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Dosage Optimization of Artificial Digestive Enzymes in Feed to Improve The Digestibility And Growth of *Osphronemus gourami*

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Abstract

Rietje J.M. Bokau*), Dian Febriani, Nur Indariyanti, and Rakhmawati. 2018. DOSAGE Optimization Of Artificial Digestive Enzymes In Feed To Improve The Digestibility And Growth Of *Osphronemus gourami*. *Aquacultura Indonesiana, 19(2): 83-89*. One of the obstacles often encountered in the cultivation of gourami is a relatively slow rate of growth compared with other freshwater fishes. This slow growth is partly due to the incomplete and unbalanced nutritional content and the low ability of the fish to digest a certain type of feed materials. The ability to digest fish feed types depends on the quality and quantity of feed and enzymes present in the digestive tract. The enzymes released by the glands in the gut have the function to digest food elements. Among the enzymes involved in digestion are amylase, protease, lipase, cellulase, pectinase, and pullulanase. To improve digestion, the use of artificial enzymes in certain doses is combined with natural food such as papaya to optimize the dose of artificial enzymes. The use of papaya is already widely used as a food supplement, especially in the gourami grow-out culture. Aside from being natural food, papaya leaves can also serve as a natural source of digestive enzymes. The objective of this study was to determine the optimal dose of artificial enzymes added to feed which can improve the digestibility and growth of gourami. In this study, treatments of artificial feeding of gourami were used addition of artificial enzymes amounting to 1% (A), 2% (B), 3% (C), papaya leaves and pellets (D), and pellets alone (E). Results after two months rearing showed that the digestibility of feed supplemented with natural enzymes in papaya (papain) can be further improve optimized by the addition of artificial digestive enzymes in a dose of 3% of the weight of the feed, the fish of this treatments group showed that faster growth compared with other treatments.

***Keywords**:*Artificial digestive enzymes ; Digestibility ; Gourami ; Growth

Introduction

One of the major obstacles in the cultivation of gourami is its relatively slow growth in comparison with other freshwater fishes, which is partly due to the incomplete and unbalanced nutritional content and the low ability of fish to digest a particular type of feed material. Digestion of feed depends on the ability of the digestive system to break down the feed into small particles and the availability of digestive enzymes that function in the feed's metabolic processes. So that the food consumed by fish should have nutrients that are easily absorbed and absorbed and can be utilized optimally. Some research results show that feed utilization can be achieved by adding exogenous digestive enzymes in feed. (Kodidjah *et al*., 2015; Yildrim and Turan, 2010; Zamini *et al*., 2012).

The ability of fish to digest a type of feed depends on the quality and quantity of feed and the enzymes present in the digestive tract. The enzymes released through the glands in the gut serve as digesters of food elements (Handayani and Widodo, 2010). The enzymes involved in digestive processes are, among others, amylase, protease, lipase, cellulase, and pullulanase.

Some efforts that have been made to improve growth include the use of artificial digestive enzymes (Said, 2006; Prabarini *et al*., 2017; Susanto *et al*., 2017), which has shown a better result. However, the use of artificial enzymes has yet to solve the problematic nature of the cultivation of gourami, especially in terms of scarcity as well as costly enzymes, which farmers cannot afford. In order to overcome these problems, artificial enzymes are combined with natural feed enzyme source such as papaya

leaves, thus reducing the dose of artificial enzymes. Papaya leaves have been widely used as supplementary foodstuff especially for the enlargement of gourami fish. Besides serving as natural food, papaya leaves can serve as a natural source of digestive enzymes (Martantyo *et al*., 2013). Aravind *et al*. (2013) explained that proteolytic papain has the ability to break down proteins and change their portion into arginine. Nevertheless the growth of carp, especially in the enlargement process has not shown optimal results. Thus an assessment of the addition of artificial enzymes in feed is needed to help and speed up the digestive process. This research aims to determine the optimal dose of artificial digestive enzymes in feed for the enlargement of gourami, which can improve the digestibility of feed and its best growth.

Materials and Methods

This rearing of gourami and the feed preparation conducted in the Laboratory of Fish Cultivation. Proximate analysis of feed (pellet) was done in the Laboratory of Food Technology whereas measurement of growth, analysis of digestibility and analysis of water quality were done in the Laboratory of Fish Environment and Health in the Study Program of Fish Cultivation, the State Polytechnic of Lampung. The research was carried out for four months.

Artificial enzymes used are complex enzymes (Enzymplex) which contain amylase, protease, lipase, and several vitamins. Enzymes are destroyed first and weighed according to the needs of each feed supply.

The making of gourami feed

The feed-making started with the preparation of raw materials according to composition (fish flour, wheat flour, soybean flour). The coarse materials were grounded first before they were sifted. Then the materials were weighed before they were mixed together, including the addition of enzymes according to the prescribed doses (1, 2, and 3%), after which hot water was added sufficiently to make harden the mixture. Next was the formation of pellets using an instrument (pelletizer) with a small diameter. The pellets were then dried in the sun or in a dryer (oven). The feed had a protein level of 30% made according to the composition and the addition of artificial enzymes (Table 1).

Proximate Analysis

1	Water (%)	11.09	10.87	10.54	10.03	10.03
2	Ash (%)	13.47	13.56	13.21	13.11	13.11
3	Protein (%)	30.26	30.38	30.59	30.03	30.03
4	Fat (%)	11.27	11.36	11.43	11.26	11.26
5	Carbohydrat(%)	20.59	20.36	20.62	20.72	20.72
6	Coarse fibre (%)	7.38	7.23	7.21	7.43	7.43

Table 1. Composition of artificial feed for gourami

No.	Raw material	Composition of treatment feed (%)				
		A	B	C	D	E
1.	Fish flour	39.0	38.5	38.0	39.0	39.0
2.	Wheat flour	12.5	12.5	12.5	12.5	12.5
3.	Soybean flour	43.0	42.5	42.0	44.0	44.0
4.	Mineral	2.0	2.0	2.0	2.0	2.0
5.	Vitamin	2.0	2.0	2.0	2.0	2.0
6.	Fish oil	0.5	0.5	0.5	0.5	0.5
7.	Artificial enzyme (Enzymplex)	1.0	2.0	3.0	-	-
Total (%)		100.0	100.0	100.0	100.0	100.0

Research Design

The use of artificial enzymes in this study aims to supplement the enzymes naturally contained in papaya leaves. Martyanto *et al*. (2013) explained that the enzymes on papaya leaves are identical with thick white sap (proteolytic), where in 10 grams papaya leaves contain 0.53 grams of papain enzyme. The average of giving papaya leaves every day is 150-200 grams.

The design used in this research was the Completely Randomized Design with five treatments and three replicates. Treatments consisted of a combined dose of artificial digestive enzymes in the artificial feed and natural food as a source of digestive enzymes (papaya leaves). The combinations of treatment were as follows: Treatment A : Artificial feed with the addition of 1% enzymplex and fresh papaya leaves. Treatment B: Artificial feed with the addition of 2% enzymplex and fresh papaya leaves. Treatment C: Artificial feed with the addition of 3% enzymplex and fresh papaya leaves. Treatment D: Artificial feed without enzymplex and with the addition of papaya leaves. Treatment E: Artificial feed only.

Research Process

In this research, tarpaulin tanks were used as media for rearing gourami. As many as 15 tanks were randomly placed outdoors and filled with water up to 60 cm in height, and left for 2-3 days to allow plankton to grow. Selected fish were scattered into the tanks: 30 fish per tank proceeded by an acclimatization process. The fish went through a period of adaptation to the feeding pattern for approximately 7- days, and then they were weighted to obtain a preliminary data on their weight.

After the adaptation period, the fish were given treatment feed during the rearing for about 60 days. The main feeds given were pellets that had been formulated with artificial digestive enzymes at a protein level 30%.

The feed given per day was 5% of the body weight of fish. Besides artificial feed, natural feed was given, i.e. fresh papaya leaves (which have been widely used for gourami) to complement the artificial digestive enzymes in the feed (pellets). In order to maintain the water quality of the media, siphoning was done during rearing period to remove the left-over feed and dirt in the bottom of the tank and at the same time to replace the water. To determine growth rate, measurement was done every 10 days by measuring the length and the weight through sampling of 20 fishes.

After the rearing for 40 days, the rearing continued with providing feed that contained 0.6% Cr_2O_3 indicator. This treatment was done to the test the digestibility of feed. Collection of faeces was done after seven days of the period of adaptation to feed that contained Cr_2O_3. The faeces was collected by siphoning and then put into a filmy bottle and kept in an icebox. The faeces was dried and the Cr_2O_3 content was measured.

Testing Parameters

The parameters measured were the body-weight (g) and length (cm). Daily specific growth rate of fish [% = (Ln final weight – Ln initial weight) x t^{-1} x 100%], Feed Conversion Ratio [FCR = [estimated total dry feed intake (g) x wet weight $gain^{-1}$ (g)]; and total digestibility [% = 100 – (100 × % Cr_2O_3 in feed / Cr_2O_3 in faeces)]. All data were statistically analysed using one-way analysis of variance (ANOVA) and multiple comparisons among treatment means were made with Duncan's multiple comparison tests using the Statistical Analysis Software Program of SPSS version 21 for Windows. All probability values were considered statistically significant at the level of $P< 0.05$.

Results and Discussion

Based on the results of research during the 40 days of rearing, the analysis variance on the whole showed that addition of artificial enzymes in different doses in feed could significantly affect the weight growth, FCR and total digestibility of feed in gourami and did not significantly affect the growth of length and the daily growth rate of length and weight (Table 2).

The greatest growth of length in treatment C was 1.6167 ± 0.54, and in treatments B and A 1.6167 ± 0.54 and 1.2733 ± 0.21 respectively. The greatest growth of weight in treatment A was 16.9067 ± 1.46^{a} i.e. in a dose of enzyme of 10 grams/kg of feed, followed by treatment C (16.667 ± 0.83^{a}) and treatment B (14.7133 ± 1.74^{ab}) each in a dose of 30 and 20 grams/kg of feed. This result showed that addition of artificial in feed did not significantly affect the growth of length, whereas in the growth of

weight it significantly affected (Table 2 and Figure 1). Digestion of feed began with the oral intake and then the stomach and intestine to undergo the digestive process. Digestion is the process of breaking down the feed into micro molecules through a series of physical and chemical processes, thus enabling them to be absorbed through the intestinal wall into the capillaries. The ability to utilize the nutrients consumed by fish depends on the physiological and biochemical capacity of the digestive system (Susilo *et al*., 2015). The absorbed nutrients will play a role in the process of growth. Each type of fish needs nutrition with complete nutritional contents, i.e proteins, fats, carbohydrates, vitamins and minerals. As sources of energy, most of them are used for building and maintaining the tissues of the body and some as a source of energy for the fish activity. The amount of nutritional content of feed is very much affected by the measurement and size of fish, feed,the better the efficiency value of feed environmental condition feeding level, and reproductive stadia (Handayani and Widodo, 2010). Protein is the major element in its function to sustain the body tissues as well as a source of energy in the fish growth. In this research, artificial feeds were used at a protein level of 30% and a feeding level of 5% of the biomass and considered optimal for the requirement for the enlargement of gourami. The result of the daily growth rate (SGR) of the length was greatest in treatment C, i.e 0.3582 ± 0.13 followed by treatments B and A, i.e. 0.2812 ± 0.17 and 0.2657 ± 0.04 respectively. Likewise, the daily growth rate (SGR) of the weigth was greatest in treatment C, i.e 1.5275 ± 0.08 followed by treatments A and B, i.e 1.4562 ± 0.18 and 1.3893 ± 0.17 respectively. These results showed that dose addition of artificial enzymes in feed have a positive effect on the growth rate of gourami.

The Feed Conversion Ratio is the index of the total feed input for growth or the amount of grams required to produce one gram of the wet weight of fish. The lower the conversion value of feed, the better the efficiency value of feed (Prabarini et al., 2017). The result of analysis of digestive enzymes in feed significantly affected the feed conversion ratio in the enlargement of gourami. The feed in the treatments of artificial enzymes (A, B, and C) resulted in a significantly different conversion from treatments without artificial enzymes (D and E). Treatment B with dose addition of 20 grams/kg of feed resulted in the lowest conversion value compared with other treatments and likewise with treatments D and E (without enzymes) (Figure 2). This showed that in that dose the artificial digestive enzymes were able to enhance the efficiency of the utilization of feed with the enzymes found in papaya leaves as natural feed for gourami. Digestibility is a parameter that indicates how much of the consumed part can be absorbed by the body because in a digestive process there is always part of the food that is indigestible and discharged in the form of feces. The presence of enzymes in feed can help and speed up the digestive process so that nutrients are sufficiently available for the growth and survival of fish (Amalia et al., 2013).

With the benefit of modern technology, digestive enzymes prepared in one package called enzyme complex becomes available. Arafat et al. (2015) pointed out that the advantage of this enzyme is that it is covered with a substance that is protect the enzyms form damage during the pelleting process. The artificial enzymes used contain amylase, protease, and lipase that functions to break down carbohydrates, proteins, and fats, repectively, in the digestive process (Kiha *et al*., 2012). The enzyme complex is also complemented with pullunase, which is not found in an animal's digestive system. Said (2006) pointed out that addition of several doses of enzymplex in feed for gourami showed a significant result, but a dose of 2% caused the best growth.

The test result of total digestibility (Table 2) showed that addition of artificial enzymes in feed had significant effect on increasing total digestibility in a dose of 1-3% ($P < 0.05$), i.e. in range of 84.6300% - 87.096%. This value is still lower than the value of digestibility in other types of fish that are carnivorous. Gourami tends to be herbivorous and its ability to digest a particular kind of feed is low. Mudjiman (2004) stated that the digestibility of protein is generally very high and reaches 90%. Marzuqi and Anjusary (2013) even stated that in grouper fish the digestibility is 96%, Tilapia with prebiotic addition produces total digestibility of 86.77% and protein digestibility of 96.82% (Afzriansyah et al., 2014).

In this research, the gourami was given additional feed of papaya leaves once a day. Papaya leaves contain papain, which is a proteolytic enzyme. According to Martantyo et al. (2013) isolation of papain from papaya leaves

produces 200 MCU/g or 5.3%/100 gram materials. In addition, papain contains other enzy enzymes, i.e. chymoprotein and lyzozyme.

whereas feed D was without enzyme addition but with papaya leaves and E with only artificial feed. This result showed that artificial enzymes

Table 2. Data on length and weight growth, daily growth rate (%/day) (SGR), Feed Conversion Ratio (FCR) and Total Digestibility (%) of gourami during research.

Treatment	Parameters measured					
	Length (cm)	Weight (g)	SGR of length (%)	SGR of weight (%)	FCR	KT (%)
A	1.2733±0.21	16.9067±1.46^{a}	0.2657±0.04	1.4562±0.18	2.7200±0.74^{ab}	85.2967±0.59^{a}
B	1.2733±0.74	14.7133±1.74^{ab}	0.2812±0.17	1.3893±0.17	2.2400±0.08^{b}	86.8567±0.31^{b}
C	1.6167±0.54	16.6667±0.83^{a}	0.3582±0.13	1.5275±0.08	2.7833±0.27^{a}	87.0976±0.46^{b}
D	1.2533±0.59	13.4033±1.48^{bc}	0.2999±0.14	1.3031±0.22	3.5767±0.14^{c}	84.7033±0.71^{a}
E	0.6600±0.10	11.3667±1.78^{c}	0.1519±0.03	1.0896±0.20	4.0533±0.41^{c}	84.6300±0.45^{a}

Note: Different superscripts indicate significant differences (P < 0.05)

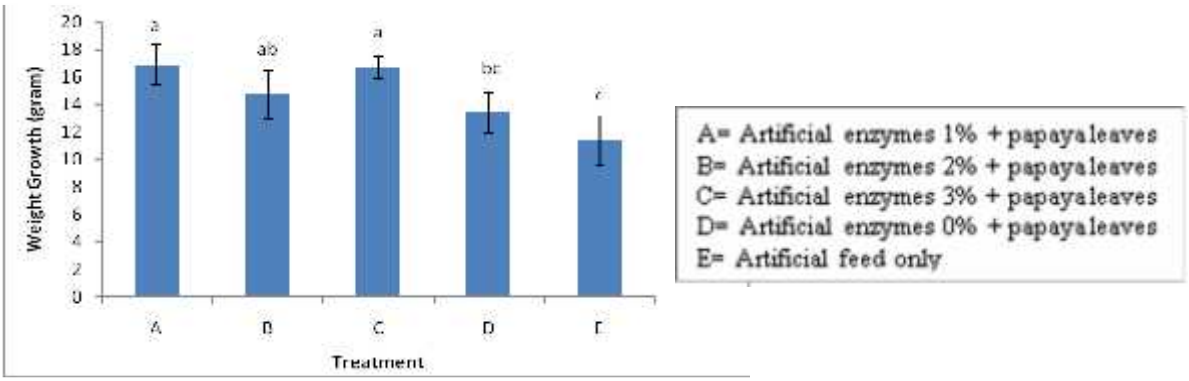

Figure 1. Weight growth of gourami in treatment with feed with the addition of artificial enzymes

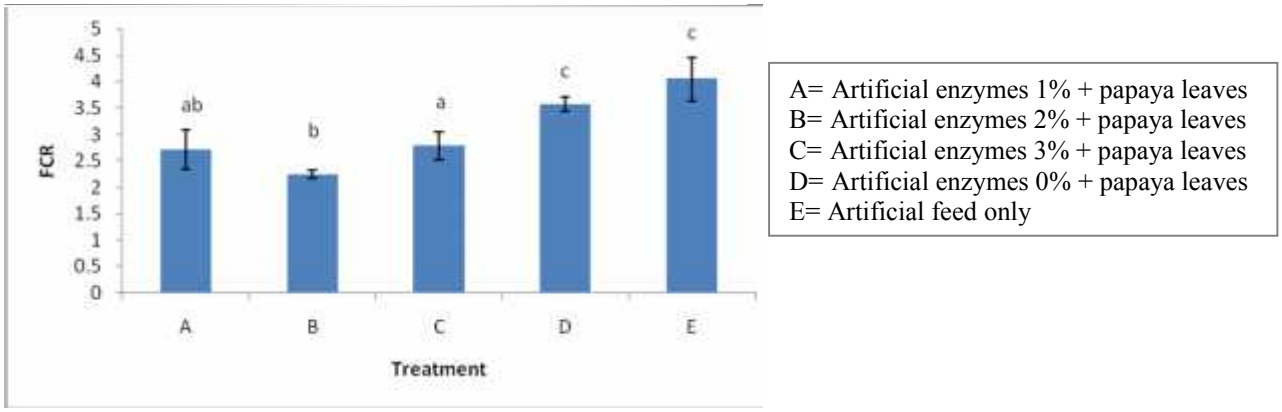

Figure 2. FCR value of gourami feed in treatment with feed with the addition of artificial enzymes

Therefore, gourami feed can be optimized with papaya leaves and supplemented with artificial digestive enzymes.

The highest value of total digestibility was obtained in treatment C (87.0976 ± 0.46b) in a dose of 3% enzyme/kg of feed compared with treatment A (1%/kg) and treatment B (2%/kg), and natural enzymes (papaya leaves) were able to increase digestibility, which was significantly different from treatments D and E (without artificial enzymes) (Figure 3): the higher the dice the higher the digestibility. The activity of enzymes in feed depends on the level of

acceptability and the enzymes properties. Besides, the activity of enzymes is also influenced by the concentration of enzymes and substrates, temperature, pH, and inhibitor. The feed in this research used the same protein level, i.e. 30%. Mudjiman (2004) further stated that the activities of amylase, lipase, and protease are extremely influenced by the composition of food. Feed digestibility is influenced by three factors, i.e. the presence of enzymes in the digestive tract, the activity level of digestive enzymes and the length of time during which the feed react with the digestive enzymes (Hepher, 1998). Each of the factors is influenced by secondary factors, associated with fish species, age, and size, environmental condition and the composition and amount of feed consumed. The value of feed digestibility can represent the ability of fish in digesting a particularfeed. So that increasing feed utilization through increasing digestibility can be functioned with the addition of enzymes (Mareta et al., 2017).

During rearing, management of water quality was carried out by siphoning every three days and total water exchange was done every five to seven days. The rapid growth of moss necessitated routine siphoning and replacement of water particularly in the media with treatment of artificial enzymes and papaya leaves. The parameters of water quality measured, i.e. temperature, pH, and DO showed that the condition of water in the rearing media was appropriate for the life and growth of gourami fingerlings in the process of enlargement (Table 3).

The digestive tract in fish, by its nature, already contains enzymes that function in the digestive processes, and their efficiency depends on the physiological capacity of fish to digest and change nutrition (Hassanatabar et al., 2013). In this research, the natural enzymes were supplemented with artificial enzymes from outside. The results of research showed that the added digestive enzymes could significantly affect the observed parameters. Generally, the higher the dose of artificial enzymes the higher the growth value, growth rate, total digestibility, and the lower the FCR value in which the feedbecame efficient. Accordingly, the optimal dose was in Treatment C (30 grams/kg of feed)

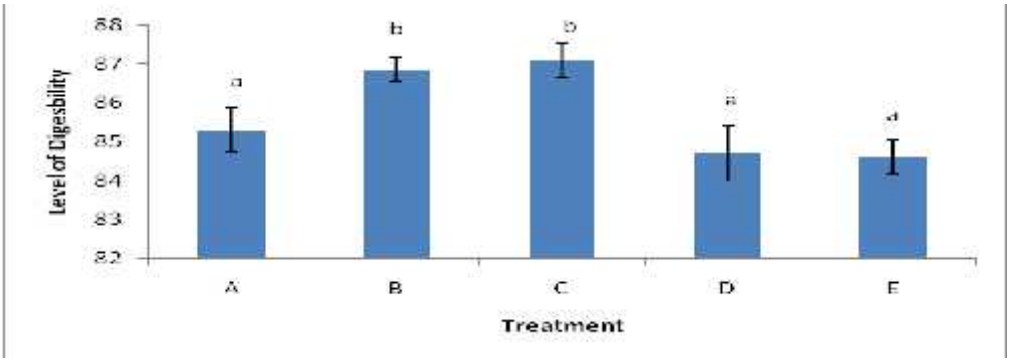

Figure 3. Level of total digestibility of feed in gourami in treatment with artificial feed and enzymes

Table 3. Results of Measurement of Water Quality Parameters During Gourami Rearing with

Treatment	Water Quality Parameters		
	Temperature (°C)	pH	DO (ppm)
A	27.0 – 29.0	6.9 – 7.7	5.0 – 6.7
B	28.5 – 29.0	7.2 - 7.7	5.0 – 6.4
C	27.5 – 29.0	7.2 - 7.8	6.2 - 8.1
D	26.5 – 29.0	7.1 - 7.8	5.5 - 7.9
E	26.0 – 29.0	6.8 - 7.8	6.3 - 7.8

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References

Afzriansyah, and Saifullah, A. N. Putra. 2014. Prebiotic application to increase feed digestibility of tilapia (*Oreochromis niloticus*). *J. Perikanan dan Kelautan*, Vol. 4 No 4: 235-242.

Amalia, R., Subandiyono, and E. Arini. 2013. The effect of papain on dietary protein utility and growth of african catfish (*Claria gariepinus*). *J. Aquaculture Management and Technology*, 2 (1):136-143.

Arafat, M.Y., N. Abdulgani and Rendro D.D. 2015. Effect of adding enzymes to fish feed on the growth of tilapia (*Oreochromis niloticus*). *Jur Sains and Seni*, ITS Vol 4 1: B21-B25.

Aravind, G., Debjit Bhaownik, Duraivel, S., and Harish, G. 2013. Tradisional and medicinal uses of carica papaya. *Jour. Of Medicinal Plants Studies,* Vol 1 issue 1: 7-15.

Handajani, H. and W. Widodo.2010. fish nutrition. UMM press, Malang

Hasan, O D S. 2000. The effect of the administration of papain enzymes in artificial feed on protein utilization and growth of seed gurame (*Osphronemus gourami*). (Tesis). Program of Pascasarjana Bogor of Agricultural Institute, Bogor, 71 pp.

Hassanatabar, F., H. Ouraji, A. Esmaeili and S.S. Babaei. 2013. Study on the activities of digestive enzymes, amylase and alkaline phosphate, in kutum larvae, *Rutilus frisiikutum* fed *Artemia nauplii*. *World J. of Fish and Marine Sciences*, 5 (3): 266-270.

Kiha, A F., W. Murningsih and Tristiarti. 2012. the effect of feed ripening with papaya leaf extract on fat digestibility and energy metabolism of broiler chickens. Animal Agricultural Journal, Vol. 1 No 1:265-276

Khodidjah, D., D. Rachmawati and Pinandoyo. 2015. Growth performance of sangkuriang seeds (*Clarias gariepinus*) through the addition of papain enzymes in artificial. *Journal of Aquaculture Management and Technology* 4 (2):35-43.

Mareta, R.E., Subandiyono, and S. Hastuti. 2017. Effect of papain and probiotics in artificial feed efficiency utilization level and growth rate of gourame (*Osphronemous gourami*). *Jurnal Sains Akuakultur Tropic* : 1 (2017) 1: 21-30.

Martantyo, D., P. Tamara, dan U. Arga. 2013. enzyme isolation from fruit and leaves extract of papaya. *Jur T Chemical Engineering,* Fak. Of Engineering, Univ Diponegoro.

Marzuqi, M. and Anjusary, D.N. 2013. Nutrient digestibility feed with different levels of protein and lipid on coral rock ggrouper (*Epinephelus coralicola*) Juvenile. *J. Ilmu dan Kelautan Tropis*, 5(2): 311-323.

Prabarini, D., E. Harpeni and Wardiyanto. 2017. Addition on enzyme composition in commercial feed to growth performance and survival rate of baung fish (*Mystus nemurus*) in tarpaulin pondsdi kolam terpal. *Jour. Sains Teknologi Akuakultur* 1 (2): 120-127.

Rachmawati, D. and S. Istiyanto. 2014. Addition of phytase artificial feed to increase digesting, specific growth and survival rate of nile tilapia fingerling (*Oreochromis niloticus*). *J. Saintek Perikanan*, 10(1): 48-55

Said, A. 2006. Studi of effect of the addition of different persentage ezympleks on feed ingredients on growth and survival of gourame seeds in the general water studi das musi. Proceeding of 3rd annual national seminar of fisheries and marine research, July 27th 2006: 332-339.

Susilo, U., E. Yuwono, F. N Rachmawati, S. Priyanto , and Hana.2015. Characteristics of the digestive enzyme protease and amylase of gurame (*Osphronemus gourami* Lac.). Biosfera 32 (2) Mei 2015: 137-142.

Yidirim, Y.B and F. Tutran. 2010. Effect exogeneus enzim supplementation in diet on growth and feed utilization in africa catfish (*Clarias gariepinus*). *Journal of Animal and Veterinery Advance* 9 (2): 327-331.

Zamini, A.A., H.G Kanani, A A Esmaeili, S. Ramesani and S. J Zoriezahra. 2012. Effects of two dietary exogeneous multi-enzym supplementation, natuzyme and beta-mannanase (Hemicell) on growth and blood parameters of caspian salmon (*Salmon trutta caspius*). Comp Clin Patrol, 23: 187-194.