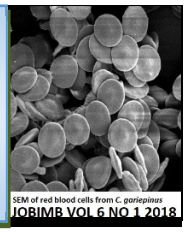
HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index



The Effects of Synbiotics and Probiotics Supplementation on Growth Performance of Red Hybrid Tilapia, *Oreochromis mossambicus x Oreochromis niloticus*

Muhamad Amin Jahari[1], Shuhaimi Mustafa[1,2*], Muhamad Akhmal Hakim Roslan[3], Yazid Abd Manap[1], Dhilia Udie Lamasudin[1,2] and Farahiyah Ilyana Jamaludin[4]

[1]Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]Malaysian Agricultural Research and Development Institute, 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Prof. Dr. Shuhaimi Mustafa
Halal Products Research Institute,
Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia
Email: shuhaimi@upm.edu.my
Tel: +03-89471952
Fax: +03-89439745

HISTORY

Received: 11th March 2018
Received in revised form: 11th of April 2018
Accepted: 20th of May 2018

KEYWORDS

synbiotics
probiotics
prebiotics
tilapia
growth performance

ABSTRACT

The purpose of this study was to evaluate the influence of a synbiotics between *Lactobacillus plantarum* and mannan-oligosaccharides (MOS) extract and probiotics (*Lactobacillus plantarum*) on growth performance in red hybrid tilapia (*Oreochromis mossambicus x Oreochromis.niloticus*). The fishes with initial average weight 11.9 ± 0.7g were randomly assigned to three dietary treatments which were fed on a diet containing probiotics only and a diet combination of probiotics and prebiotic (synbiotics), with four replicates for each treatment. The control group was fed without supplementation of any probiotics or prebiotics for the same duration. All the diets were isocaloric and isonitrogenous. All fish were fed daily at 3% of the body weight per day in two equal instalments. The feeding rate was kept at 4% body weight day-1 for the whole rearing period of 30 days, and the amount of feed was adjusted every tenth day following a bulk weighing of each group of fish. All diets contained about 32% of crude protein. Significantly ($P < 0.05$) high growth performance (percent gain body weight, weight gain and feed intake) were observed in the group fed diet containing synbiotics as compared to probiotics and control group. Similarly, significantly ($P < 0.05$) low FCR was recorded in synbiotics as compared to probiotics and control group. This result revealed that a feeding regime with synbiotics for 30 days led to a significant increase in growth performance, survival rate and feeding efficiency in red hybrid tilapia fingerlings.

INTRODUCTION

Fish farming, also known as aquaculture, whereby equipped with high-end technology is shown to be a prominent and emerging field to meet the increasing demands of fish as food, commercial products or rehabilitate endangered species. It was investigated that the majority cost of manufacturing farming operations was mainly from the production of feeds, covering about 66% to 84% of the total production cost [1]. As such, multiple kinds of research were thoroughly conducted to further increase fish production. Thus, the utilization of synbiotics and probiotics are one of the many promising solutions [2]. A synbiotic product is defined as an integration of probiotics and prebiotics which aims to improve the animal's rate of survival by introducing nutrient additives for microbes into the gastrointestinal tract of the host. This, in turn, incites the development or metabolism of health-stimulating bacteria and further enhances its well-being [1], behaving like health or nutritional supplement. Besides promoting the functional welfare of the animal, it has additional benefits such as capable of improving profitability as synbiotics are seen to enhance growth in aquaculture [1].

Prebiotics are indigestible food components aimed to initiate development and bacterial activity in the digestive system to confer health benefits to the host, while probiotics are either monocultures or mixed cultures of microorganisms whereby when applied to humans or animals, it confers health benefits. Authors in [3] defined probiotics as "live microorganisms, which

when administered in suitable amounts, confer benefits to the health of the host by improving the balance of the microbiota in the intestine." Most probiotics are supplied in food which must have the ability to survive passage through intestinal tract [4]. The benefit of the host may arise as a nutritional effect, whereby the bacteria can break down toxic or otherwise non-nutritious components of the diet, which the host can then digest [5].

Furthermore, contagious diseases faced by aquatic animals also have been known to be a common challenge faced by many in the world when conducting aquaculture [2]. Though this may seem to be a detrimental health effect towards the particular species of the aquatic animals in the farm, this challenge may be easily overcome through the use of antibiotics which helps to reduce the consequences of pathogens infecting the aquatic animals [2]. However, the European Parliament resolution of 2012 (2015/C 434/06) stated that it had banned the use of sub-therapeutic antimicrobials in animal farming due to the possibility of side effects such as it may incur antibiotic resistance of the pathogen through either chromosomal mutation or acquisition of plasmids.

Therefore, the focal point of the present study was to analyze the usage of a specific oligosaccharide found within palm kernel cake as the source of prebiotic and *Lactobacillus plantarum* as probiotics to assemble the synbiotics concept which involves exertion of its synergistic effects and its effects on the growth performance of tilapia.

MATERIALS AND METHODS

Probiotics and synbiotics preparation

Lactobacillus plantarum was obtained from Halways Sdn. Bhd. The culture was maintained and propagated using nutrient broth media. Cultures were incubated anaerobically at 37ºC in an aerobic bottle on a rotary shaker set at the speed of 100 rpm for fermentation and propagation purpose. As for synbiotics diet, the culture was mixed with palm kernel cake (PKC) before dried with filtered air-flow and post-mixed with the feed with 0.4 g/1kg.

Experimental design and culture technique

A 3 x 4 factorial experiment was designed to study the effect of probiotics (*Lactobacillus plantarum*), prebiotic (MOS), and their synbiotics interactions on growth performance and survival rate of the *O. mossambicus x O. niloticus*. The fish were obtained from the local hatchery at Semenyih, Selangor and were acclimated for two weeks at the Alternative Protein Source Laboratory, Faculty of Biotechnology and Science Biomolecule, Universiti Putra Malaysia, Malaysia. During this period, fish were fed with a commercial diet (32% crude protein) twice a day to be adapted to pelleted feed and surroundings water according to [6].

The experiment was conducted in 8 aquaria (36"×18"×16"). The aquaria were supplied with freshwater from Syarikat Bekalan Air Selangor (SYABAS), governate by pump machines of each aquarium. Each aquarium was stocked with 30 fish with initial weight ranging 11.9-12.6 g. Four replicates were randomly assigned to each treatment, before the start of the experiment. During the test, fish were hand-fed their respective diets at a level of 4% of their body weight. The daily ratio was divided into three equal amounts and offered three times a day (0900, 1200, 1500 h). Fish for each aquarium were weighed biweekly, and the amount of daily diet was adjusted accordingly. About one-third of water in each aquarium renewed manually once a week. All aquaria were provided with continuous aeration to maintain the dissolved oxygen level near saturation and fish held under natural light. Total ammonia, nitrite, and nitrate were measured once a week using API Freshwater Master Test Kit, while pH was monitored every two days by taking water samples from each replicate and measured it using pH meter [7]. Water quality criteria were suitable within the acceptable limits for rearing the red hybrid tilapia fingerlings.

Experimental diets

The basal diet was formulated to contain approximately 32% crude protein, 5% crude fat, and 6% crude fiber. The processing of the feed begins with crushing of the ingredients by the fish feed crusher. Powdery feed materials then mixed thoroughly in the mixer to ensure a high quality of nutritional feed. After mixing, feed pellet extruder will compress feed powder into sized pellet and stored at the warehouse in the Pulau Indah until use. As for probiotics diet, *Lactobacillus sp*. was supplemented separately to the basal diet after it was pelleted by spraying onto of it. While for synbiotics diet, the pellets were sprayed with synbiotics cultures of *Lactobacillus sp.* and extract of PKC.

Proximate analysis of feed

The crude protein was determined using 2000 Digestion System and Kjeltec Auto Analyser 2400 (FOSS TECATOR AB, Sweden). The method used was the Kjeldahl method. The moisture and crude fiber contents of pellet were analysed according to the standard procedure of [8] using the FiberTech 2010™ Hot Extractor (FOSS TECATOR AB, SWEDEN). This protocol was carried out based on the method described by [9].

Physical Properties

Bulk Density (BD)

The feed pellets for each replicate was filled into 1L measuring cylinder until full. The pellets in the measuring cylinder were then weighed on the electronic balance to determine the ratio of the feed weight per one liter as bulk density. The unit used for the density of the feed is gram per liter. The measuring cylinder was not tapped to standardize the method prior of weighing [10].

Expansion Ratio (ER)

Ten pellets from each replicate of experimental diet were taken. The initial diameter of the pellets was measured using the caliper and the initial average width (E_i) was calculated. The pellets then immersed in 50 ml beaker containing distilled water. The pellet was let to absorb water and become expanded for 20 minutes. After 20 minutes, the water is removed, and the diameter of the expanded pellet was measured using caliper and final average diameter (E_f) was calculated. The ER was determined as the ratio of final average diameter to the initial average diameter of the pellet. The calculation was as follow;

$$ER = \frac{Ef - Ei}{Ei}$$

Floatability

Ten pellets of feed for each replicate were immersed into 100 ml beaker containing distilled water for 20 minutes at room temperature. After 20 minutes, the pellets were observed for its floatability. The percentage of floatability was calculated by using the following formula:

$$Percentage\ of\ floatability = \frac{Number\ of\ pellets\ floated\ after\ 20\ mins}{Total\ number\ of\ pellets}\ x\ 100$$

This protocol was carried out based on the method described by [9].

Bacterial count

The bacteria were added to experimental diet and stored in 25℃ for 30 days. The initial bacterial count by colony forming unit (CFU/g) was taken after 24 hours incubation of the experimental diet with *Lactobacillus sp*. Bacterial count was determined every 15 days until day 30th of the incubation period. The method used was standard plate count method.

Growth performance analysis

Growth performance and feed utilization parameters were measured using the following equations:

$$\text{Net weight gain} = \text{Final fish weight} - \text{Initial fish weight}$$

$$\text{Feed intake} = \text{Initial feed weight} - \text{Final feed weight}$$

$$\text{Feed conversion ratio} = \frac{Feed\ intake\ (g)}{Weight\ Gain\ (g)}$$

The growth performance analysis was carried out according to protocol cited by [7].

Statistical analysis

All experiments were carried out in four replicates. Minitab software version 17 (2013, Minitab Pty Ltd, Sydney Australia) were used for t-test and ANOVA analysis. All significant differences were considered at the significant level of $P < 0.05$.

RESULTS AND DISCUSSION

Nutritional profile of experimental diets

The nutritional profile for all diets is shown in **Table 1**.

Table 1. Nutritional profile of experimental diets.

Chemical composition	Control diet	Probiotics diet	Synbiotics diet
Crude protein (%)	32 ± 1.2[a]	32 ± 1.2[a]	32 ± 1.17[a]
Crude fat (%)	5 ± 0.01[a]	5 ± 0.01[a]	5 ± 0.03[a]
Crude fiber (%)	6 ± 0.05[a]	6 ± 0.05[a]	6 ± 0.012[a]
Moisture content (%)	8 ± 0.08[a]	8 ± 0.08[a]	8 ± 0.07[a]

Values are expressed as mean ± standard deviation
[a,b] Means within a row with different superscripts are significantly different ($P < 0.05$)

Table 1 shows that the nutritional profile of the feed is similar for all treatments in term of crude protein, crude fat, crude fibre and moisture content with the percentage of 32%, 5%, 6% and 8% respectively. Statistically, all diets have no significant different ($P > 0.05$) in term of the chemical composition. All diet treatments were formulated to be the same regarding nutritional for standardization purpose. Different in the nutritional profile would render the result of growth performance to be inaccurate and unacceptable as a valid result. Therefore, the results depict that feed contents have been standardized as a control variable and will not affects results of the experiment. Hence the effectiveness of probiotics and synbiotics supplementation can be evaluated efficiently.

Probiotics Quantification

The colony forming unit (CFU) counts were taken every 15 days including the first day the probiotics were mixed up together with the feed. The reason why the probiotics were mixed up with the feed before feeding trial was that to ensure probiotics able to adapt with feed and to confirm that feed was not toxic to probiotics. Another reason was to evaluate the mortality of probiotics in the feed to determine the shelf life of feed when it has been mixed with probiotics.

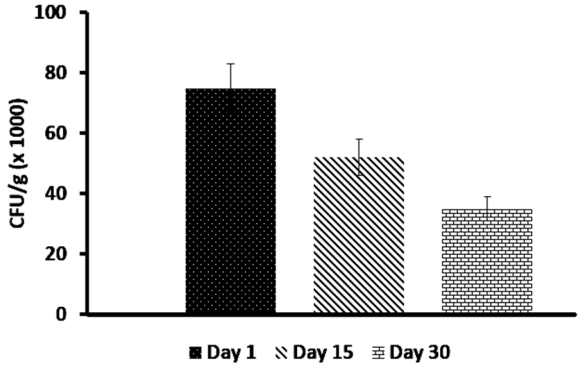


Fig. 1. Total *Lactobacillus* sp. viability (colony forming unit (CFU)/g) of synbiotics diet given to red hybrid tilapia, *Oreochromis sp*. (n = 4). Bacteria were added to feed and stored in 25 ℃ for 30 days. The error bars represent the range of sample variation between replicates and the standard deviation.

From the graph, the initial total *Lactobacillus* sp. on day 1 was 7.5 x 104 CFU/g; day 15 was 4.5 x 104 CFU/g and day 30 was at 2 x 104 CFU/g. The result shows that the viability of *Lactobacillus* sp. in the diet when incubated at 25℃ decreases significantly ($P < 0.05$) from day 1 until day 30 (**Fig. 1**), suggesting that the probiotics were degenerating or die when it was mixed with the feed at the rate of 10,000 CFU/g every week. Therefore, it is necessary to use the feed combined with probiotics immediately. In the previous study done by [4], they used directly feed that was added probiotics *Lactobacillus lactis AR21* in *Brachionus plicatilis* culture. This is because the probiotics bacteria were active on a diet, however, had continuously died due to inability to gain the nutrient resource from diet to remain survived. This reason was speculated as the diet are still intact and durable even after several days after probiotics addition, suggesting that the nutrient inside the diet was not being degraded by the bacteria. From the result, it was expected that maximum time for the probiotics to remain viable on a diet was at least within the range of 5 weeks. On the other hand, authors in [4] found that the probiotics-activity declined over an eight-week period when incorporated in the diets. Authors in [11] were the first to report the used of probiotics dose over such a wide range (10^4 – 10^{11} CFU/g). In this study, the concentration of probiotics included in the diets was 10^4 CFU/g. Most of the prior studies used probiotics concentration on feed at 10^7 CFU/g [4, 12, 13, 14]. The viability of probiotics in the feed over a period is higher with a higher probiotic initial concentration of feed.

Physical Properties

The physical properties of experimental diets were analyzed on expansion ratio (ER), bulk density (BD) and floatability (F). The result of the analysis is shown in **Table 2**.

Table 2. Physical Properties of experimental diets.

	Control diet	Probiotics diet	Synbiotics diet
Expansion ratio, ER	1.4 ± 0.07[a]	1.4 ± 0.04[a]	1.4 ± 0.04[a]
Bulk density, BD (g/L)	445 ± 0.22[a]	450 ± 0.25[a]	449 ± 0.23[a]
Floatability, F (%)	100% ± 0.00[a]	100 ± 0.00[a]	100% ± 0.00[a]

Values are expressed as a mean ± standard deviation
[a,b] Means within a row with different superscripts are significantly different ($P < 0.05$)

Table 2 indicates no significant differences among the physical parameter tested ($P < 0.5$) with values for expansion ratio, bulk density, and floatability were 1.4, 445-450 g/L and

100% respectively. Previously, authors in [9] have analyzed the physical properties of fish pellets containing broke rice starch and found that the expansion ratio, bulk density, and percentage of floatability are 1.2, 450 g/L and 80% respectively. Based on the comparison between those pellets in term of physical properties, the experimental diets used in the study meet the standard requirement needed as their results almost identical. This analysis is to ensure the physical properties of the diets are not significantly different to reduce the errors during the feeding trial.

The ER was analyzed due to its effect towards fish as fish is known to consume an enormous amount of diet when fed. In the stomach, the feed would expand due to absorption of water and could cause the fish stomach to swell, and eventually will suffocate the fish. According to authors in [15], BD is an index of the expansion of puffing which considers extension in all direction. The BD was analyzed to identify to choose the suitable packaging volume. Lastly, the floatability analysis was conducted to ensure that the feed pellets were not sinking immediately after it being fed to the fish in glass tanks. The experimental diets prepared was for floating type feed as most fish preferred to feed at the water surface. Authors in [9] stated that the capacity of feeds to float for a period is related to BD and also water stability. Authors in [16] reported that extruded feed would float if the BD is below than 530 g/L. This experiment proves that BD of the experimental diets below than 530 g/L, thus the floatability of the diet was expected to exceed 90% and from the result shows that 100% of the feed was floating.

Growth Performance

Results in **Table 3** indicated that the highest final body weight (BW), weight gain (WG) and feed intake (FI) were recorded by fish fed synbiotics (25.1 ± 0.76, 12.6 ± 0.11, 19.2 ± 0.33 g/fish respectively) while the lowest one was shown by fish fed with control diet (21.6 ± 0.45, 9.7 ± 0.04, 17.8 ± 0.61 g/fish respectively). The lowest FCR also recorded by fish fed synbiotics diet (1.53 ± 0.03 g/g) as compared to probiotics (1.56 ± 0.036 g/g) and control group (1.85 ± 0.034 g/g), indicating the fish fed synbiotics diet had best growth performance while control group displayed worst performance.

Table 3. Effect of dietary inclusion of synbiotics and probiotics on red hybrid tilapia, *Oreochromis sp*. final weight, net weight gain, feed intake and feed conversion ratio (FCR).

	Control diet	Probiotics diet	Synbiotics diet
Initial weight (g fish^{-1})	11.9 ± 0.7^{a}	11.6 ± 0.6^{a}	12.0 ± 0.5^{a}
Final weight (g fish^{-1})	21.6 ± 0.45^{a}	23.7 ± 0.95^{b}	25.1 ± 0.76^{b}
Weight gain (g fish^{-1})	9.7 ± 0.04^{a}	12.2 ± 0.01^{b}	12.6 ± 0.11^{b}
Feed intake (g fish^{-1})	17.8 ± 0.61^{a}	19.1 ± 0.42^{a}	19.2 ± 0.33^{a}
FCR (g g^{-1})	1.85 ± 0.034^{a}	1.56 ± 0.036^{b}	1.53 ± 0.038^{b}

Values are expressed as mean ± standard deviation (n = 4)

^{a,b} Means within a row with different superscripts are significantly different (P < 0.05)

The study by authors in [17] showed that after 60 days rainbow trout (*Oncorhynchus mykiss*) fed diets containing different levels of synbiotics (Biomin IMBO) (0.5%, 1.0%, and 1.5%) increased in body weight about 50%, 59%, and 53%, respectively and improved SGR and FCR in comparison with control group. Authors in [18] reported that Japanese flounder fed diet supplemented with fructo oligosaccharides (FOS), mannan oligosaccharides (MOS) and *Bacillus clausii* increased WG. Also, authors in [19] indicated that at every dietary FOS level, supplemented by 1.35 x 10^{7} CFU g^{-1} *B. subtilis* significantly increased SGR and feed efficiency ratio (FER) when compared to the control group. Similarly, Authors in [20] reported that synbiotics application of *Lactobacillus sp*. and mannan oligosaccharides in feed diet was able to improve the growth performance and enhance the survival of European lobster *Homarus gammarus*.

Such an increase in the growth in aquatic animals that were fed probiotics supplemented diets may be attributed to the improved digestive activity due to enhancing the synthetic vitamins and enzymatic activities [21]; consequently, improving digestibility and growth performance. Probiotics have been shown to produce digestive enzymes such as amylase, protease, lipase which may enrich the concentration of intestinal digestive enzymes and prebiotics may become the nourishment for probiotics to exert their functions more efficiently. This combination of synergistic effects of probiotics and prebiotics (namely synbiotics) further enhance the growth performance of the fish. Also, probiotics inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by the competition for nutrients and the alteration of the microbial metabolism [22]. It also improves the nutrition by detoxifying the potentially harmful compounds in feeds by producing vitamins such as biotin and vitamin B12 [23], and by stimulating host immunity [24]. The author in [25] indicated that *Lactobacillus sp.* were able to increase the chance of turbot to survive in rotifers culture. Authors in [26] also illustrated that African catfish *Clarias gariepinus* that were fed the *Lactobacillus acidophilus* showed a better growth performance than the control fish group. Authors in [27] also reported that application of *Enterococcus faecium* as a probiotic was found to enhance the growth performance of Nile tilapia, *O. niloticus*. Similarly, the supplementation of basal diet with *Bacillus subtilis*, significantly (P<0.01) improved BW, BL, WG, and SGR of *O. niloticus* [28].

CONCLUSION

The results of the present study depicted that the supplementation of *Lactobacillus plantarum* not only provide the alternative for usage of antibiotics, but also enhanced the growth performance and fed utilization of red hybrid tilapia. Moreover, the supplementation of MOS extract had significant beneficial effects and their significant interactions between dietary *Lactobacillus plantarum* and MOS extract.

ACKNOWLEDGEMENT

The fund granted by the Universiti Putra Malaysia, under the Putra Grant – Putra Graduate Initiative (IPS) (Project number: UPM/700-2/1/GP-IPS/2017/9554400) and Universiti Putra Malaysia for the facilities are acknowledged.

REFERENCES

1. Huynh T, Shiu Y, Nguyen T, Truong Q, Chen J, Liu C. Current applications, selection, and possible mechanisms of actions of synbioticss in improving the growth and health status in aquaculture: A review. Fish Shellfish Immunol. 2017; 64:367-382. doi: https://doi.org/10.1016/j.fsi.2017.03.035.
2. Ringø E, Song SK. Application of dietary supplements (synbioticss and probiotics in combination with plant products and β-glucans) in aquaculture. Aquac Nutr. 2016; 22:4-24.
3. Vershuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotics bacteria as biological control agents in aquaculture. Microbiol Mol Biol Rev. 2000; 64 (4):655-671.
4. Irianto A, Austin B. Probiotics in aquaculture. J Fish Dis. 2002; *25*(11):633-642.
5. Fuller R. History and development of probiotics. Aquaculture. 1992;232:1-18.

6. Hassaan MS, Soltan MA, Agouz HM, Badr AM. Influences of calcium/phosphorus ratio on supplemental microbial phytase efficiency for nile tilapia (Oreochromis niloticus). Egypt J Aquat Res. 2013; 39:205-213.
7. Standen BT, Peggs DL, Rawling MD, Foey A, Davies SJ, Santos GA, et al. Dietary administration of a commercial mixed-species probiotics improves growth performance and modulates the intestinal immunity of Tilapia, *Oreochromis niloticus*. Fish Shellfish Immunol. 2016; 49:427-435.
8. AOAC (Association of Official Analytical Chemists). Official methods of analysis of the Association of Official Analytical Chemists. 1980; 13th ed. Washington, DC.
9. De Cruz CR, Kamarudin MS, Saad CR, Ramezani-Fard E. Effects of extruder die temperature on the physical properties of extruded fish pellets containing taro and broken rice starch. Anim Feed Sci Technol. 2015; 199:137-145.
10. Aas TS, Oehme M, Sørensen M, He G, Lygren, I, Åsgård T. Analysis of pellet degradation of extruded high energy fish feeds with different physical qualities in a pneumatic feeding system. Aquacult Eng. 2011; 44(1):25-34.
11. Nikoskelainen S, Ouwehand A, Salminen S, Bylund G. Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. Aquaculture. 2001; 198(3):29-236.
12. El-Haroun ER, Goda AS, Chowdury K. Effects of dietary probiotics Biogen® supplementation as a growth promoter on growth performance and feed utilization in Nile tilapia. *Oreochromis niloticus*. Aquacult Res. 2006; 37(14):1473-1480.
13. Aly SM, Mohamed MF, John G. Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (*Oreochromis niloticus*). Aquacult Res. 2008; 39(6):647-656.
14. Zhou X, Tian Z, Wang Y, Li W. Effects of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. Fish Physiol Biochem. 2010; 36(3):501-509.
15. Singh RR, Majumdar RK, Venkateshwarlu G. Optimum extrusion-cooking conditions for improving physical properties of fish-cereal based snacks by response surface methodology. J Food Sci Technol. 2014; 51(9):1827-1836.
16. Glencross B, Rutherford N, Jones B. Evaluating options for fishmeal replacement in diets for juvenile barramundi (*Lates calcarifer*). Aquac Nutr. 2011; 17(3):722-732.
17. Mehrabi Z, Firouzbakhsh, Jafarpour A. Effects of dietary supplementation of synbiotics on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Orcorhynchus mykiss*) fingerlings. J Anim Physiol Anim Nutr. 2011; 96:474-481.
18. Ye JD, Wang K, Li FD, Sun YZ. Single or combined effects of fructo and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounde *Paralichthys olivaceus*. Aquac Nutr. 2011; 17(4):902-911.
19. Ai Q, Xu H, Mai K, Xu W, Wang J. Effects of dietary supplementation of *Bacillus subtilis* and fructo oligosaccharides on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture. 2011; 317:155-161.
20. Daniels CL, Merrifield DL, Boothroyd DP, Davies SJ, Factor JR, Arnold K E. Effect of dietary *Lactobacillus* sp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut. Aquaculture. 2010; 304(1):49-57.
21. Ding X, Li ZQ, Chen YQ, Lin HZ, Yang YY, Yang K. Effects of probiotics on growth and activities of digestive enzymes of *Pennaus vannamei*. J Fish Sci China. 2004; 1(6):580-584.
22. Gatesoupe FJ. The use of probiotics in aquaculture. Aquaculture. 1999; 180:147-165.
23. Hoshino T, Ishizaki K, Sakamoto T, Kumeta H, Yumoto I, Matsuyama H, et al. Isolation of a *Pseudomonas* species from fish intestine that produces a protease active at low temperature. Lett Appl Microbiol. 1997; 25:70-72.
24. Gibson GR, Saavendra JM, MacFarlane S, MacFarlane GT. Probiotics and intestinal infections. In: Probiotics 2. Springer; 1997. p. 10-39.
25. Gatesoupe FJ. Lactic acid bacteria increase the resistance of turbot larvae *Scophthalmus maximus*, against pathogenic Vibrio. Aquat living resour. 1994; *7*(4):277-282.