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Environmentally Friendly Catfish Cultivation Engineering: Probiotic Use in Feed With Addition of Spices

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***Abstract*—The objective of the study was to examine production performance with probiotic use in a feed with an addition of spices as an environmentally friendly catfish cultivation engineering by measuring growth based on looking at SGR (Specific Growth Rate), FCR (Feed Conversion Ratio), RP (Protein Retention) and SR (Survival Rate). The research design used was Completely Randomize Design with 4 treatments: without probiotics (0%), probiotic 5%, 10% and 15% of feed and 3 replications. Each 300 catfish seeds (total length 7-9 cm) were stocked in 12 in 200 lit. Probiotics were administered via commercial feed with 33% protein content for 8 weeks at doses of 0.5%, 10%, 15% of total feed. Feed is given in adlibitum as much as 5% of body weight with the frequency twice a day. Analyze the data using ANOVA, followed by Duncan test. The results showed that probiotic in feed and aquaculture had the significant effect on SGR, FCR and SR (P<0,05). Giving 10% probiotic yield SGR 3.3867 ± 0.24440%, FCR 1.5733 ± 0.0987, PR 38.949 ± 0.500 and highest SR equal to 81.4567 ± 1.9985%. Water temperature 26.5-28.7 °C, pH 6.5-7.4 and DO 3.3-5.9 mg / ml.**

Keywords—probiotics; SGR; FCR; PR; SR; spices; environmentally friendly; catfish cultivation

I. INTRODUCTION

At this time the development of fish cultivation, especially catfish intensively increased followed by feeding in large quantities and operational costs for feed can reach 70% of the total cost [1]. Feed is indispensable in the cultivation of catfish because it provides the nutrients needed for maintenance, growth, and reproduction. Catfish brood stock gives high-quality feed, especially containing animal and vegetable protein also contains lipids to produce good eggs and fry [2-4]. Fry will express his performance if supported by the provision of good feed, especially protein [5] and maintained with good management will also produce rapid growth [6][7].

The impact of intensive catfish cultivation is the residual feed and the residual metabolism results in the form of ammonia-nitrogen accumulated in aquaculture waters [8][9] so that the quality of aquaculture waters decreases [10-12]. In order for the feed can give maximum effect and produce bigger fish biomass weight also reduce feed cost and can improve the quality of aquaculture water, hence need to look for a solution by doing engineering of feed and aquaculture engineering.

One effort to improve the efficiency of feed, growth and environmental quality in fish farming by means of probiotic utilization. The use of probiotics has been widespread and widely used by fish farmers, especially for enlarging catfish. Probiotics are one of engineering in aquaculture management to improve the quality of nutrients in a fish feed [13]. Provision of probiotics in the feed proved to decrease feed costs [14]. At present the utilization of probiotics in fish farming, especially catfish is more popular because it can increase the growth, feed efficiency, improve the quality of aquaculture [15-19] and affecting fish survival [20] [21].

This study uses a commercial liquid probiotic that is Effective Microorganism 4 (EM4) contains bacterium Lactobacillus sp., Acetobacter, and Yeast. This propagation of probiotics is added spices such as red ginger, white turmeric and *Curcuma xanthorriza* containing bioactive such as gingerol, curcumin compounds, essential oils and oleoresin which play a role as antimicrobials can increase immunity for organisms such as microbes and for fish and increase fish appetite [22-24]. The study used probiotics from EM4 which were reproduced themselves in this special medium so that catfish farmers easily make it and the cost is relatively cheap, it can also make it in large quantities when doing catfish cultivation in an environmentally friendly production cycle.

Provision of probiotics in feed and in the waters of cultivation of environmentally friendly catfish can improve environmental quality such as temperature, pH and DO (demand oxygen), ammonia-nitrogen. This is confirmed by the susceptibility of fish culture to the accumulation of organic materials with high nitrogen compounds such as ammonia, nitrite, and nitrate can cause high mortality [19]. The use of probiotics in fish farming can improve the quality of the environment [15][25].

Based on the descriptions of the above background, this study was designed to evaluate the effects of probiotics in feed and environment on the medium with the addition of spices as a commercially available environmentally friendly catfish culture technique containing Lactobacillus sp. Acetobacter and Yeast at several inclusion levels on production performance by measuring feed intake, feed efficiency by looking at FCR and RP, growth by looking at SGR, SR and water quality.

II. METHODS

This experimental study used a Completely Randomized Design consisting of 4 levels of probiotic volume of 0, 5, 10 and 15% of the total feed given mixed in the feed for fermentation feed. The main equipment used in this research is drum plastic capacity of 100 lit for fermentor, drum plastic of 200 lt capacity as much as 12 for catfish farming, aerator, nets large and small, digital analytical scale. The material used is size 7-9 catfish seeds as much as 3600 seeds. Feed seeds with 33% protein content, and EM4 liquid as commercial probiotic EM4 liquid containing bacteria Lactobacillus sp., Acetobacter, and Saccharomyces sp. grown in medium consisting of red ginger 5 kg, white turmeric 5 kg, *Curcuma xanthorriza* 5 kg, brown sugar 5 kg, fresh cow's milk 5 lt, molasses 5 lt, 2 lt rice bran and 3 kg pineapple.

Propagation of probiotics. The study does multiplication probiotic using a formula consist of spices: Red ginger, white turmeric and *Curcuma xanthorriza* washed and thinly sliced. Further mashed using a blender. Red sugar sliced thinly. Spices and brown sugar and then added as much as 10 lt of water in the heat until the temperature reaches 100 °C. Pineapple peeled, sliced and mashed using blenders. Red sugar sliced thinly. Pineapple peeled, sliced and mashed using blenders. Fresh milk, drops, rice bran and pineapple given about 10 lt of water is heated until the temperature reaches 60 °C. These materials in hot conditions are included in the 100 lt capacity drum plastic. Furthermore, boiled water to reach 100 lt of volume. After 1 × 24 hours are given starter EM4 (probiotics) as much as 2 lt and drum plastic closed and fermented for one month. Furthermore, probiotic inclusion into plastic bottles with a capacity of one liter and ready for use.

Preparation of fish culture medium and adaptation. Plastic water pool fill (60 cm) high, then given chlorine 100 gr chlorine for sterilization and left for one week. Check whether there is a plastic pool leak. After sterilization for one week just put probiotics into 12 glasses of water of catfish cultivation as much as 100 ml per plastic barrel and left for one week to give microbe chance of probiotics to grow. Next, put the catfish seeds as much as 300 heads per plastic barrel and fed the factory without being given probiotics

Treatment of test fish. Two days before the adaptation was completed the preparation of fermentation feed with probiotics 0, 5, 10 and 15% of the total feed given from the total biomass for each treatment. One day before the implementation of this research the seeds are fastened. Feeding at week 0, 1 and 2 for each treatment is given as much as 8% of the total weight of the biomass. One day before the implementation of this research the seeds areis fastened. Feeding at week 0, 1 and 2 for each treatment is given as much as 8% of the total weight of the biomass. For the 3rd week until the 6th week, the feed is given 5% of the total weight of its biomass given twice daily which is around 08.00-08.30 and the afternoon at 17.00-1.30. Parameters tested.

The observed parameters were amount of feed consumption, protein retention (PR), FCR, SGR, SR, water quality and how to measure it as follows. Protein retention was calculated using the method as follows [26]:

$$\text{PR}=\frac{\text{increased body protein}}{\text{protein consumed}}\times 100 \quad (1)$$

The Increment of fish body protein was calculated by multiplying the dry weight of fish body end of the study with body protein content end of the study, minus the initial dry body weight of the research multiplied initial protein content of the study. The consumed protein is calculated by multiplying the consumed feed with the protein content of the feed.

Feed Conversion Ratio (FCR) by calculating the amount of the feed given during the study and catfish biomass at the end of the study. The feed efficiency or FCR is calculated as follows [27]:

$$FCR = \frac{W_t - W_0}{F} \quad (2)$$

with:
W_t = Final body weight
W_0 = Initial body weight

To calculate Specific Growth Rate (SGR) by weighing the body at the beginning and end of the study can be calculated using the following formula [28]:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\% \quad (3)$$

Survival rate (SR) of the test fish was determined by counting the live fish at the end of the study and compared with the number of fishes at the start of maintenance, calculate using the formula [29]**:**

$$SR = \frac{N_t}{N_0} \times 100\% \quad (4)$$

with:
N_t = Number of total fish
N_0 = Number of dead fish

Aquaculture quality of catfish can be known by using a set of water quality digitally: water temperature and pH with pH meter and DO use DO meter. Data analysis. Research data in the form of SGR, FCR, PR, and SR were analyzed by ANOVA, if the results were significantly followed by Duncan's Multiple Rank Test.

III. RESULT AND DISSCUSION

The results of this study showed that probiotics in feed and waters on the growth of catfish seeds seen from the specific growth rate (SGR), Protein Retention (PR), Feed Ratio Conversion (FCR) and Survival Rate (SR) for 8 weeks can be seen in Table I the following.

TABLE I. PROBIOTICS IN FEED AND WATERS ON THE GROWTH OF CATFISH SEEDS

Concentrations Probiotics (%)	SGR±SD (%)	FCR±SD	PR±SD	SR±SD (%)
0	2.5133 ± 0.35907[a]	3.3267 ± 0.0841[a]	30.571 ± 0.700[a]	61.9300 ± 0.6080[a]
5	3.2600 ± 0.36428[b]	2,4000 ± 0.3372[b]	35.734 ± 0.370[b]	71.5900 ± 0.6029[b]
10	3.3867 ± 0.24440[b]	1.5733 ± 0.0987[c]	38.949 ± 0.500[c]	81.4567 ± 1.9985[d]
15	3.3667 ± 0.06110[b]	2.0200 ± 0.0954[b]	36.512 ± 0.380[b]	75.5900 ± 7.4864[c]

Different letter notations in the same column show no significant effect

[a.] SGR: Somatic Growth Rate

[b.] FCR: Feed Conversion Rate

[c.] SR: Survival Rate

[d.] PR: Protein Retention

Somatic Growth Rate (SGR) of catfish seeds observed every 7 days for 8 weeks after being treated showed that the highest SGR value was obtained from 10% probiotic concentration with an average SGR of 3.3867 ± 0.24440%, and the lowest was obtained without probiotics (control) of 2.5133 ± 0.35907%. Provision of 10% probiotics in the feed is optimal treatment in response to SGR (Table I). Pursuant to result of analysis of variance indicates that giving of probiotic volume at commercial feed influence significant to the growth rate (SGR) of catfish seed (P <0.05). Feeding the volume of 10% of probiotics in the diet gave the highest response to SGR, followed by 15%, 5%, and 0% volumes.

Influence of probiotic contain EM 4 is heterotrophic bacteria with different volume through feed and water to SGR of seed catfish which maintained for 8 weeks showed the significant difference (P <0.05). This suggests that during the maintenance of catfish capable of utilizing fermented feed to grow. The growth of catfish is seen from the increase in body weight and SGR during 8 weeks of maintenance. Growth is a complex biological process, it can occur when there is excess energy coming from the consumed feed. Quantification for growth can be body weight or body nutrient content such as: protein, fat, carbohydrate derived from the feed is used for fish metabolism activities. Quantification of growth depends on the quality of feed consumed by catfish.

Treatment that gives high level SGR effect is 10% probiotic in feed with SGR value 3,3867 ± 0, 24440%. Probiotics are propagated in a medium containing spice, molasses, rice bran, red sugar, pineapple, and milk. These probiotics already contain the necessary nutrients for life and the growth and development of microbes in this EM4. In addition, the nutrient content in the feed also supports the growth and development of microbes in this EM4. This is due to the provision of probiotics in the fermentation feed will provide a growing substrate for microbes in EM4 as a source of energy for microbial metabolism in the process of fermentation of feed.

The presence of probiotic bacteria metabolism process on the feed before it is given to fish causes an increase in the number of probiotic bacteria so it is suspected to cause more acidic atmosphere because probiotic bacteria is a lactic acid bacterium. Increased acidity and increase the number of probiotic bacteria that produce protease enzymes that are able to hydrolyze feed proteins can accelerate protein denaturation so easily absorbed and stored in the digestive tract of fish.

The media to grow this EM4 probiotic contains a considerable source of carbon derived from molasses, red sugars, rice bran as a medium of micro floc growth (from high probiotics will decompose nutrients in feed and nutrients in waters to be converted by proteases and amylases into proteins, and simple sugars used for metabolic activity and growth and development of catfish. This suggests that micro floc growth can provide essential nutrients to improve growth performance. Supported by the opinion of [30] states that micro floc growth can provide essential nutrients to increase growth activity. Probiotics works by recycling nutrients by maintaining the C/N ratio so as to stimulate heterotrophic bacteria that convert ammonia into microbial biomass thereby enabling proteins to be eaten twice (in feed and microbe) by aquaculture fish [31].

A. Feed Conversion Ratio

Feed Conversion Ratio (FCR) is the ratio of the amount of feed needed to produce 1 kg of fish meat, the conversion value of catfish feed on probiotic treatment is lower than the control. Efficiency of feed use shows the value of feed that can be utilized by fish by converting it to the fish body weight gain. Feed efficiency can be seen from several factors where one of them is FCR. FCR results of fermented feed with probiotics in catfish fish for 8 weeks showed that giving probiotics gave a significant effect (P <0.05). Based on Table 1 it was found that probiotic gave the best result of FCR with other treatments with FCR value of 1,5733 ± 0,0987 for 10% probiotic in feed followed by 15% probiotic with FCR 2.0200 ± 0.0954 and 5% with FCR of 2,4000 ± 0,3372 and for control of FCR 3,3267 ± 0,0841. From the results of this study there is a higher tendency of giving probiotics in the feed resulting in higher FCR values and subsequent decline. It is suspected that the higher probiotic treatment has a negative impact on the performance of catfish. This agrees with [32], states that the provision of probiotics has a threshold in order to obtain high feed efficiency. The addition of probiotics beyond the threshold (> 2 g.kg-1) may result in a negative impact on fish performance. Furthermore, FCR values of 1.5 to 2.0 are considered good for the growth of almost all organisms in the raring phase [27].

The best feed efficiency rate will be achieved at the lowest feed conversion value. In this treatment the feed quality condition is better than the other treatment. Good feed quality conditions result in the energy obtained in catfish more used for growth, so fish with a small feed is expected to obtain rapid growth rate. Factors that affect the high feed efficiency is the type of nutrient source and the amount of each nutrient source component in the feed. The amount and quality of feed given to fish have an effect on fish growth. The higher the value of feed efficiency then the response of the fish to the feed the better showed with rapid growth of fish.

The use of probiotics in fermented catfish seed feed and in aquaculture waters is causing the availability of feed in addition to the given feed is the presence of probiotic that can be used as a source of feed in situ. The value of FCR feed catfish test maintained for eight weeks is presented in Table I.

The use of probiotics containing heterotrophic microbes in EM4 with different doses significantly affected FCR ($P < 0.05$). This is thought to be influenced by feed fermentation and nutrient probiotic content. Fermentation can cause more digestible feed, and can increase the nutritional value of feed and the rate of nutrient absorption, so that the use of feed by the body more efficient. The treatment of probiotics resulted in better feed conversion ratio than control, since the addition of probiotics in the feed could improve feed utilization more efficiently than controls [19].

Provision of probiotics containing EM4 microbes in the feed and waters has the lowest FCR compared to other treatments, meaning that the low amount of feed can increase the weight of fish. A a significant reduction in fish feed use by 20% decreases the total production cost of micro floc growth ponds, the dynamics of biological, chemical and physical interactions allowing microbial communities in ponds [31]. This is as a result of the work of heterotrophic bacteria in EM4 that can increase the protein content of feed and feed utilization. Probiotics are living microbial agents capable of providing benefits to the host by modifying microbial communities or associating with host, improving nutritional value and feed utilization [19].

Probiotics are on the market a lot and used by fish farmers, especially catfish. But probiotics used in this study is different from the probiotics on the market that contain lots of bioactive derived from spices. Multiplying the EM4 Probiotics to be used in this study by adding "bio-spices" which contains lots of bioactive and fruits rich in vitamin C and rich in bromelain enzymes such as pineapple also added rice bran, molasses and brown sugar as a source of energy. Derived from vegetable as well as the addition of milk as a protein source as an appropriate medium for growth and microbial propagation added to the feed. Thus, this probiotic has a better ability to degrade macromolecules from fermented feeds such as proteins, carbohydrates and fats into simpler components in order to be absorbed by the fish's small intestine.

B. Protein Retention

The contribution of feed proteins consumed by catfish can reflect the magnitude of the body's increased protein value referred to as protein retention (PR) [33]. This study showed that the highest PR score resulted from giving 10% probiotic level in feed and aquaculture of 38.949 ± 0.500 and followed by 15% probiotic level equal to 36.512 ± 0.38; 5% probiotic level of 35.734 ± 0.370 and probiotic 0% level of 30.571 ± 0.700 ($P < 0.05$) (Table I). Increased PR on treatment results due to the addition of probiotic microbes proved able to help decipher the feed ingredients. Provision of probiotics as much as 10% through the feed is the best.

PR can reflect the magnitude of the body's additional protein from the consumed feed protein. Protein from feed consumed by fish is used for maintenance, metabolic activity and growth [34]. Microbes contained in EM4 are a single source of cell protein that can help the process of absorption of nutrients in the intestine feed and in aquaculture waters because it can describe the source of carbohydrates and proteins used for metabolic activity, then can be used to reproduce themselves. Thus, these proteins and carbohydrates can be used by fish for their activities such as: maintenance, protein synthesis stored in the body, growth and development. The bacteria in this probiotic is a single cell protein source so that the self-propagation of bacteria can increase feed proteins and decrease crude fiber [35]. Expected bacteria is release enzyme saccharolytic and pectinolytic which results in digest crude fiber so that decrease crude fiber in feed [36].

The results of research conducted suggests that PR is a parameter to indicate the magnitude of the contribution of protein consumed in the diet to the increase in body protein [33]. PR is a factor that needs to be considered to see the contribution of proteins consumed in the feed to the fish body weight gain [26]. The PRvalue also shows the quality of protein in the feed, the higher the PR value the better the feed [37]. The relationship between the PR and the specific growth rate of catfish. Feeding rations feed 10% of probiotic microbes produced the best specific growth rate.

The growth of fish depends on the quality of feed given so it can be seen from the daily weight gain. Protein is the main energy source for fish so that high protein in feed can affect fish growth. The energy produced by fish is used for its activities such as for basal metabolism, growth and development of fish. Probiotic in aquaculture is an effort to integrate the technique of zooplankton and microflora formation as a source of feed for fish and improve its environment [38].

The high PR on the treatment of 10% probiotics in the diet caused by increased protein and carbohydrate content by probiotic microbial activity was added. In addition, the role of probiotics is proven to help the use of energy in the process of digestion of feed ingredients. The impact of giving microbes in probiotics as much as 10% provides energy savings for the process of digestion (metabolism) which further result in the best PR in the body. PR values with probiotic addition tend to be higher than control treatment. This suggests that the protein from the feed with the addition of probiotics is more dominant to be absorbed and stored in the body compared with feed without the addition of probiotics. The results showed that the addition of probiotics 10% can increase the activity of enzyme such protease enzyme from test fish.

Protein that has been ingested some of which is stored in the body and those directly used as a source of energy for growth. Thus, the feed that has been given probiotics proved that the fish has been able to digest the protein which is then stored in the body in the form of retention value.

C. Survival Rate

Survival Rate (SR) catfish seeds observed every 7 days for 8 weeks were treated showed that the SR fluctuated. Based on Table 1 it is known that SR is highest in obtaining from 10% probiotic concentration with SR average $81.4567 \pm 1.9985\%$ and the lowest obtained at 0% probiotic is $61.9300 \pm 0.6080\%$. Pursuant to result of ANOVA indicate that giving of probiotic at commercial feed influence significant to SR catfish seed ($P < 0.05$). Provision of 10% probiotics in the diet gave the highest response to SR compared with other treatments.SR treatment group of 10% probiotic in feed and in aquaculture water has the highest SR value ($81{,}4567 \pm 1.99855$) compared with

negative control that is feed without given probiotic and positive control that is given 5% and 15% probiotics % (P <0.05).

Increased cultivation production has implications for increasing the density and amount of feed used. This will lead to the accumulation of organic matter in the cultivation environment. The accumulation of organic matter results in a decrease in water quality due to the high content of inorganic nitrogen compounds, whether derived from metabolic waste (excretion), uneaten feed, faces, dead algae and other organic materials [39][40]. Fish only assimilates 20-30% of the amount of feed given, the rest is excreted into pond water. Approximately 5.5 of the nitrogen that enters the pond (which comes from the feed) will be converted to ammonia. High ammonia can lead to high nitrite content in toxic waters. The nitrite is a product of nitrifying bacteria that utilizes ammonia in the process. The accumulation of ammonia is overcome and managed by manipulating the use of a heterotrophic probiotic solution that can be performed to control water quality such as: inorganic nitrogen [41][40]. The results of this study indicate that the provision of EM4 (probiotics) in feed and water cultivation trigger the growth and development of heterotropia microbes that are able to grow micro floc so that catfish can improve survival. Along with a particular research, micro floc can improve the utilization of natural feed and biota survival [42]. Furthermore, in the treatment of micro floc, the fish showed no signs of stress so that the health status of the fish on the treatment of micro floc was supposedly better [43]. The presence of micro floc can improve the health status of fish, so that the survival of fish is higher than the control [44].

Based on the results of this study showed that the provision of probiotics in the water cultivation of catfish able to improve the quality of fish farming water. This is because in the media to ferment EM4, among others, the raw material is spices composed of *Curcuma xanthorriza*, white turmeric and red ginger. These spices contain bioactivity, among others, can increase the resistance of the fish body to attack disease and increase the appetite of fish. Thus, catfish become healthier and increase appetite. In addition, the raw materials for bacterial growth medium in EM 4 also contain carbohydrate sources such as molasses, rice bran and red sugar as a source of energy and milk as a much-needed source of protein for the growth and development of microbes in EM4.

Provision of probiotics containing EM4 is proven to improve water quality of catfish farming. This can be seen from the pond cultivation of this catfish, the death of catfish is attacked by without disease, in general catfish death this treatment due to attacked fellow catfish, and proved that the provision of probiotics in water cultivation catfish can improve the quality of water cultivation. Based on the result of this research, the temperature of aquaculture waters is 26.5-28.7 °C, the pH ranges between 6.5-7.4 and DO 3.3-5.9 mg/ml. Water quality data in this study can be categorized as normal. The results of this study indicate that the SR obtained from the treatment of probiotics 0% to 15% of the amount of feed given ranges between 61.9300 ± 0.6080% to 81.4567 ± 1.9985 %. Here SR can be proven that resulted from giving probiotics in feed equal to 71.5900 ± 0.6029% until 81.4567 ± 1.9985% and without giving of probiotic in feed of SR equal to 61.9300 ± 0.6080%. Provision of probiotics containing EM4 can kill pathogenic bacteria that exist in the water cultivation and can break the chain of disease. Thus, before it is eaten by the catfish has been cut the chain of disease by microbial pathogens.

Enhancement of immunity and disease resistance in the aquaculture by the probiotic [45]. Similar results are also shown by other researchers, mentioned that the presence of optimum microbial cell concentrations in probiotic can improve the health status of fish and bacterial cells there is accumulation of poly-β-hydroxybutyrate (PHB) with alleged role in inhibition of pathogenic microbes in cultivation catfish [43]. The contents of PHB in micro floc consumed by the fish can boost the immune system so that fish can be more resistant to environmental disturbances during treatment [46]. Therefore, the development of a heterotroph system from micro floc can be one of the solutions that can be done to control water quality such as inorganic nitrogen [30][41][47]. This heterotrophic system is based on bacteria. Bacteria play an important role in the decomposition of organic nutrients in aquaculture production activities and pond sediments [48][49]. This bacteria able to increase the level of growth performance and survival of fish farming [19][50].

IV. CONCLUSION

To production performance with probiotic use in feed with addition of spices as an environmentally friendly catfish cultivation engineering had effect on SGR, FCR and SR significant. Provision of 10% probiotics on commercial feed and in aquaculture is best to produce SGR 3.3867 ± 0.24440%, FCR of 1.5733 ± 0.0987, PR of 38.949 ± 0.500 and SR 81.4567 ± 1.9985 and safe used for cultivation of environmentally friendly catfish.

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REFERENCES

[1] L.A. Muzinic, K.R. Thompson, A. Morris, C.D. Webster, D.B. Rouse and L. Manomaitis, "Partial and total replacement of fish meal with soybean meal and brewer's gains with yeast in practical diets for Autralian red claw crayfish (Cherax quadricarinatus)," Aquac., vol. 230, pp. 359-376, 2004.

[2] T.D. Sink, R.T. Lochmann, C. C. Pohlenz, A.A. Buentello, and D. Gatlin, "Effects of dietary protein source and protein–lipid source interaction on channel catfish (Ictalurus punctatus) egg biochemical composition, egg production and quality, and fry hatching percentage and performance," Aquac., vol. 298, pp. 251–259, 2010.

[3] M.S. Izquierdo, H. Fernandez-Palacios, and A.G.J. Tacon, "Effect of broodstock nutrition on reproductive performance of fish," Aquac. Vol. 197, pp. 25–42, 2001.

[4] T. Lovell, Nutrition and Feeding of Fish, New York: Van Nostrand Reinhold, pp. 260, 1989.

[5] C.T. Da, T. Lundh, and J.E. Lindberg, "Evaluation of local feed resources as alternatives to fish meal in terms of growth performance,

feed utilisation and biological indices of striped catfish (Pangasianodon hypophthalmus) fingerlings," Aquac., vol. 364–365, pp. 150-156, 2012.

[6] L.T. Phan, T.M. Bui, T.T.T. Nguyen, G.J. Gooley, B.A. Ingram, H.V. Nguyen, P.T. Nguyen, and S.S. De Silva, "Current status of farming practices of striped catfish, Pangasianodon hypophthalmus in the Mekong Delta," Aquac., vol. 296, pp. 227–236, 2009.

[7] A.M. Kelly, "Broodfish management," in: Biology and Culture of Channel Catfish, vol. 34, C.S. Tucker, J.A. Hargreaves, Amsterdam: Elsevier Science, pp. 129–144, 2004.

[8] L. Cao, W. Wang, Y. Yang, C. Yang, Z. Yuan, S. Xiong, and J. Diana, "Environmental Impact of Aquaculture and Countermeasures to Aquaculture Pollution in China," Env. Sci. Pollut. Res., vol. 14, pp. 452–462, 2007.

[9] J.M. Ebeling, M.B. Timmons, and J.J. Bisogni, "Engineering analysis of the stoichio metry of photoautotrophic, autotrophic and heterotrophic removal of ammonia–nitrogen in aquaculture systems," Aquac., vol. 257, pp. 346-358, 2006.

[10] P.V. Krishna, G. Gopi, V. Panchakshari, and K. Prabhavathi, "Effects of probiotics on the survival and growth of Catla catla, Labeo rohita, Cirrhinus mrigala and Pangasius hypophthalmus under polyculture system," Int. J. of Adv. Res., vol. 3, pp. 625– 632, 2015.

[11] B.R. Kiran, "Physico-chemical characteristics of fish ponds of Bhadra project at Karnataka," RASĀYAN J. of Chem., vol. 3, pp. 671-676, 2010.

[12] M.E. Sanders, L. Morelli, and T.A. Tompkins, "Sporefor mers as human probiotics: Bacillus,Sporolactobacillus, and Brevibacillus," Compr. Rev. in Food Sci. and Food Saf., vol. 2, pp. 101-110, 2003.

[13] Y.B. Wang, J.R. Li. and J. Lin, "Probiotics in aquaculture: challenges and outlook," Aquac., vol. 281, pp. 1-4, 2008.

[14] J.L. Balcazar, I. de Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell and J.L. Muzquiz, "The Role of Probiotics in Aquac.," Rev. Veterin. Microbiol., vol. 114, pp. 173-186, 2006.

[15] T.N. Tuan, P.M. Duc, and K. Hatai, "Review Article. Overview of the use of probiotics in aquaculture," Int. J. of Res. in Fisheries and Aquac., vol. 3, pp. 89-97, 2013

[16] D.M. de Souza, G.B. Martins, S.R.N. Piedras, J.L.O.F. Pouey, R.B. Robaldo, and F.P.L Leite, "Short Communication. Probiotic actions of Bacillus cereus var. toyoi and Saccharomyces boulardii in silver catfish (Rhamdia quelen) larvae culture," R. Bras. Zootec., vol. 41, pp. 815-819, 2012.

[17] M.M. Mohideen, A. Kader, T.S. Mohan, S.P. Mohamed and M.I.Z. Hussain, "Effect of Probiotic Bacteria on the Growth rate of Fresh Water Fish, Catla catla," Int. J. of Biol. Tech., vol. 1(2), pp. 113-117, 2010.

[18] G. Burr, D. Gatlin, and S. Ricke, "Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture," J. of the World Aquac. Society, vol. 36, pp. 425-436, 2005.

[19] L. Verschuere, G. Rombaut, P. Sorgeloos, W. Verstraete, "Probiotic bacteria as biological control agents in aquaculture," Microbiol. Mol. Biol. Rev., vol. 64, pp. 655–671, 2000.

[20] S.M. Aly, A.M. Abd-El-Rahman, G. John, M.F Mohamed, "Characterization of Some Bacteria Isolated from Oreochromis niloticus and their Potential Use as Probiotics," Aquac., vol. 277, pp. 1-6, 2008.

[21] L. Villamil, C. Tafalla, and A. Figueras, "Evaluation of Immunomodulatory Effects of Lactic Acid Bacteria in Turbot (*Scophthalmus maximus*)," Clin. Diagn. Lab. Immunol., vol. 9, pp. 1318-1323, 2002.

[22] D.Y. Chen, J.H. Shien, L. Tiley, S.S. Chiou, S.Y. Wang, T.J. Chang, Y.J. Lee, K.W. Chan and W.L. Hsu, "Curcumin inhibits influenza virus infection and haemagglutination activity," Food Chem., vol. 119, pp. 1346-1351, 2010.

[23] P. Anand, "Bioavailability of Curcumin: Problems and Promises," J Mol Pharm., vol. 4(6), pp. 807-18, 2007.

[24] S. Chrubasic, M.H. Pittler, and B.D. Roufogalis, "Zingiberis rhizoma: A Comprehensive Review on the Ginger Effect and Efficacy Prolfiles," J. of Phytomedicine, vol. 12(9), pp 684-701, 2005.

[25] Z. Qi, Z. Xiao-Hua, N. Boon, and P. Bossier, "Probiotics in aquaculture of China — Current state, problems and prospect," Aquac., vol. 290, pp. 15–21, 2009.

[26] T. Watanabe, Fish Nutrition and Mariculture, Tokyo: Tokyo University of Fisheries, 1988.

[27] S. Craig, and L.A. Helfrich, "Understanding fish nutrition, feeds, and feeding," Virginia Coop. Ext., no. 420-256, 2009.

[28] E.A. Huissman, Principles of Fish Production. Department of Fish Culture and Fisheries, Nederland: Wageningen Agricultural University, 1987.

[29] S. Goddard, Feed Management in Intensive Aquaculture, 3rd ed., London: Chapman and Hall, 1996.

[30] R. Crab, "Bioflocs Technology: an integrated system for the removal of nutrients and simultaneous production of feed in aquaculture," PhD thesis, Ghent University, pp. 178, 2010.

[31] E.O. Ogello, S.M. Musa, C.M. Aura, J.O. Abwao, and J.M. Munguti, "An Appraisal of the Feasibility of tilapia production in ponds using biofloc technology: A review," Int. J. of Aquat. Sci., vol. 5(1), pp. 21-39, 2014.

[32] B. Grisdale-Helland, S.J. Helland, and D.M. Gatlin Iii, "The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmo salar)," Aquac., vol. 283, pp. 163-167, 2008.

[33] R.D. Ballestrazzi, L. ED'agoro, A. Mion, "The effect of dietary protein level and source on growth and body composition, total ammonia and relative phosphate excretion of growing sea bass Dicentrarchuss labrax," Aquac., vol. 127, pp. 197–206, 1994.

[34] Y.X. Cui, S.W. Liu, and S. Chen, "Growth and energy budget in young grass carp Ctenopharyngodon idella Val., feed plant and animal diets," J. of Fish Biol., vol. 41, pp. 231–238, 1992.

[35] H.G. Schlegel, and K. Schmidt, General Microbiology, 6th ed, Cambridge: Cambridge University Press, 1986.

[36] C.F. William and D.C. Westhoff, Food Microbiology, 4th ed. New York: Mc.Graw-Hill, 1988.

[37] J.E. Halver, Fish Nutrition, 2nd ed., New York: Academy Press, 1989.

[38] R. Crab, Y. Avnimelech, T. Defoirdt, P. Bossier, and W. Verstraete, "Nitrogen removal in aquaculture towards sustainable production," Aquac., vol. 270, pp. 1-14, 2007.

[39] R.M. Duborow, D.M. Crosby, and M.W. Brunson, Ammonia in Fish Ponds, Stoneville: Southern Regional Aquaculture Center, 1997.

[40] R. Crab, T. Defoirdt, P. Bossier, and W. Verstraete, "Biofloc technology in aquaculture: Beneficial effects and future challenges," Aquac., vol. 356-357, pp. 351 –356, 2012.

[41] D. Willet and C. Morrison, "The buildup of toxic inorganic forms of nitrogen (especially ammonia-NH_3) is an inherent," [Online]. Available: http://era.daf.qld.gov.au/2072/2/15._Qld_Aqua_News_Issue _28%2C_page_6_%26_7-sec.pdf. [Accessed: 28 July 2018].

[42] M. Asaduzzaman, M.A. Wahab, M.C.J. Verdegem, R.K. Adhikary, S.M.S. Rahman, M.E. Azim, and J.A.J. Verreth, "Effects of Carbohydrate Source for Maintaining a High C: N Ratio and Fish Driven Re-suspension on Pond Ecology and Production in Periphyton-based Freshwater Prawn Culture Systems," Aquac., vol. 301, pp. 37-46, 2010.

[43] M.E. Azim, and D.C. Little, "The Biofloc Technology (BFT) in Indoor Tanks: Water Quality, Biofloc Composition and Growth and Welfare of Nile tilapia (Oreochromis niloticus)," Aquac., vol. 283, pp. 29-35, 2008.

[44] J.A Hargreaves, "Bioflocs production system for aquaculture," Southern Regional Aquaculture Center (SRAC) Publication No. 4503, 2013.

[45] D.Y. Tseng, P.L. Ho, S.Y. Huang, S.C. Cheng, Y.L. Shiu,C.S. Chiu, , and C.H. Liu, "Enhancement of immunity and disease resistance in the white shrimp, Litopenaeus vannamei, by the probiotic, Bacillus subtilis E20," Fish Shellfish Immunol, vol. 26, pp. 339–344, 2009.

[46] L. Michaud, J.P. Blancheton, V. Bruni, and R. Piedrahita, "Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters," Aquac. Eng, vol. 34, pp. 224-233, 2006.

[47] G. Luo, J. Wang, N. Ma Z. Liu, and H. Tan, "Effects of Inoculated Bacillus subtilis on Geosmin and 2-Methylisoborneol Removal in Suspended Growth Reactors Using Aquacultural Waste for Biofloc Production," J. Microbiol. Biotechnol, vol. 26, pp. 1420–1427, 2016

[48] J.A. Hargreaves, "Nitrogen biogeochemistry of aquaculture ponds," Aquac., vol. 166, pp. 3-4, 1998.

[49] J.A. Hargreaves, "Photosynthetic suspended-growth systems in aquaculture," Aquac. Eng, 34, pp 344-363, 2006.

[50] O. de Camp, D.J.W. Moriarty, and P. Lavens, "Probiotics for shrimp larviculture: review of field data from Asia and Latin America.," Aquac. Res., vol. 39, pp. 334-338, 2008.