area sized 100 m^2.

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 m^2 larvae of *larasati* red tilapia and 10 m^2 / m^2 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^{b}	176.19±4.46^{ab}	178.97±5.87^{ab}	185.75±0.50^{a}
2. Absolute growth of white shrimp (g)	21.72±0.54^{b}	21.90±0.53^{b}	23.29±0.94^{ab}	25.25±0.95^{a}
3. Survival rate of red tilapia of larasati (%)	75.48±0.47^{c}	88.55±1.24^{b}	91.49±1.66^{b}	97.25±0.33^{a}
4. Survival rate of white shrimp (%)	75.99±0.55^{c}	89.58±1.23^{b}	91.88±1.72^{b}	95.75±0.52^{a}
5. FCR of red tilapia larasati and white shrimp.	3.16±0.11^{a}	2.32±0.07^{b}	1.95±0.05^{c}	1.22±0.13^{d}

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^2 larvae of *larasati* red tilapia and 10 m^2 / m^2 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^2 for red tilapia of *Larasati* and 10 individuals per m^2 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^2 of white shrimp and 30 individuals per m^2 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 m^2 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 m^2 for red tilapia of *Larasati* and 10 individuals per m^2 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 m^2 and 30 individuals of milkfish per m^2 that had survival rate of 75±0.13 % . this shows the Effect of the difference in density of white shrimp *L. van*namei 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 m^2 of *Larasati* red tilapia of and 10 individuals per m^2 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±1.02^a %). 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±1.02^a %), 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 [5][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 red tilapia *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 ng 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 polyculuture 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 (^{0}C)	26.5 – 29,5	25,5 – 35 ^{0}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 m^2 larvae and 10 m^2 / m^2 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.

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