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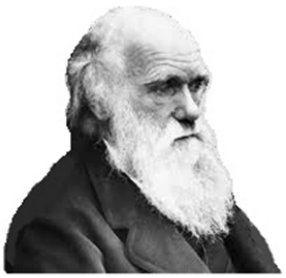

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Effect of Probiotic Addition in Commercial Feed to the Growth and Survival Rate of Sangkuriang Catfishes (*Clarias gariepinus* (Burchell, 1822))

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ABSTRACT

This study was conducted to determine the effect of probiotic addition in a commercial feed to the growth and survival rate of Sangkuriang catfish (*Clarias gariepinus*). This research was conducted at the Ciparanje Experimental Pond Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jatinangor, West Java. The method used in this research was Completely Randomized Design with four treatments and four replications. The treatment was the variances of probiotic addition which consisted of control (without probiotics), probiotic addition at as much as 0.5 grams / kg of feed, 1 gram / kg of feed and 1.5 grams / kg of feed. The parameters observed were specific growth rate (SGR), absolute biomass, survival rate (SR), and feed conversion ratio (FCR). Data were analyzed using Variant Analysis, at 95% confidence level, and continued with Duncan's Multiple Range Test. The results showed that the increase in the daily growth rate of Sangkuriang catfish was straight-line with the addition of probiotics. Furthermore, commercial probiotics with a dose of 1 gram / kg of feed resulted in a specific growth rate of 4.22%, absolute weight of 11 grams, survival rate (SR) of 97.5%, and FCR of 0.89.

***Keywords*:** probiotic, Sangkuriang catfish, feed conversion ratio, specific growth rate, survival rate, *Clarias gariepinus*

1. INTRODUCTION

Sangkuriang catfish is one of the superior varieties of African catfish. Sangkuriang catfish is a crossing between female dumbo catfish (F2) with male dumbo catfish (F6). Genetic

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improvement through cross-breeding is the result of breeding from Balai Besar Pengembangan Budidaya Air Tawar (BBPBAT) Sukabumi. Catfish cultivation techniques that are easy, inexpensive, and favored by the society, make catfish become one of the most cultivated fish (**Figure 1**).

Figure 1. Sangkuriang Catfish (*Clarias gariepinus* (Burchell, 1822))

According Elumalai *et al*. (2013), live microorganisms in fish farming can prevent disease, thereby increasing production and reducing the loss of economists. Furthermore, Fuller (1987) is a product composed of microbial culture or microscopic natural feed that is beneficial and has an impact on improving the microbial balance of the host's intestinal tract. Probiotics can be used through feed fish and water, the use of probiotics has a big efect on the growth of fish because of the nature of probiotics that can convert complex compounds into simple compounds, thus making it easier for fish to digest feed. The use of probiotics is an internal solution to produce optimal growth of fish, feed fish efficiency, and reduce production costs (Iribarren *et al*. 2012).

According Wirdani *et al*. (2012), probiotics work by controlling the development and microbial population harmful so as to produce an optimal growing environment for beneficial microbes, until finally these microbes will dominate and make habitat more suitable for the growth of living things in the environment. Probiotics are useful for regulating the microbial environment in fish intestines and blocking intestinal pathogenic microbes and can improve feed efficiency (Dhingra, 1993; De Schryver *et al*., 2008; Faizullah *et al*., 2015). Furthermore, Irianto *et al*. (2003) state that the use of *Bacillus* sp. is able to improve water quality through balancing the microbial population and reducing the number of pathogens and simultaneously reducing use chemical compounds and increase the growth of aquatic animals. The addition of probiotic bacteria in fish feed can improve nutrient absorption by digestion of fish because of the enzymatic activity of probiotic bacteria and their presence is also able to stimulate the host (test animals) to produce more enzymes. Kennedy *et al*. (1998) say, the *Bacillus* sp. is able to improve the quality and survival of *Centropomus undecimalis*. *Bacillus* sp. is able to increase feed absorption through increased concentration of proteases in the digestive tract, improve growth and reduce the number of potentially pathogenic bacteria in the intestine.

Gatesoupe (2008) states that probiotics are microbial cells given in a certain way to enter into the gastrointestinal tract which has a beneficial effect on the host animals which consume it through balancing the microbial flora of the intestine and with the aim of improving health.

The probiotic bacteria used in this study are bacteria commonly used in fish feed including *Bacillus* sp., *Lactobacillus* sp. and Nitrosomonas. *Bacillus licheniformis* makes the protein digestibility of fish better (Mao *et al*., 1992). Probiotic bacteria produce enzymes that can break down complex compounds into simpler compounds that make digestion easier for fish. One of the good probiotics for digestion is *Bacillus* sp. Ecoenzim, which is secreted by the bacteria not only to meet the energy needs but also to provide benefits for fish by increasing the activity of digestive enzymes, so that it can increase feed protein and reduce coarse fiber (Schlegel and Schmidth, 1985).

According to Holt *et al*. (2000), *Lactobacillus* sp. used in aquaculture plays a role in suppressing the growth of pathogenic bacteria. Microorganisms in probiotics compete with pathogens in the channel digestion to prevent pathogens from taking the nutrients needed to live fish (Cruz *et al*., 2012). In an attempt to improve the feed fish nutrition, bacteria found in probiotics have a mechanism to produce several enzymes for digestion of fish feed, such as amylase, protase, lipase, and cellulase (Wang *et al*., 2008). Research on probiotics has been carried out to increase the aquaculture production as a food supplement, increase disease resistance, and increase growth performance (Nayak 2010). The use of powder probiotics and effective doses in feed to increase the quality of Sangkuriang catfish seeds are not widely known. Therefore, it is necessary to conduct study on the addition of probiotics in fish feed for the growth and survival rate of Sangkuriang catfish seeds.

2. MATERIALS AND METHOD

The maintenance of Sangkuriang catfishes has been carried out at the Ciparanje Experimental Pond Laboratory hatchery, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Indonesia. The time for the study began in July - August 2018. The time spent was 30 days during the study.

The tool used in the study is a glass aquarium measuring 55 cm × 30 cm × 40 cm as many as 16 pieces, as a maintenance container for test animals with a volume of 30 L / aquarium, aerator, aeration stone, aeration hose, to supply oxygen to the maintenance container – as many as 16 pieces. Aquila Q6 brand blowers were used with 4.5 watts power to regulate the air pressure that will be flowed into the maintenance container, with 1-inch paralon pipe, as a medium for channeling air to the aerator hose. Next, Heater Rising Heat 9000-100 W brand with a power of 100 watts to regulate and stabilize the temperature; Lutron brand pH meter with PH-201 type to measure water pH in maintenance media; DO Hanna brand type HI 9146 to measure Dissolved Oxygen (DO); thermometer to measure the temperature of the maintenance medium; analytical scales of accuracy of 0.001 grams, for weighing probiotics, Scout pro-digital scales with accuracy of 0.01 grams, to weigh fish feed and test animals; Tea spoon, to take probiotic fish feed; Label paper to provide information on maintenance containers; Millimeter blocks with 1mm accuracy to measure the length of the Sangkuriang catfishes; Scoop Net to take Sangkuriang catfishes; Digital cameras, as a tool to document every activity on research results were used in the study. The size of 5-7 cm catfishes seeds of 400 were obtained from the Cijengkol Catfishes Seed Center, the food used was the commercial pellet type obtained from the fish feed store, the probiotics used were commercial probiotics in powder form obtained from online stores, sand molasses as a trigger for probiotics. The research method was carried out experimentally using a Completely Randomized Design (CRD)

method. This study consisted of four treatments with four replications, namely the addition of probiotics to dry preparations in feed namely, control (K): without the provision of probiotics, treatment A: The amount of probiotics as much as 0.5 grams / kg of feed, treatment B: The amount of probiotics as much as 1 gram / kg of feed, treatment C: The amount of probiotics of 1.5 grams / kg of feed.

2. 1. Culture Preparation

Aquaria, hoses, and aeration stones were cleaned with detergent, then washed again with fresh water, installation of aeration equipment in each aquarium, 30 L of water in each aquarium, treatment was randomly placed and marked according to its layout.

2. 2. Research Implementation

The study was carried out for 30 days, observations carried out every 10 days. Calculation of the growth in length and weight for sampling, then enter the Sangkuriang catfishes into each aquarium; Fish feed three times a day at 08.00, 12.00, 16.00 WIB with a feeding rate of 5% of body weight; Siphon the remaining food and waste from metabolism in the aquarium every day in the morning before feeding, by sucking the water at the bottom of the aquarium as much as ± 50% of the water in the aquarium using a hose and measuring the weight of Sangkuriang catfishes every 10 days. Sample was taken from 5 catfishes from each aquarium and weighed to determine the growth rate of Sangkuriang catfishes. Weighing was carried out in wet conditions, namely by entering Sangkuriang catfishes into a water-sided container so that its weight is known. Previously, the scales that had been filled with water were first tared so that the value of the scales would be 0. Doing water quality measurements which included DO, temperature, and pH meters were carried out every 7 days before chiffon was done and if there were fish that died.

The parameters observed, namely the daily growth rate; growth could be seen from the addition of fish weight from each treatment that was weighed during the study. According to Effendie (1997), to calculate the daily growth rate of fish, the formula is used:

$$G = \frac{(\ln Wt - \ln Wo)}{t} \times 100\%$$

where:

G = Specific Growth Rate (%)
Wt = The average daily weight of fish at the end of the study (g)
Wo = Average daily weight of fish at the beginning of the study (g)
t = Observation time (days).

Absolute biomass growth, calculation of absolute biomass growth, using the Effendie (1997) formula:

$$W = Wt - Wo$$

where:

W = absolute growth (gram)

Wt = biomass weight at the end of the study (gram)
Wo = biomass weight at the beginning of the study (gram).

Feed conversion ratio, calculation of feed conversion ratio or FCR (Feed Convertion Ratio), is the amount of weight of fish feed given to form a unit in the fish, so that the measured included the weight of fish feed given during the maintenance process and the weight gain of fish from the beginning of the spread of fries to harvest. The FCR calculation, according to Effendi (1997) is as follows:

$$\text{FCR} = \frac{F}{(Wt+D)-Wo} \times 100$$

where:

FCR = Feed conversion ratio
Wt = weight of fish biomass at the end of observation (g)
Wo = Weight of fish biomass at the beginning of observation (g)
D = Weight of fish that died during the study (g)
F = Food weight given during the study (g).

The supporting test parameters in this study are water qualities which include temperature, DO, and pH meter.

2. 3. Data analysis

Analysis of the data used, namely analysis of variance (ANOVA) with F test at 95% confidence interval, was used to determine whether the treatment had an effect on growth, survival and feeding efficiency. If the treatment has a significant effect, then further testing is done with Duncan's multiple range test (Gasperz, 1995), then the results of the data obtained from the results of the study are compared with the data in the catfishes case study to find out whether the results have a significant effect or not.

3. RESULTS AND DISCUSSION

3. 1. Specific Growth Rate

Growth rate is one of the important parameters to be considered in cultivation activities. The success and effectiveness of breeding time in the cultivation business is obtained by looking at growth period of the fish. Several factors to achieve a success of high growth rate are the management of fishes breeding time, both when giving food and control routine of the breeding, also maintaining the water to avoid all kinds of diseases and parasites that can interfere fish cultivation media, which can cause the growth rate decrease, or not good and fish becomes sick or die. Based on the results of daily growth rate data, which were analyzed by using statistical rules, the daily growth rate of Sangkuriang catfishes for 30 days is obtained (see **Table 1**).

Table 1 shows the growth rate of the Sangkuriang catfishes, in the 1.5 grams treatment is the highest growth rate with a value reaching 4.31%/day, followed by 1 gram and 0.5 gram treatment, which have the value of 4.22%, and 3.76%. The lowest treatment of 0.5 gram only reaches 3.76% of value, due to the energy contained in the first feed, it was used to suffice the

needs for body maintenance energy and if there is a residual of new energy used to meet its growth needs.

Table 1. Specific Growth Rate Sangkuriang Catfishes

Handling	Specific Grotwh Rate (%/Day)
Addition of probiotics 0 grams (K)	$3,57 \pm 0.30^{a}$
The addition of probiotics 0.5 grams (A)	$3,76 \pm 0,35^{ab}$
The addition of probiotics 1 grams (B)	$4,22 \pm 0,43^{bc}$
The addition of probiotics 1.5 grams (C)	$4,31 \pm 0.28^{c}$

* Description: The average value followed by letters is not significantly different according to Duncan's Multiple Range Test at 95% confidence level.

The results showed that 0.5 gram, 1 gram, and 1.5 grams treatments had higher growth rates compared to their controls. The high value of the growth rate in this treatment is due to the presence of *Bacillus* sp. contained in probiotics with the right concentration and dose, so as to increase the number of red blood cells and blood hemoglobin levels. This is believed to be one indicator of increasing the ability of fish in supplying nutrients throughout the body and tissue repair, so as to increase the fish growth (Rajikkanmu *et al.*, 2015).

According to Gatesoupe (1999), the activity of bacteria in the digestion will change with fatigue. If there are microbes entering through food or water, they can cause changes in the balance of bacteria that already exist in the intestine (digestive tract) with incoming bacteria. When a balance between the bacteria of the digestive tract of fish causes probiotic bacteria to be antagonistic to pathogenic bacteria, it can make the digestive tract of fish better at digesting and absorbing food extracts. Determination of the right probiotic dose in the fish feed can make bacteria in the intestine to digest food better so that the growth rate of fish becomes good and supported by nutrients in fish feed that matches the needs of fish so that it synchronizes probiotics with fish feed.

Macey and Coney (2005) which stated that supplementation of feed with probiotic bacteria increases digestion and absorption of proteins in the digestive tract due to increased activity of the protease enzyme in the intestine, so the provision of probiotics on feed becomes more effective and makes fish growth more maximal.

The number of bacterial colonies will affect the increasing number of enzymes' activities by probiotic bacteria in the digestive tract of fish which can increase the nutrient digestibility of fish feed. Good digestibility will optimize the utilization of food consumed, so that it is expected to increase the growth of Sangkuriang catfishes.

3. 2. Absolute Weight

Absolute biomass growth is a change or weight gain of fish that is maintained in units of time. Based on the results of the processing of biomass growth data on average by using

statistical rules, the absolute biomass growth data obtained from Sangkuriang catfishes in **Table 2** are as follows:

Table 2. Absolute Weight Catfishes Sangkuriang

Treatment	Absolute Weight (grams)
Addition of probiotics 0 grams (K)	$8,3 \pm 1,30^{a}$
The addition of probiotics 0.5 grams (A)	$9,6 \pm 0,79^{ab}$
The addition of probiotics 1 grams (B)	$11,0 \pm 1,62^{b}$
The addition of probiotics 1.5 grams (C)	$10,7 \pm 1,20^{b}$

* Description: The average value followed by letters is not significantly different according to Duncan's Multiple Range Test at 95% confidence level.

Table 2 shows the growth value of the absolute biomass of Sangkuriang catfishes with the biggest value in 1 gram treatment is 11 grams, followed by 1.5 grams and 0.5 treatments, respectively, with 10.7 and 9.6 grams. The lowest value was found in control, with 8.3 grams to produce a value that is significantly different.

Growth is a process of increasing the volume and weight of an organism, which can be seen from changes in the length and weight in units of time. According to Effendie (1997), growth is the addition of length or weight in units of time that are influenced by several factors, such as heredity, sex, age, parasite, fish feed, and water conditions. The growth of Sangkuriang catfishes occur due to the supply of energy contained in fish feed, meanwhile the energy exceeding the energy is needed to utilized for growth.

Zonneveld *et al*. (1991) stated that the growth occurs because of the excess energy derived from fish feed after being reduced by the metabolic energy and energy contained in feces. Long growth and absolute biomass in the treatment given by this commercial probiotic showed the role of probiotics in the growth of Sangkuriang catfishes. The role given is due to the contribution of enzymes for digestion which makes the fish digest food better. The nutrients absorbed by the digestive tract of fish will be better, resulting more and the growth will increase.

The 1-gram treatment has the highest absolute addition value compared to other treatments with a weight value of 11 grams. This is due to the fish feed added with probiotics with optimum doses that can provide maximum results in a culture. It is also presumably because the number of bacteria that enter the digestive tract of fish and live in it increases in line with the dose of probiotics given. Furthermore, the bacteria in the digestive tract of fish are secrete digestive enzymes, such as protase and amylase (Irianto and Austin 2003). In addition, Gatesoupe (1999) stated that the amount of this secreted enzyme increases according to the amount of probiotic doses given, in turn, the amount of digested fish feed also increases. Digestion enhancement means the higher the available nutrients to be absorbed by the body, so that the body's protein and growth increase. The increase in growth in aquatic animals given probiotic feed can be associated with an increase in digestive activity by enzymatic activity and vitamin synthesis so as to increase the value of digestibility and increase in weight (Liu 2009).

Probiotics *Lactobacillus* sp. which enters the body of the fish through fish feed has the metabolic ability to convert carbohydrates (glucose) to produce lactic acid, then lactic acid can create a lower pH atmosphere. *Lactobacillus* sp. can increase acidity by 1.5 to 2.0% on substrates (Sarles *et al*., 1956). In acidic conditions, *Lactobacillus* sp. has the ability to inhibit pathogenic bacteria and spoilage bacteria (Delgado *et al*., 2001). In addition, it can increase the secretion of proteolytic enzymes that are used to reform proteins into amino-acids so that they can be absorbed more quickly by the intestine.

3. 3. Survival Rate

Based on the results of processing, the survival data by using statistical rules, the SR data of Sangkuriang catfishes obtained are in **Table 3** as follows:

Table 3. Survival Rate Catfisheses Sangkuriang.

Treatment	Survival Rate (%)
Addition of probiotics 0 grams (K)	93.8 ± 4.8^{a}
The addition of probiotics 0.5 grams (A)	95.0 ± 5.8^{a}
The addition of probiotics 1 grams (B)	97.5 ± 2.9^{a}
The addition of probiotics 1.5 grams (C)	90.0 ± 7.1^{a}

* Description: The average value followed by letters is not significantly different according to Duncan's Multiple Range Test at 95% confidence level.

Survival rate in catfishes is a comparison between the number of organisms at the beginning of the study and at the end of the study, which is expressed in percent form to determine the tolerance of fish in survival. The results showed that there were no significance differences in each treatment. The highest percentage of survival reaches 97.5% with 1-gram treatment, and followed by 0.5-gram treatment with 93.8% of value. Meanwhile, 1.5-gram treatment appears to be the lowest with the percentage of 90%. However, the results of ANOVA test showed that probiotic administration against the survival of fish showed an unreal effect.

The occurrence of death in each treatment is suspected to be the uneven distribution of fish feed and triggers the growth of large fish becoming larger. Meanwhile, the smaller ones remain with the small size and nature of catfishes that belong to the type of piscivor fish or eat everything that is greedy and have cannibal properties, who want to eat smaller types of catfishes. According to Widigdo (2013), survival rate is categorized as good if the SR> 70%, for the medium category SR 50-60%, and in the low category SR <50%. This indicates that the survival rate of Sangkuriang catfishes in each treatment is still good.

3. 4. Feed Convetion Ratio

Fish growth is very much determined by the nutrition of food eaten by fish. The more perfect and complete nutrients in the fish feed and the easy digestion of fishfeed will have an effect on fish feed conversion. According to Effendi (2004), the Feed Convertion Ratio is a

measure that states the ratio of the amount of fish feed needed to produce 1 kg of cultivated fish. If the FCR = 1 means that to produce 1 kg of fish meat in the aquaculture system it takes 1 kg of fish feed. Feed Conversion Ratio, or called the FCR (Feed Conversion Ratio) catfishes seeds are used to determine the level of fish feed efficiency in each fish feed treatment. Fish feed which has the lowest FCR value is the best treatment that shows a high fish feed efficiency.

Based on the results of processing fish feed conversion ratio data by using statistical rules, the data of feed conversion ratio (FCR) of Sangkuriang catfishes obtained are in **Table 4** as follows:

Table 4. Feed Convertion Rasio Sangkuriang Catfishes

Treatment	FCR
Addition of probiotics 0 grams (K)	1,01 ± 0,13[a]
The addition of probiotics 0.5 grams (A)	0.95 ± 0,05[a]
The addition of probiotics 1 grams (B)	0,89 ± 0,17[a]
The addition of probiotics 1.5 grams (C)	0,76 ± 0,09[a]

* Description: The average value followed by letters is not significantly different according to Duncan's Multiple Range Test at 95% confidence level.

The results of the fish feed conversion rate study (Table 4), which address the control treatment, have the highest fish feed conversion value of 1.00, followed by treatment of 0.5 gram and 1 gram, respectively, with 0.95 and 0.89. Meanwhile, the lowest value is found in the treatment 1.5 gram which has a value of 0.76. The high FCR value on the control is thought to be due to the high crude fiber content that makes Sangkuriang catfishes difficult to digest. Provision of probiotics containing *Lactobacillus* sp. on 0.5-gram, 1-gram, and 1.5- gram treatment can reduce the ratio of fish feed conversion compared to control because the presence of *Lactobacillus* sp. levels of crude fiber in fish feed can be reduced.

Based on the results of ANOVA fish feed conversion test, it can be seen that there is no significant difference between the treatments. Although not significantly different, the value produced by the treatment of 0.5 gram, 1 gram and 1.5 grams is lower than the control value. It can be concluded that *Lactobacillus* sp. found in bacteria works well enough to reduce the conversion value of the feed ratio. Barrows and Hardy (2001) explained that the value of the feed conversion ratio is influenced by dietary protein, and fish feed protein that matches the nutritional needs of fish results in more efficient feeding. It is also influenced by the amount of fish feed given, with less and less fish feed given more efficient feeding. Furthermore, probiotics are also capable of acting as immunostimulants, increasing feed conversion ratios, inhibiting the growth of pathogenic bacteria, producing antibiotics, and improving water quality (Watson *et al*., 2008). Feed conversion ratio shows the value of fish feed that can be utilized by turning it into weight gain in fish. Fish feed efficiency can be seen from several factors where one of them is FCR. The best-feed conversion ratio will be achieved at the lowest feed conversion calculation value, where in the treatment conditions the quality of feed is better than

the other treatments. The condition of good fish feed quality causes the energy obtained in catfishes to be more used for growth.

4. CONCLUSIONS

Factors that influence the high and low efficiency of fish feed are the types of nutrient sources and the number of each component of the nutrient source in the fish feed. The amount and quality of fish feed given affects fish growth. Verschuere *et al*. (2000) states that the treatment of probiotics results better in feed conversion ratio than control. It is because the addition of probiotics in fish feed can improve fish feed utilization more efficiently than controls. Provision of probiotics in fish feed is expected to affect the speed of fish feed fermentation in the digestive tract that will be a great help for the process of absorption of food in the digestion of fish.

Reference

[1] Cruz, P.M., A.L. Ibanez, O.A.M Hermosillo, and H.C.R. Saad. 2012. Use of Probiotic in Aquaculture. ISRN Microbiology, doi: 10. 5402/2012/1916845

[2] Faizullah, M., Rajagopalsamy, C.B.T., Ahilan. B., and Francis, T. 2015. Impact of bofloc technology on the growth of Goldfish young ones. *Indian Journal of Science and Technology*, Vol 8 (13).

[3] Fuller, R. 1987. A review, Probiotics in Man and Animals. *Journal of Applied Bacteriology*. 66: 365-37.

[4] Gatesoupe, F.J. 1999. The Use of Probiotics in Aquaculture. *Aquaculture* 180 (2-3), 147-165.

[5] Gatesoupe, F J. 2008. Updating the Importance of Lactic Acid Bacteria in Fish farming: natural occurrence and probiotic treatments. *J. Mol. Microbiol. Biotechnol*. 14(1–3): 107–114.

[6] Irianto, A., P.A.W. Robertson, and B. Austin, 2003. Oral administration of formalin-inactivated cells of *Aeromonas hydrophila* A3-51 controls infection by atypical A. salmonicida in goldfish, *Carassius auratus* (L.). *Journal of Fish Diseases*, 26: 117–120.

[7] Iribarren, D., P. Daga, M.T. Moreira, and G. Feijoo. 2012. Potential Environmental Effects of Probiotics Used in Aquaculture. *Aquacult. Int*. 20: 779-789.

[8] Kennedy, S.B., Jr. Tucker., J.W. Neidic., L. Carole., G.K. Cooper., J.L. Jarrell, and D.G. Sennett. 1998. Bacterial management strategies for stock enhancement of warmwater marine fish: A case study with common snook (*Centropomus undecimalis*). *Bulletin of Marine Science*, 62: 573-588.

[9] Liu C.H., Chiu C.S., Ho P.L., and dan Wang S.W. 2009. Improvement in the growth performance of white shrimp, Litopenaeus vannamei, by a protease-producing probiotic, Bacillus subtilisE20, from natto. *Journal of Applied Microbiology* 107: 1031–1041.

[10] Macey, B.M., and dan V.E. Coyne. 2005. Improved Growth Rate andDisease Resistance of Farmed Haliotis Midae Through Probiotic Treatment. *Journal Aquaculture* 245: 249-261.

[11] Mao, W., R. Pan, and D. Freedman. 1992. High Production of Alkaline Protease by *Bacillus licheniformis* in a FedBatch Fermentation Using a Syntetic Medium. *J. of Industrial Microbiology*, 11: 1-6.

[12] Moriaty D.J.W. 1998. Control of luminous Vibrio species in penaeid aquaculture pond. *Aquaculture*, 164: 351-358.

[13] Nayak S.K. 2010. Probiotics and Immunity: A Fish Perspective. Review. *Fish and a Shellfish Immunologi* 29: 2-14.

[14] Rajikkannu M., Natarajan N., Santhanam P., Deivasigamani B., Ilamathi J., and Janani S. 2015. Effect of probiotics on the haematological parameters of Indian major carp (Labeo rohita). *International Journal of Fisheries and Aquatic Studies*, 2(5): 105-109.

[15] Rosenfeld, W.D. and Zobell, C.E. 1947. Antibiotic production by marine microorganisms. *Journal of Bacteriology* 54: 393- 398.

[16] Sarles, William Bowen., William Carrol Frazier, and Joe Bransford Wilson. 1956. Microbiology: General and Applied, second edition. Harper and Brothers, New York.

[17] Verschuere L., G. Rombaut, P. Sorgeloos, and W. Verstraete. 2000. Probiotic Bacteria as Biological Control Agents in Aquaqulture. *Microbiology and Molecular Reviews* 64, 4: 655-671.

[18] Wang, Y.B. Li, J.R., and Lin, J. 2008. Probiotics in Aquaculture: Challenges and Outlook. *Journal Aquaculture* 281: 1-4.

[19] Watson A.K., Kaspar H., Lategan M.J., and dan Gibson L. 2008. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture* 274: 1–14.

[20] Widarni, Ekasari, J., and Maryam, S. 2012. Evaluation of biofloc technology application on water quality and production performance of Red tilapia *Oreochromis sp*. cultured at different stocking densities. *Hayati Journal of Biosciences*. Vol. 19, No. 2, pp. 73-80.