Original article Casp. J. Vet. Sci., 2024; 1(1): 29-35

Amol University of Special Modern Technologies

Caspian Journal of Veterinary Science

doi: 10.22034/cjvs.2024.198846

Journal homepage: https://cjvs.ausmt.ac.ir/

Investigating the effect of the use of PrimaLac probiotics on the factors common carp blood serum

Mojtaba Pour Mohammadali habibi[1], Zeinab Rahimi Afzal[2], Mehdi Mohammadalikhani[3]*

[1]Faculty of Veterinary Medicine, Babol Branch, Islamic Azad University, Babol, Iran.
[2]Aquatic Animal Health & Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
[3]Science and Research Branch, Islamic Azad University, Tehran, Iran.
(*) Corresponding author: m.mohammadalikhani1982@gmail.com

Article Info

Article history:

Received: 19 November 2022

Accepted: 20 February 2023

Keywords:

Albumin

Cortisol

Cyprinus carpio

Probiotics

Weight gain

Abstract

This study was carried out to evaluate the effect of PrimaLac probiotic (based on lactic acid bacteria; LAB) addition and frequency of application on *Cyprinus carpio* blood serum factors and growth performance (weight gain). For this purpose, 84 fish with an average weight of 60 ± 10 g in 4 groups with 3 replications in each group were compared for 42 days. Control diet had not probiotic additive but treatments of 1, 2, 3 probiotic usages daily, every other day, on 2 successive days in every 4 days period of time, respectively. During the experiment period, feed intake and growth rate were recorded. At the end of experiment, experimental fishes were killed by dianthus powder and then were measured serum cortisol, albumin, and total protein. The average rates of total protein and albumin in group1 were increased ($p < 0.05$) and cortisol serum level decreased in all experimental groups compared to control group ($p < 0.05$). The average weight gain in all experimental groups that used probiotic, significantly increased ($p < 0.05$). Weight gain was increased in daily addition probiotic group compared to control and other experimental groups ($p < 0.05$). The result of this study had shown that use of probiotic based on LAB can affect on cortisol, albumin, total protein serum, and growth performance and also probiotic frequency of application can affect on probiotic efficiency.

Introduction

Population increases on the one hand and increasing public awareness about the benefits of aquatic consumption on the other hand have increased the demand and per capita consumption of aquatics in Iran in recent years (Jega *et al.,* 2018). Along with this growth, the aquaculture sector has always faced problems such as water quality changes, disease outbreaks, and etc. (Kalfagianni *et al.,* 2013). The use of antibiotics and chemicals to solve the problems of aquaculture, in addition to having marginal effects and high cost, causes the accumulation of chemicals in the environment and fish (Burridge *et al.,* 2010). Common carp is one of the economic species of the Caspian sea, which is considered an important food source (Biermann and Geist, 2019). Although this species exists natively and naturally on all the shores of the Caspian sea and enters the mouths of rivers for reproduction; but in recent years, due to overfishing and destruction of breeding places, its generation has decreased (Piri-Gharaghie *et al.,* 2022). To compensate for this problem and to restore the stocks, semi-natural reproduction of fish has been carried out. However, low survival and productivity in fish breeding and breeding centers is one of the biggest effective factors in preventing proper restoration of fish stocks (Taylor *et al.,* 2017). It seems that one of the options according to the conditions of the country is the use of growth and safety incentives (Piri-Gharaghie *et al.,* 2022). In recent years, a lot of research has been done on hormones, antibiotics, and several other chemicals as growth

stimulants, antibacterial agents to increase existing health and feed efficiency; but due to the residual effects in fish and shrimp muscles and consumers' reluctance to use them in aquatic production, it is not recommended (Thornber *et al.,* 2020; Beiranvand *et al.,* 2022). Recently, the studies conducted on the practical application of the positive effects of some probiotics in aquatic nutrition have been expanded (Wang *et al.,* 2019). Probiotics are complementary microorganisms such as bacteria, fungi, and yeasts that have beneficial effects on the host by improving the intestinal microbial balance (Wang *et al.,* 2008). According to the definition, probiotics are auxiliary foods whose side enzymes can increase the digestion process (Felicilda-Reynaldo and Kenneally, 2016). These microorganisms not only reduce pathogenic microbes in the environment and living organisms, but also by creating and strengthening useful microorganisms in the digestive system, they provide health benefits or increase the growth rate in living organisms (Piri-Gharaghie *et al.,* 2022). The positive effects of probiotics on farmed aquatic animals such as optimizing the physical and chemical parameters of the breeding environment, preventing and fighting pathogenic agents, and improving the growth performance of farmed aquatic animals have been confirmed in numerous researches by fisheries researchers (Meena *et al.,* 2014). It is believed that many probiotic bacteria have a positive effect on the growth of organisms, which is important through the production of vitamins, increasing the ability to absorb minerals and trace elements, and the production of digestive enzymes (Ghajari *et al.,* 2020; Ghajari *et al.,* 2022). Probiotics can prevent the proliferation of fish diseases in the digestive tract through competition for obtaining nutrients, changes in the metabolism of bacteria, or stimulation of the host's immune system (Ghajari *et al.,* 2022). In general, probiotics are divided into three main groups: bacterial, algal, and yeast (fungi) (Piri Gharaghie *et al.,* 2021; Das *et al.,* 2022; Goh *et al.,* 2022). The probiotic studied in this research was called PrimaLac, which has four bacterial strains with equal proportions, including *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidum*. The positive effects of each of these bacteria on different animals have been investigated. Therefore, the present study was designed and implemented to determine the effect of different levels of PrimaLac probiotic on growth, blood biochemistry, survival, and resistance to salinity stress in common carp.

Materials and Methods

Preparation of fish and experimental design

This research was conducted in the fall of 2014 at the Aquaculture Research Center of the Faculty of Veterinary Medicine, Islamic Azad University of Babol. Carps with an average weight of 60 ± 10 g were obtained from a farm located in Simorgh city, Mazandaran province. Eighty four common carp were placed in four experimental groups with three replications. In each repetition, there were seven fish with an average weight of 60 ± 10 g. The samples were transferred in groups of 7 to 12 tubs of 100 liters with a drainage volume of 80 liters. The tubs were randomly arranged and equipped with an aerator to maintain the temperature, pH, and oxygen levels.

Food ration preparation

According to the manufacturer's protocol, probiotics were added to the basic diet based on the composition of Table 1, with the ratio of 908 grams per ton. To maintain the quality, fish food was prepared every week for the same amount of seven days, and in order to control the bacterial load of the rations of the experimental groups, gloves and separate containers were used when preparing the rations. Also, this standard diet contained 28.17% protein, 16.6% fiber, 14.9% fat and 3216 kcal per kg. In order to adapt the fish to the basic diet, the fish were fed with the basic diet for one week before the experiment. The fishes were kept for 42 days and during this period feeding was done twice a day (9 am and 2 pm) at the rate of 1% of body weight according to Table 2.

Table 1. The composition of the basic ration used.

Row	Diet components	Amount of consumption (% by weight)
1	Wheat flour	55.12
2	Fish meal	32.88
3	Fish oil	10
4	Vitamins and minerals	2

Table 2. How to consume feed in different groups.

Experimental group	How to use probiotics	Consumption
Control	No probiotics	Two shifts a day (9 am and 2 pm)
1	Daily	Two shifts a day (9 am and 2 pm)
2	Every other day	Two shifts a day (9 am and 2 pm)
3	Two days in between	Two shifts a day (9 am and 2 pm)

Monitoring of environmental conditions

The tubs were continuously oxygenated, and in order to remove waste and food residues on the bottom of the tubs, one third of the water in each tub was slowly siphoned daily without stress, and with the water that had been in the tank for 24 hours before, 300 litter had been heated, it was replaced. Aeration and water exchange are common ways to reduce ammonia in water. Also, to prevent the influence of environmental conditions on the experiment, the physical and chemical factors of water were measured at specific times; Thus, pH and water temperature were determined using a multi-portable device, oxygen through an oxygen meter, and water salinity using a relevant measuring device. The measured water factors of the reservoirs were recorded in Table 3.

Table 3. Measured factors of reservoir water.

Row	Parameters	Amount
1	Temperature (centigrade)	23 ± 2
2	dissolved oxygen (mg per liter)	7 ± 0.5
3	pH	7
4	Salinity (mg per liter)	1.25 ± 0.05

After the weight measurement, blood was taken from the anesthetized fish. Blood sampling was done from the caudal stem of unconscious fish. The blood of each fish was entered into a sterile test tube to evaluate serum blood factors. After the end of the blood collection operation, the blood samples were transferred to the laboratory at a temperature of 4°C inside the container and in the vicinity of the ice.

Blood tests

Blood serum factors including cholesterol, triglyceride, glucose, alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), amylase, and cortisol were measured.

Statistical analysis

This research was planned and executed in the form of a completely random design. Before analysing the data related to different factors, the Shapiro-Wilk normality test was performed, and if it was normal, the ANOWA test was used to determine the existence of significant differences among the groups. The Deccan test was used to determine the existence of significant differences among the factors of different groups under SPSS version 18 software.

Results

Environmental conditions

The environmental conditions of fish keeping were regularly monitored. According to the results of Table 3, during the storage period, the temperature was 23 ± 2°C, dissolved oxygen 0 ± 7.5 mg/liter, salinity 25.05 ± 1 mg/liter, and pH equal to 7.

Weight gain

The rate of weight gain, weight gain percentage, and protein efficiency ratio increased significantly in fish treated with PrimaLac compared to the control group ($p < 0.05$). This showed a significant decrease in the food conversion ratio of fish treated with PrimaLac compared to the control group. There was no significant difference in specific growth rate, condition factor, daily food intake, and survival in the experimental treatments compared to the control group.

Investigation of fish growth indicators

Growth indicators were shown in Table 4. In this study, the growth factors in fish fed with PrimaLac food supplement improved compared to the control group.

Table 4. Blood serum factors and weight index of common carp fed with PrimaLac probiotic at different times (mean ± standard deviation).

Parameters	Experimental groups			
	Control group	Daily (group 1)	Every other day (group 2)	Two days in between (group 3)
Cholesterol (mg/dL)	148.66± 19.50	160.00±7.0 0	157.00±1 1.00	150.00±21. 00
Tri glyceride (mg/dL)	216.00± 32.00	223.00±28. 00	223.00±1 3.00	220.00±28. 00
SGOT (U/L)	152.00± 7.00	140.00±8.0 0	141.00±1 2.00	147.00±19. 00
SGPT (U/L)	32.66±2. 51	31/00±4/0 0	32.00±3.0 0	32.00±1.00
Amylase (U/L)	249.00 ± 9.00	264.00±7.0 0	260.00 ± 7.00	259.00±10. 00
Glucose (mg/dL)	94/33±7 /02	103.33±14. 01	100.00±1 3.52	97.66±25.0 0

- The presence of non-identical letters in each row on the numbers indicated a significant difference.

Comparison of blood factors

The study of the biochemical indices of common

carp blood showed that the amount of total protein increased in groups 1, 2, and 3 compared to the control group, and this increase was significant in group 1 ($p < 0.05$) and not significant in groups 2 and 3. Also, the amount of albumin in group 1 has increased compared to other groups ($p < 0.05$). It showed a constant value. On the other hand, cholesterol and triglyceride levels in all three groups (1, 2, and 3) increased compared to the control group ($p < 0.05$) (Fig. 1).

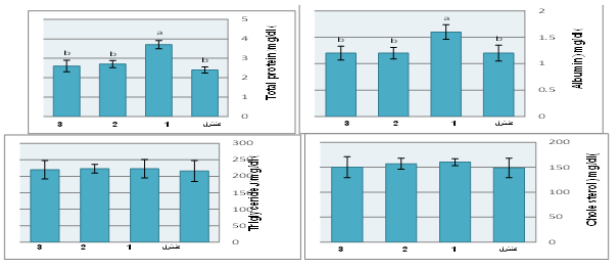

Fig. 1. Diagram of changes in blood biochemical indices in carp fish.

As for liver SGOT and SGPT enzymes, according to Fig. 2, the amount of these enzymes decreased in all three groups (1, 2, and 3) compared to the control group ($p < 0.05$) and the highest decrease was observed in group 1. The level of amylase enzyme and blood glucose showed a significant increase in all three groups compared to the control group, and the highest increase in both factors was observed in group 1.

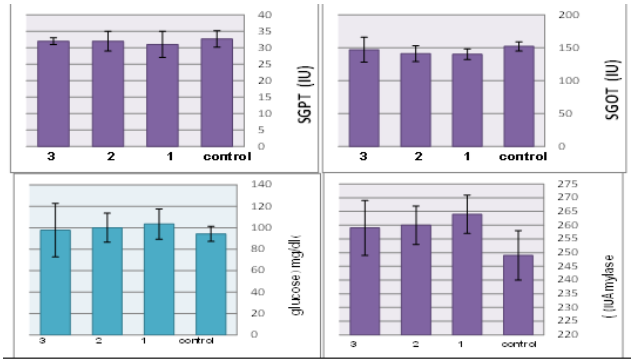

Fig. 2. Diagram of body enzymes changes in carp fish.

Discussion

In the present study, PrimaLac probiotic played a very positive role in promoting the growth of carp fish in all experimental treatments compared to the control group. All diets supplemented with probiotics led to better growth efficiency and food consumption compared to the basic diet in the group. Similar results were observed by a research group on Indian carp (Swain *et al.,* 1996; Hatlen *et al,* 2005). It seems that the increase in growth is due to the increase in appetite and enzyme secretion or the improvement of fish health because of controlling infection and increasing the digestibility of food. In this study, the specific growth factor and weight gain in the treatments receiving PrimaLac compared to the control treatment showed a significant increase. Since bacteria can promote digestion activity by producing vitamins and cofactors or by improving enzyme activities (Pham *et al,* 2021), therefore, the presence of PrimaLac supplement in the diet can be considered as the reason for this positive performance of fish in this feature. Similar results were obtained in other studies for probiotics based on *Streptococcus faecium* in carp, and groups fed with probiotics had the highest weight and specific growth factor (Frizzo *et al,* 2008). In the present study, the treatments receiving the probiotic PrimaLac showed a higher food conversion ratio and higher protein consumption efficiency than the control group. Fish feed supplemented with probiotics improves diet and protein digestion, which justifies higher growth and better feed conversion ratio (Ramos *et al,* 2017). Growth is directly related to nutrition (Piri Gharaghie *et al,* 2020). The use of high-quality food rations causes the fish to reach the market weight by consuming less food in a shorter period, and in this way, the production costs are significantly reduced (Bernal *et al,* 2015). Therefore, to improve the growth indicators in a certain period, the survival rate and the efficiency of the consumed ration, the use of growth stimulants and nutritional supplements can be beneficial (Bernal *et al,* 2015). Probiotic bacteria are live microbial food supplements that have beneficial effects on the host animal by creating microbial changes in the intestine (Hernández-Mendoza *et al,* 2022). A study has been conducted on Nile tilapia fish, in which a decrease in food conversion factor was observed in the group that consumed food supplemented with probiotics. Therefore, it was suggested that the utilization of food even under stress conditions is increased by probiotics (Samson, 2022). The results obtained in Japan regarding the status index of probiotics showed that probiotics cause an increase in the status index (Shimizu *et al,* 2105), but they obtained different results in rainbow salmon and salmon that did not have such an effect on their status index (Nimalan *et al,* 2022). This difference can be the way of feeding fish with probiotics. The present study, in line with the previous studies, showed that the way of feeding has a significant effect on the change of physical indicators, so that the fish that received the daily probiotic diet showed better physical indicators. Research has shown that some aerobic bacteria such as *Bacillus circulants* and *B.* subtilis isolated from the

microbial population of the digestive system of farmed fish such as Katla 1, Roho 2, common carp, silver carp 3, grass-eating carp 4, marigal 5, and tilapia 6 have extracellular enzymes such as lipase and protease (Nimalan *et al.,* 2022). It has also been reported that probiotic bacilli in relation to Indian white shrimp caused a significant increase in digestive enzymes including protease and lipase in control treatments (Gómez *et al.,* 2008). Also, increasing the exploitation of carbon hydrates has basically provided part of the maintenance energy in these fishes, and finally, due to less consumption of protein in providing the energy needed by the body; we have an increase in protein (Gómez *et al.,* 2008). Cholesterol and triglyceride levels increased in all three groups receiving probiotics compared to the control group ($p < 0.05$). In relation to both fat factors, the highest increase was observed in the group that received the probiotic daily and the lowest increase in the group that received the probiotic every other day. In research conducted by Hosseini *et al*. (2013) to determine the effect of probiotics based on *Pediococcus lactic* 1 on the growth and blood and serum factors of Caspian salmon, cholesterol and triglyceride levels were the highest in the probiotic treatment. The addition of photosynthetic bacteria and *bacillus* sp. as probiotics to the diet of cultured carp showed that the number of digestive enzymes increased in the experimental treatments compared to the control group (Hosseini *et al*., 2013). Probiotics have increased the specific activity of this enzyme in the digestive tract of fish by secreting more and more lipase enzyme. Serum enzymes are mainly influenced by physiological and environmental factors. For example, the type of diet, ambient temperature, fish age, and salinity are effective in the level of serum enzymes and their activity (Assan *et al*., 2022). The amount of SGOT and SGPT enzymes in the liver decreased in the three groups that received probiotics compared to the control group ($p < 0.05$). Rahmani Asgarabadi (2013) showed a positive effect on the level of liver enzymes. The amount of amylase enzyme and glucose increased in all three probiotic groups compared to the control group ($p < 0.05$). For both factors, the highest amount was observed in the group that received daily probiotics. An increase in amylase can be justified by an increase in glucose. In fact, with the increase of carbohydrate, its decomposing enzyme (amylase) also increases (Peyrot *et al*., 2016).

External factors such as diet, age, time of the last meal, season, and temperature can affect glycogen reserves and increase or decrease blood sugar in fish. In fact, the change in blood glucose level is not only dependent on the action of cortisol and various factors are effective in it (Peyrot *et al*., 2016). According to the results obtained from this research, the amount of cortisol hormone was the highest in the group that was not fed with probiotics, and it was lower in the groups that received probiotics. This difference is significant in all three probiotic groups compared to the control group ($p < 0.05$). The lowest amount of cortisol was observed in the group that received probiotics daily. In a study, it was observed that *Lactobacillus delbrueckii* on sea jasmine reduced cortisol levels from 6.3 ng/g to 1.5 ng/g (Alverson *et al*., 1994). One of the many effects of the cortisol hormone is to increase the body's resistance during stress by reducing the absorption of glucose and intensifying the need for sodium and water in the body (Stachowicz *et al*., 2016). Blood sugar is one of the important factors that is usually influenced by hormones and hormonal control, and increasing the amount of stress and secretion of cortisol hormone can affect fish plasma glucose (Hafez *et al*., 2013). As for the weight gain index, it was higher in the groups that received probiotics than the control group ($p < 0.05$). Among the groups receiving probiotics, the group that received it daily showed a higher amount than the other two groups that received it one day in between and two days in between ($p < 0.05$). In line with the results of the present study, in a study that was conducted to investigate the effects of PrimaLac probiotic on the growth and survival factors of Iranian fish, the groups that received probiotics had a higher weight gain (Salaghi *et al*., 2013).

Based on the findings of this research, PrimaLac probiotic can be considered as a useful substance that affects the serum factors of fish blood, and the amounts of total protein and albumin. The daily consumption of probiotics has increased compared to the control group, which shows the positive effect of PrimaLac probiotic on these two factors effective in growth. Cortisol factor has decreased in all three groups compared to the control group, and this decrease in serum cortisol level improves the function of the immune system. Therefore, it seems that the presence of probiotics can stimulate the production of digestive enzymes and thereby improve the digestion of protein, starch, fat and cellulose, which ultimately causes better growth.

Acknowledgment

This research has been financially supported by Science and Research Branch, Islamic Azad University, Tehran, Iran.

Conflict of Interest

None declared.

References

Alverson, DL and Larkin, PA (1994). Fisheries: Fisheries science and management-Century 21. Fisheries: Fisheries Science and Management-Century 21, PP: 150-167.

Assan, D; Kuebutornye, F.K; Hlordzi, V; Chen, H; Mraz, J; Mustapha, UF and Abarike, ED (2022). Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: A mini review. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 257: 110653.

Beiranvand, S; Piri-Gharaghie, T; Dehganzad, B; Khedmati, F; Jalali, F; AsadAlizadeh, M and Momtaz, H (2022). Novel NAD-independent Avibacterium paragallinarum: Isolation, characterization and molecular identification in Iran. Vet. Med. Sci., 8: 1157-1165.

Bernal, MG; Campa-Córdova, ÁI; Saucedo, PE; González, MC; Marrero, RM and Mazón-Suástegui. JM (2015). Isolation and in vitro selection of actinomycetes strains as potential probiotics for aquaculture. Vet. World, 8: 170.

Biermann, G and Geist, J (2019). Life cycle assessment of common carp (*Cyprinus carpio* L.)–A comparison of the environmental impacts of conventional and organic carp aquaculture in Germany. Aquac., 501: 404-415.

Burridge, L; Weis, JS; Cabello, F; Pizarro, J and Bostick, K (2010) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquac., 306: 7-23.

Das, TK; Pradhan, S; Chakrabarti, S; Mondal, KC and Ghosh, K (2022). Current status of probiotic and related health benefits. Appl. Food Res., 2: 100185.

De, C; Meena, DK; Behera, BK; Das, P; Das Mohapatra, PK and Sharma, AP (2014) Probiotics in fish and shellfish culture: Immunomodulatory and ecophysiological responses. Fish Physiol. Biochem., 40: 921-971.

Felicilda-Reynaldo, RF and Kenneally, M (2016). Digestive enzyme replacement therapy: Pancreatic enzymes and lactase. Med. Surg. Nurs., 25: 182.

Frizzo, LS; Zbrun, MV; Soto, LP and Signorini, ML (2011). Effects of probiotics on growth performance in young calves: A meta-analysis of randomized controlled trials. Anim. Feed Sci. Technol., 169: 147-156.

Ghajari, G and Moosavi, R (2022). Evaluation of the effects of Diazinon toxin on some reproductive parameters in male rats. Pers. Med. J., 7: 30-35.

Ghajari, G; Heydari, A and Ghorbani, M (2022). Mesenchymal stem cell-based therapy and female infertility: Limitations and advances. Curr. Stem Cell Res. Ther., 18: 322-338.

Ghajari, G; Nabiuni, M and Amini, E (2021). The association between testicular toxicity induced by Li2Co3 and protective effect of Ganoderma lucidum: Alteration of Bax & c-Kit genes expression. TISSUE CELL., 72: 101552.

Goh, JX; Tan, LT; Law, JW; Ser, HL; Khaw, KY; Letchumanan, V and Goh, BH (2022). Harnessing the potentialities of probiotics, prebiotics, synbiotics, paraprobiotics, and postbiotics for shrimp farming. Rev. Aquac., 14: 1478-1557.

Gómez, RG and Shen, MA (2008). Influence of probiotics on the growth and digestive enzyme activity of white Pacific shrimp (Litopenaeus vannamei). J. Ocean Univ. China., 7: 215-218.

Guo, X; Chen, DD., Peng, KS; Cui, Z.W; Zhang, XJ; Li, S and Zhang, YA (2016). Identification and characterization of Bacillus subtilis from grass carp (*Ctenopharynodon idellus*) for use as probiotic additives in aquatic feed. Fish Shellfish Immunol., 52: 74-84.

Hafez Amini, P; Arian, Sh and Prior, K (1381). Investigating the effects of sodium chloride stress on sugar and cortisol hormone in common carp (*Cyprinus carpio*). Iran. J. Fish. Sci., 3: 35-42.

Hatlen, B; Grisdale-Helland, B and Hellan, SJ (2005). Growth feed utilization and body composition system of juvenile rainbow trout (*Oncorhynchus mykiss*) in the Southern Hemisphere. Aquac., 221: 581-591.

Hernández-Mendoza, A; González-Córdova, AF and Martínez-Porchas, M (2022). Influence of probiotics on the animal gut microbiota and their impact on the bioavailability of toxic agents: An opinion paper. Front. Nutr., 9: 870162.

Hosseini, A; Oraji, H; Yegane, S; Shahabi, H (2013). Effect of *Pediococcus lactic* acid probiotic on growth, blood and serum factors in Caspian Sea salmon. Iran. J. Fish. Sci., 23: 35-44.

Jega, IS; Haque, MM and Miah, M.I (2018). Analogical viewpoint of fisheries and aquaculture between Bangladesh and Nigeria: potential of knowledge transferability: Analogical perspective between Bangladesh and Nigeria Fisheries and Aquaculture. J. Bangladesh Agri. Univ., 16: 523-532.

Kalfagianni, A and Pattberg, P (2013). Fishing in muddy waters: Exploring the conditions for effective governance of fisheries and aquaculture. Mar. Policy., 38: 124-132.

Nimalan, N., Sørensen, S.L., Fečkaninová, A., Koščová, J; Mudroňová, D; Gancarčíková, S and Sørensen, M (2022). Mucosal barrier status in Atlantic salmon fed marine or plant-based diets supplemented with probiotics. Aquac., 547: 737516.

Peyrot des Gachons, C and Breslin, PA (2016). Salivary amylase: Digestion and metabolic syndrome. Curr. Diab. Rep., 16: 1-7.

Pham, VT; Dold, S; Rehman, A; Bird, JK. and Steinert, RE (2021). Vitamins, the gut microbiome and gastrointestinal health in humans. Nutr. Res., 95: 35-53.

Piri Gharaghie, T; Beiranvand, S and Hajimohammadi, S (2021). Comparison of antifungal effects of aquatic and alcoholic extract of *Mentha pulegium* L. with fluconazole on growth of *Candida albicans*. Deve. Biol., 13: 7-18.

Piri Gharaghie, T; Beiranvand, S; Doosti, A; Ghadiri, AH and Haji Mohammadi, S (2020). A review of the epidemiology and clinical signs of SARS-COV-2. NCMBJ., 11: 103-120.

Piri-Gharaghie, T; Beiranvand, S; Riahi, A; Shirin, N.J; Badmasti, F; Mirzaie, A; Elahianfar, Y; Ghahari, S; Ghahari, S; Pasban, K and Hajrasouliha, S (2022). Fabrication and characterization of thymol-loaded chitosan nanogels: Improved antibacterial and anti-biofilm activities with negligible cytotoxicity. Chem. Biodiversity., 19: e202100426.

Piri-Gharaghie, T; Doosti, A and Mirzaei, SA (2022). Identification of antigenic properties of *Acinetobacter*

baumannii proteins as novel putative vaccine candidates using reverse vaccinology approach. Appl. Biochem. Biotechnol., 194: 4892-4914.

Piri-Gharaghie, T; Jegargoshe-Shirin, N; Saremi-Nouri, S; Khademhosseini, S.H; Hoseinnezhad-Lazarjani, E; Mousavi, A; Kabiri, H; Rajaei, N; Riahi, A; Farhadi-Biregani, A and Fatehi-Ghahfarokhi, S (2022). Effects of Imipenem-containing Niosome nanoparticles against high prevalence methicillin-resistant *Staphylococcus epidermidis* biofilm formed. Sci. Rep., 12: 5140.

Rahmani Asgarabadi, Farshad (2013). Investigating the growth performance and biochemical parameters of the body extract of common carp (*Cyprinus carpio*) larvae in supplementation with probiotic bacilli. Master's thesis, Gonbad Kavos University - Gonbad Faculty of Agriculture.

Ramos, MA; Batista, S; Pires, MA; Silva, AP; Pereira, LF; Saavedra, MJ and Rema, P (2017). Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. Anim., 11: 1259-1269.

Salaghi, Z; Imanpuor, M and Taghizadeh, V (2013). Effect of different levels of probiotic primalac on growth performance and survival rate of Persian sturgeon (*Acipenser persicus*). Glob. Vet., 11: 238-242.

Samson, JS (2022). Effect of probiotic *Bacillus* spp.-supplemented feed on the growth, length-weight relationship, and condition factor of Nile tilapia (*Oreochromis niloticus*). J. Exp. Biol. Agric. Sci., 10: 90-96.

Shimizu, M; Hashiguchi, M; Shiga, T; Tamura, HO and Mochizuki, M (2015). Meta-analysis: Effects of probiotic supplementation on lipid profiles in normal to mildly hypercholesterolemic individuals. PLoS One., 10: e0139795.

Stachowicz, M and Lebiedzińska, A (2016). The effect of diet components on the level of cortisol. Eur. Food Res. Technol., 242: 2001-2009.

Swain, SK; Rangacharyulu, PV; Sarkar, S and Das, KM (1996). Effect of a probiotic supplement on growth, nutrient utilization and carcass composition in mrigal fry. J. Aquac., 4: 29-35.

Taylor, JJ; Rytwinski, T; Bennett, JR; Smokorowski, KE and Cooke, SJ (2017). The effectiveness of spawning habitat creation or enhancement for substrate spawning temperate fish: a systematic review protocol. Environ. Evid., 6. 10.1186/s13750-017-0083-1.

Thornber, K; Verner-Jeffreys, D; Hinchliffe, S; Rahman, MM; Bass, D and Tyler, CR (2020). Evaluating antimicrobial resistance in the global shrimp industry. Rev. Aquac., 12: 966-986.

Wang, A; Ran, C; Wang, Y; Zhang, Z; Ding, Q; Yang, Y; Olsen, RE; Ringø, E; Bindelle, J and Zhou, Z (2019). Use of probiotics in aquaculture of China—a review of the past decade. Fish Shellfish Immunol., 86: 734-755.

Wang, YB; Li, JR and Lin, J (2008). Probiotics in aquaculture: Challenges and outlook. Aquac, 281: 1-4.