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To cite this article: Diana Rachmawati *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **530** 012037

IOP Conf. Series: Earth and Environmental Science **530** (2020) 012037 doi:10.1088/1755-1315/530/1/012037

The effects of exogeneous papain enzyme in the feed on growth and blood profiles of Sangkuriang catfish (*Clarias* sp.) cultivated in the pond

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Abstract. The objectives of the study were to evaluate the effects of exogeneous papain enzyme supplemented feed on the growth and blood profiles of Sangkuriang catfish (*Clarias* sp). The intensive culture system of Sangkuriang catfish decreases water quality that hinders fish growth. The cultivation practices also develop diseases that further decreases the production, including through feeding that contain immunostimulant. The fish sample was Sangkuriang catfish with the average weight of 12.84 (±0.69) g per fish. The experiment used papain enzyme supplemented feed with 5 (five) treatments. Those treatments were A (0 g papain per kg feed), B (0.5 g papain per kg feed), C (1 g papain per kg feed), D (1.5 g papain per kg feed), E (2 g papain per kg feed). Parameters observed were diet usage efficiency (EFU), ratio of protein efficiency (PER), relative growth rate (RGR), survival rate (SR), blood profiles and water quality. The results show that papain enzyme supplementation in the feed significantly ($P<0.01$) affected on EFU, PER, and RGR; however, it did not significantly influence SR and blood profiles of Sangkuriang catfish. The optimal dose of the papain enzyme for EFU, PER and RGR were 1 g per kg feed. The water quality during study had been in feasible condition for Sangkuriang catfish cultivation.

1. Introduction

Sangkuriang catfish (*Clarias* sp.) is one of the freshwater fish which has good economic value. The catfish can be cultivated in high stock density. The catfish also has advantages, such as: high growth, resistance to environmental adversity, and being able to cultivate in low water quality [1].

Along with the development of super intensive catfish culture, the environment has decreased so that it hindered the fish growth due to the diseases. One of the efforts to increase the catfish growth and to prevent the diseases is by supplementing immunostimulant, vitamin and hormone [2]. Immunostimulant supplementation is to boost immune system in the fish by adding microbes, such as β-glucan and liposacharides or dead bacterium cell [3]. One of the constraints in using this

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immunostimulant is expensive; therefore, one needs to find an alternative which is cheap and easy to handle. The alternative is exogenous papain enzyme that is produced from extracted papaya leaves.

The need of papain enzyme depends on the type of fish and the weight [4]. Papain enzyme has proteolytic activities and antimicrobials, while alkaloid carpain acts as antibacterial [4]. Studies on the exogeneous papain enzyme supplemented feed have been done by some researchers. The addition of papain enzyme in the feed could hydrolyze protein into amino acids to support the fish growth [5]. Moreover, the papain in the feed makes the feed easy to digest, absorb, and increase the fish growth and immune system [6]. The papain enzyme supplementation at the dose of 0.1% could give the highest growth in *Macrobrachium rosenbergii* [4]. The dose of 27.5 mg per kg feed resulted in the highest growth of Kuereling fish [7]. According previous study [8], the dose of 10 g per kg feed made *Labeo rohita* grow the best, meanwhile the dose that generated the best growth was 6 g per kg feed in the Sangkuriang catfish [1]. *Cherax quadricarinatus* has the highest growth when it was given with the 0.3 % papain enzyme supplemented feed [9]. According previous study [10] also discovered that papain enzyme as immunostimulant with the dose of 30 mg per l could improve the immune respond in the *Litopenaeus vannamei*. However, the studies on the exogeneous papain enzyme supplemented feed in the Sangkuriang catfish as immunostimulant are still limited. Since the papain enzyme supplemented feed in the Sangkuriang catfish can increase the fish immune system, it makes the fish disease resistance and in turn it can increase production. The objectives of study were to evaluate the effects of exogeneous papain enzyme supplemented feed on growth and blood profiles in the fingerlings of Sangkuriang catfish (*Clarias* sp).

2. Methodology

2.1. Preparation of Sangkuriang Catfish Fingerlings

The amount of 40,000 Sangkuriang catfish that had average weight of 12.84±0.69 g per fish was used in the study. The sampled fingerlings were chosen based on the organ completeness, healthiness, size homogeneity, and swimming activity [11]. To adapt to the feed and the environment, the fingerlings of the Sangkuriang catfish was acclimated for one week and the fingerlings were let one day fasting to get rid of the previous metabolism. Papain enzymeused in the study was procured from the Brackishwater Aquaculture Development Center of Jepara, Central Java, Indonesia.

2.2. Preparation of Tested Feed

The artificial feed used in the study was a manufactured feed contains of 30% protein, 4% fat, 6% raw fiber, 14% ash and 11% water. The papain enzyme was diluted into 100 ml water for every kg feed [8]. By weighing the enzyme according to the treatments, those papain enzyme and the feed were mixed. The mixing was by spraying the diluted papain enzyme into the feed that was based on the previous study [12] method. The mixture of the enzyme and feed was dried at the room temperature for around 2 (two) hours [13]. Then it was labeled and placed in the storage until ready to use.

2.3. Blood Profile

The blood profiles indicated the level of immune system in the fish. The observed blood profiles of Sangkuriang catfish were glucose (mg per dl), hematokrit (%), erytrochit (x10^6sel per mm^3) and leucosit. The observation was based on previous study [14] method in which it was observed at the starting and at the end of the study.

2.4. Pond Preparation

The containers for cultivation were dirt ponds with the dimension of 6x10x1 m^3. There were 5 (five) ponds. Each pond was divided into 3 (three) sections for Sangkuriang catfish cultivation. The water came from the local spring water. The water used in the ponds was controlled.

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2.5. Methodology

The Completely Randomized Design was implememted in the experiments. There were 5 (five) treatment with 4 (four) replicates in every treatment. The treatments in the study were by supplementing immunostimulant papain enzyme into the feed. The treatments had various doses. Those doses were 0 g papain per kg feed (treatment A), 0.5 g papain per kg feed (treatment B), 1 g papain per kg feed (treatment C), 1.5 g papain per kg feed (treatment D), 2 g papain per kg feed (treatment E).

2.6. Research Procedure

The study was conducted at the Sangkuriang catfish farmers association, Tambak Sari Village, Rowosari District, Kendal Regency, Central Java, Indonesia on May to July 2019. The fingerlings were scaled to measure the initial weight. The fingerlings were stock into the dirt ponds with the stock density of 2.000 fingerlings in each section of the ponds. The feeding method used a fixed feeding rate with the feed amount of 5% per biomass weight per day. The fingerlings were fed 3 (three) times a day, i.e., in the morning, noon and afternoon. To measure the weight gain, the fingerlings were weighed every 7 (seven) days for 49 days.

The variables that were observed were efficiency of feed usage (EFU), ratio of protein efficiency (PER), relative growth rate (RGR) and survival rate (SR). The observation of those variables based on the [13] method, while blood profile observation based on the [14] method and water quality based on the [15] method. The equations of the observed variables were as follows:

$EFU = 100\,(W_2 - W_1)F^{-1}$

$RGR = 100\,(W_2 - W_1)(T x W_1)^{-1}$

$FCR = 100\,((FI\,(g))(WG)^{-1}\,(g))$

$PER = 100\,((WG\,(g))\,(PI)^{-1}\,(g))$

$SR = 100\,((FC)\,(IC)^{-1})$

W_1 is initial weight, W_2 is final weight, F is the amount of feed consumed and T is the number of days. FI is feed intake, WG is weight gain, PI is protein intake, FC is final count and IC is initial count.

2.7. Statistical Analysis

Analysis of Variance (ANOVA) and Duncan test were implemented to evaluate the variable [16]. To calculate the optimal dose, so SAS9 and Maple12 were utilized. Water quality in the study was compared to the references to determine the vibility.

3. Results and Discussion

Variable data from the study can be seen in the Table 1.

Table 1. The values of EFU, FCR, PER, RGR and SR of Sangkuriang Catfish.

Experiment Data	**Treatments (g papain per kg feed)**				
	A (0)	**B (0.5)**	**C (1)**	**D (1.5)**	**E (2)**
EFU (%)	48.38 (±0.53)^{e}	58.74 (±0.62)^{d}	75.86 (±0.83)^{a}	65.75 (±0.32)^{b}	60.18(±0.72)^{c}
PER	1.89 (±0.28)^{d}	2.57 (±0.39)^{d}	4.68 (±0.47)^{a}	3.58 (±0.28)^{b}	3.07 (±0.25)^{c}
RGR (% per day)	2.53 (±0.32)^{d}	3.24 (±0.53)^{c}	5.48 (±0.38)^{a}	4.98 (±0.25)^{b}	4.57 (±0.36)^{b}
SR (%)	88.33 (±3.65)^{a}	90.33 (±2.62)^{a}	92.33 (±3.24)^{a}	90.33 (±2.75)^{a}	90.33 (±2.68)^{a}

Note: Mean values with different superscript letter in the same row showed significant difference ($P < 0.05$)

The efficiency of feed utilization was one of indicators of feed quality [13]. The high efficiency of the feed pointed out that the feed was well utilized to grow by the fish. The supplementation of papain enzyme in the feed that yielded the best efficient feed utilization was at the treatment C with value of EFU 75.86 %, followed by the treatments D (65.75 %), E (60.18 %) and A (48.38 %). The increase of the EFU showed that the exogeneous papain enzyme supplemented feed could boost

IOP Conf. Series: Earth and Environmental Science **530** (2020) 012037 doi:10.1088/1755-1315/530/1/012037

digestibility, in turn the fish was able to absob the feed better. Proteolitic enzyme benefit on protein digestibility [4]. Proteolitic enzyme transformed protein into simple peptides and amino acids so that it was easily absorbed by the fish. The EFU values were considered in good condition since the values were higher than 50% [17]. They stated that the values of EFU were considered good if the values were greater thtn 50%.

As mentioned before that the best dose of papain enzyme to generate the best EFU (75.86%) was at the dose of 1 gram papain for every kg feed. It was suggested that the addition of papain enzyme in the feed could hydrolyze polypetide into amino acids so that it was easily digested by the Sangkuring catfish. Moreover, the energy obtained from the feed utilization could regenerate body tissue, fish activity and growth. The more the hydrolized nutrient causes the higher the feed utilization in the Sangkuriang catfish [9][18]. They found that papain protease enzyme was a key enzyme to hydrolyze protein into peptides; therefore, it could increase protein digestibility, absorption and growth. The lowest value of EFU (48.38%) was attained in the treatment A. It was suspected that the protein was not well absrobed by the fingerlings. The effects of the exogeneous papain enzyme supplemented feed that could increase the EFU values were also discovered in previous studies [1, 7, 18, 19].

The measurement of the optimal doses was obtained through Polynomial Orthogonal test. The polynomial orthogonal test produced a quadratic relationship between the exogeneous papain enzyme supplemented feed and EFU. The relationship was as in the equation of $Y = -16.883x^2 + 39.888x + 47.219$ with $R^2 = 0.85$ (Figure 1). The optimal dose of papain enzyme to yield the highest value of EFU (75.86 %) was 1 g per kg feed.

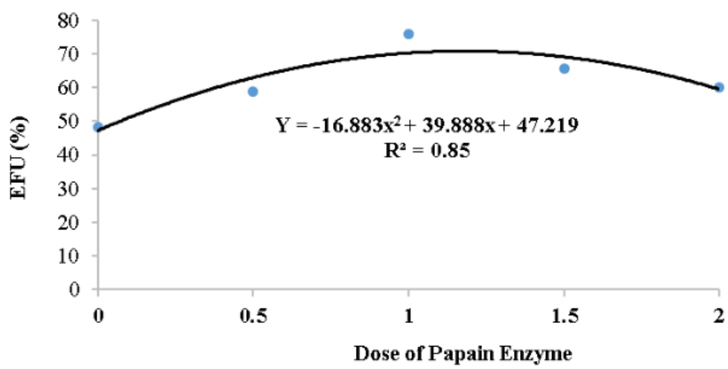

Figure 1. The Relationship between the Exogeneous Papain Enzyme Supplemented Feed and EFU in the Sangkuriang Catfish.

The ratio of protein efficiency was an indicator to measure the level of protein digestibility to provide essential amino acids [18]. The exogeneous papain enzyme supplemented feed was proven to improve ratio of protein efficiency of Sangkuriang catfish. The highest value of PER (4.68) was attained in the treatment C (1 g papain per kg feed). It was suggested that the dose of papain enzyme in the treatment C was the right amount to optimally hyrdrolyze protein to increase PER, in turn it increased the growth. It was supported by Patil and Singh [4]. They detected that the papain enzyme supplemented feed could increase protein digestibility compared to those without papain enzyme supplementation. It was due to increasing of protein content and protease enzyme from the feed. Papain enzyme is a type of protease enzyme that is a key factor to improve protein digestibilty and absorption; in turn it boosts the fish growth [8]. The papain enzyme supplemented feed could increase ratio of protein efficiency in the fish [9]. Papain enzyme could become biological catalyst that could improve digestibility of low quality feed; therefore, the cost of feed could be reduced. Papain enzyme could also reduce negative factor of phytate acid originated from plant based nutrient. The lowest value of PER (1.89) was obtained in the treatment A.

The low value of PER was because of the absent of papain enzyme in the feed that could help protein digestibility, especially plant based protein in turn not all energy from the feed can be absorbed and utilized. According to Mo et al. [20] the slow growth of the fish especially in which the fish was given the feed made from plant based protein was due to the existence of anti-nutrient that could hinder amino acids absorption, such as methionine. While Singh et al. [18] suggested that the exogeneous papain enzyme supplemented feed could effectively reduce anti-nutrient or mitigate the effect of phytate acid made of plant based nutrient. This element could reduce the growth of the fish. Furthermore [8] also reported that the exogeneous papain enzyme supplemented feed could raise PER. The addition of 10 gram of papain enzyme in the feed for *Labeo rohita* fish could result in the PER of 2.30%. The similar findings were reported by [9] who discovered that the dose of 0.3 % papain enzyme per kg feed generated 3.75 of PER in the *Cherax quadricarinatus* fish. Moreover, Singh et al. [18] identified that the dose of 2% papain enzyme for every kg feed could yield 2.24 of PER in the *Cyprinus carpio*.

The value of PER highly depends on the quality and the amount of the feed consumed by the fish. The higher the PER the better the protein to be efficiently utilized by the fish. As Hepher [21] reported that the higher the PER the better the feed quality to be efficiently utilized. The PER could influence growth, but the value of PER was influenced by the fish digestibility on the feed. One of the factors in the digestibility is feed composition in which the higher protein was utilized by the fish the more efficient protein was.

The papain enzyme optimal dose for PER of Sangkuriang catfish was calculated through Polynomial Orthogonal test. The Polynomial Orthogonal test for PER resulted in a quadratic equation as $Y = -1.5971x^2 + 3.8683x + 1.6854$ with $R^2 = 0.76$ (Figure 2). From the equation, the highest value of PER was 4.68 that was generated at the papain enzyme optimal dose of 1 g papain for every kg feed.

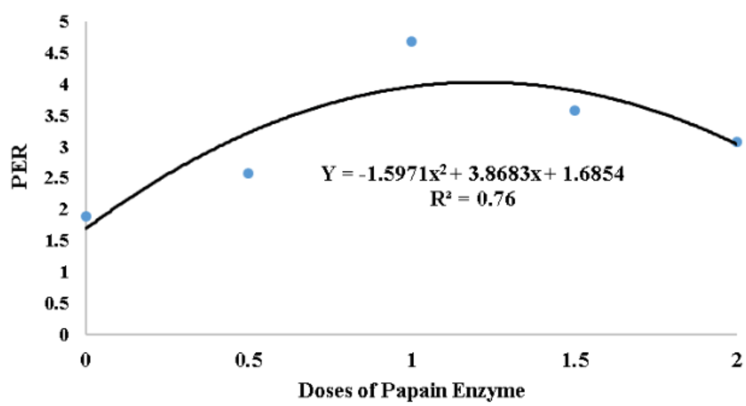

Figure 2. The Relationship between the Exogeneous Papain Enzyme Supplemented Feed and PER in the Sangkuriang Catfish.

The results of analysis variance showed that the papain enzyme fortified feed significantly ($P<0.05$) influence on RGP of Sangkuriang catfish. The highest value of RGP (5.48 % per day) was attained in the treatment C at the dose of 1 g papain per kg feed, followed by treatments D (4.98% per day), E (4.57 % per day) , B (3.24 % per day), and A (2.53 % per day) at the doses of 1g per kg feed, 2 g per kg feed, 0.5 g per kg feed, and 0 g per kg feed respectively. The treatment C that generated the highest RGR was due to having high EFU. It indicated that the value of RGR was directly proportional with the value of EFU; therefore, the higher the EFU the higher the RGR was. It was in accordance with the findings by previous study [21] who stated that the high value of EFU was related with the high growth. The high value of EFU showed that since just a little of nutrients was converted to energy, so it was mostly converted to grow. The addition of exogenous papain enzyme could increase nutrient absorption to boost fish growth [5]; however, the addition could only be to certain amount since the more papain enzyme was fortified in to the feed, as in the treatment C, D, and E, it caused the decrease in RGR. The decrease of the value of RGR was suspected due to excess of the supplementation of papain enzyme in the feed. The decrease of the value of RGR was due to the excess of protein hydrolysis; therefore, it affected synthesis regulation and tripsine secretion that negatively affected on growth and survival rate [22]. Moreover, Kazerani and Shahsavani [23] reported that the excess of papain enzyme could free monosaccharide too much and cause hyperglichemic hindering the growth. The addition of papain enzyme that could increase growth was also discovered by Dawood et al. [4] who studied in the *Macrobaicum rosenbrigri* fish. The dose of papain enzyme of 0.1% brought about the highest growth of *Macrobaicum rosenbrigri* fish as high as 1.41±0.03% per day. The enrichment of 10 g papain enzyme per kg feed generated the best growth in the fingerlings of *Labeo rahita* as high as 3.35% per day [8], meanwhile Rachmawati et al. [9] discovered that the addition of papain enzyme of 0.3 % per kg feed resulted in the highest growth in the *Cherax quadricarinatus*.

The results showed that the effects of the papain enzyme supplemented feed on RGR was ambiguous, it was due to the optimal dose of papain enzyme depending on the enzyme mechanism. The enzyme mechanism was indirectly explained by Khattak et al. [24] that the enzyme specifically catalyzed one substrate and worked only on one substrate. The statements indirectly mentioned that if there is no a substrate available, there is no enzyme activity. It happened at the treatment that has excess enzyme but the availability of substrate was limited. The excess addition of papain enzyme with limited substrate could not increase enzyme activity, because enzyme activity would stop when the subtract has exhausted. Furthermore, the increase of substrate could increase enzyme activity until reaching the maximal activity [25]. The condition indicated that substrate molecule attached to the enzyme molecule at the active side until all active sides used. This condition is in maximal state.

The optimal dose of papain enzyme for RGR of Sangkuriang catfish was obtained from Polynomial Orthogonal test. Polynomial Orthogonal test for RGR has a quadratic relationship i.e., $Y= -1.4229x^2 + 4.0097x + 2.2846$ with $R^2 = 0.86$ (Figure 2). The optimal dose of papain enzyme for RGR (5.48 % per day) was 1 g papain per kg feed.

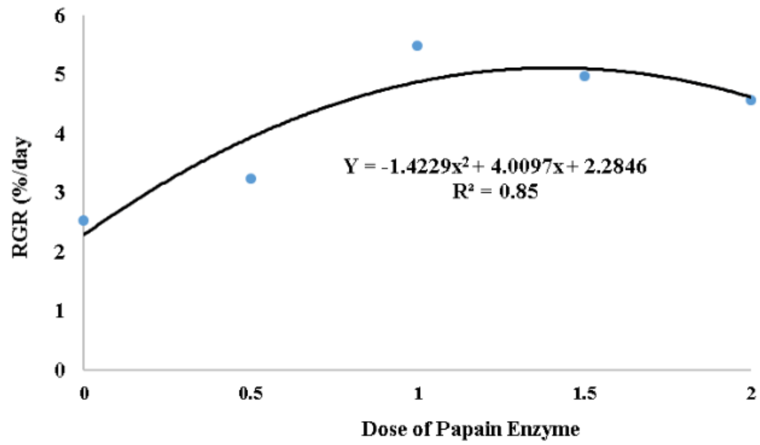

Figure 3. The Relationship between the Exogeneous Papain Enzyme Supplemented Feed and RGR in the Sangkuriang Catfish.

The results of analysis variance showed that the inclusion of papain enzyme insignificantly (P>0.05) affected on the RGR of Sangkuriang catfish. The highest RGR in the study was found at the treatment C (1 g papain per kg feed) with the RGR value of 92.33%, followed by D (1.5 g papain per kg feed), E (2 g papain per kg feed), B (0.5 g papain per kg feed) and A (0 g papain per kg feed) with the RGR values of 90.33%, 88.33% respectively. All treatments in the study resulted in the good RGR values that were higher than 85%. The RGR is considered good if the value is higher than 80% [26].

The blood profiles of Sangkuriang catfish were displayed in the Table 2.

Table 2. Glucose (mg per dl), Hematocrit (%), Erythrocytes (x10^6cells per mm^3) and Leukocytes (cells per mm^3) of Sangkuriang Catfish

Treatments (g papain per kg feed)	Glucose (mg per dl)	Hematocrit (%)	Erythrocytes (x10^6sel per mm^3)	Leukosyte (sel per mm^3)
A (0)	62.00(±2.35)^a	13.46(±0.14)^a	1.43(±0.46)^a	102.21(±4.54)^a
B (0.5)	60.15(±3.21)^a	14.32(±0.52)^a	1.60(±0.30)^a	101.35(±5.32)^a
C (1)	67.00(±2.24)^a	15.24(±0.94)^a	1.97(±0.86)^a	102.42(±4.63)^a
D (1.5)	60.23(±2.38)^a	13.67(±0.22)^a	1.50(±0.75)^a	102.54(±3.67)^a
E (2)	61.37(±2.37)^a	14.46(±0.26)^a	1.43(±0.23)^a	100.64(±5.63)^a

Notes: Mean values with different superscript letter in the same coloum showed significant difference ($P < 0.05$)

The results of analysis variance of the papain enzyme fortified feed insignificantly ($P > 0.05$) influenced on the glucose level of Sangkuriang catfish. Every treatment has different glucose levels. The glucose levels of treatments A, B, C, D, and E were 62.26 mg per dl, 60.15 mg per dl, 67.20 mg per dl, 62.23 mg per dl and 61.37 mg per dl respectively. The highest level of glucose was 67.20 mg per dl that was found in the treatment E, while the lowest level of glucose was 60.15 mg per dl that was obtained in the treatment E. The glucose levels in the study were considered normal as mentioned by Nasichah et al. [27] who informed that the normal level of glucose was 40-90 mg per dl. Moreover, Hartanti et al.[28] suggested that the normal level of glucose of the fish ranged from 41 mg per dl to 150 mg per dl. If the level of glucose was above the normal level, it indicated that the fish was under stress condition, as reported by Nasichah et al. [27] who suggested that in the fish under stress condition the receptor organ would accept information that would be conveyed to the brain, in hypothalamus, then chromatin cell would excrete catecholamine hormone. This hormone would suppress insulin secretion which helps to supple glucose into the cells; therefore, the level of glucose in the blood hiked.

The results of analysis variance of the the exogeneous papain enzyme supplemented feed insignificantly ($P > 0.05$) influenced on leukocytes level of Sangkuriang catfish. The level of leukocytes in the treatments was still in normal categories, as supported by Hartanti et al. [28]. They mentioned that the amount of leukocytes in the teleostei fish was between 20-150x10^3 cells per mm^3. Factors that affected the amount of leukocytes were environment condition and fish healthiness[29]. The increase of leukocytes in the fish was a sign of stress; otherwise the low of leukocytes was a sign of low immunity of the fish. These phenomena were also discovered by Moyle and Cech [30] who found that the level of leukocytes was affected by cortisol that suppress immune system.

The results of analysis variance of the papain enzyme supplemented feed insignificantly (P > 0,05) influenced on hematocrit and erythrocytes of Sangkuriang catfish. The levels of hematocrit in all the treatments in the study were in a normal range as suggested by previous studies [31] [32] who reported that the normal levels of hematocrit ranged from 20% to 30%. The levels of erythrocytes were also in a normal range as suggested by Roberts [33] who reported that erythrocytes in the teleostei fish was in normal range when the erythrocytes ranged from 1,05 x 10^6 cells per mm^3 to 3,0 x 10^6 cells per mm^3.

4. Conclusion

The addition of exogenous papain enzyme in the feed had significant (P<0,05) effects on EFU, PER, RGR, but it had insignificant effects on SR and blood profile of Sangkuriang catfish. The optimal dose of the papain enzyme for EFU, PER and RGR was 1 gram for every kg feed.

Acknowledgement

The authors want to thank to the Mr. Imam Santosa as chairman of the Sangkuriang Catfish Farmers Association in Tambak Sari Village, Rowosari District, Kendal Regency, Central Java, Indonesia, which has provided a place for applied research.

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