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Application of probiotic bacteria in aquaculture for safe fish production

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By

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ABSTRACT

Aquaculture is one of the largest food producing sectors of the world. During the last few years world aquaculture has immensely grown and becoming an economically significant zone. But in the time of culture aquatic organisms are subjected to stress condition. Fish immune system hampered by this. It also increases the susceptibility to diseases. Thus, affects both economic development and socio-economic status of the local people in many countries. Disease control in fisheries industry has been achieved by different methods. These are traditional ways, synthetic chemicals and antibiotics. Application of antimicrobial drugs and pesticides has carried to the evolution of resistant strains of bacteria. Therefore, substitute methods are much more necessary to maintain a healthy microbial environment in the aquaculture systems. So probiotics may become alternative to antibiotics. Various bacteria have been seen used as probiotics in different experiments, mainly as a supplement in feed at different concentration in which beneficial effect like better growth with reduced production cost, better reproductive performances, immunology, disease resistance have found for probiotic bacteria treated fishes. Different sectors of the aquaculture industry would benefit if cultured organisms were conferred with high growth performance, feed efficiency and disease resistance. As such, the cost of medicine and production costs could be reduced and consumer perceptions would be improved. This article outlines importance of probiotic bacteria is for safe food production in aquaculture.

Keywords: Aquaculture, probiotic, bacteria, fish, resistance, antibiotics

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[1]A seminar paper for the course GFB 598, Summer’ 2020.

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CHAPTER 1

INTRODUCTION

Probiotic is a cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal (microbial) balance (Irianto and Austin, 2020). The probiotics are live microbial incorporated feed which provide positive effect on the host animal by altering its intestinal microbes (Fuller, 1989). The mode of action of the probiotics is rarely investigated. But possibilities include competitive exclusion, i.e. the probiotics inhibit the colonization of potential pathogens in the digestive tract by their antimicrobial compound or competition for nutrients and/or space (Irianto and Austin, 2020)

Aquaculture is one of the fastest growing agriculture sectors in the world, providing food and nutritional security to millions of people (Gatlin, 2002). Compared with farm animals, fish is a better source of high-quality protein, micronutrients, particularly phosphorus, selenium and iron, and essential fatty acids, especially long-chain polyunsaturated fatty acids (LC-PUFA) (Tacon & Metia, 2013). However, disease outbreaks are a constraint to aquaculture production, thereby affecting the socio-economic status of people in many countries (Gatlin, 2002). Due to intensive farming practices, infectious diseases are a major problem in finfish and shellfish aquaculture, causing heavy loss to farmers (Cabello, 2006; Romero et al. 2012). For instance, disease is now considered to be the limiting factor in the shrimp farming industry. Disease control in the aquaculture industry has been achieved using various methods, including traditional means, synthetic chemicals and antibiotics (Panigrahi and Azad 2007). In the 1970s and 1980s oxolinic acid, oxytetracycline (OTC), furazolidone, potential sulphonamides (sulphadiazine and trimethoprim) and amoxicillin were the most commonly used antibiotics in fish farming. However, the indiscriminate use of antibiotics in disease control has led to selective pressure of antibiotic resistance in bacteria, a property that may be readily transferred to other bacteria (Cabello, 2006; Romero et al. 2012). Traditional methods are ineffective against controlling new disease in large aquaculture systems. Therefore, alternative methods need to be developed to maintain a healthy microbial environment in aquaculture systems, thereby maintaining the health of the cultured organisms. The use of prebiotics, probiotics and immune nutrients is growing as means of producing healthy organisms (Panigrahi and Azad 2007).

Probiotics are micro-organisms or their with health benefit to the host. It has found use in aquaculture as a means of disease control, supplementing or even in some cases replacing the use of antimicrobial compounds. The use of probiotics has been accompanied by a concomitant reduction in the levels of antimicrobial compounds (particularly antibiotics) used in aquaculture and in improved appetite and/or growth performance of the farmed species. The former is obvious in so far as if the animals are otherwise healthy then there will not be any need to use antimicrobial compounds (Irianto and Austin, 2020).

The demand for food fish is increasing day by day. Use of antibiotics for fish growth promotion has been banned. Public awareness also increased for healthy fish production. These led to an increased interest in the potential of functional feeds as health promoters. Probiotics are improves fish in feed efficiency, growth, immune status, digestive enzyme activities, gut morphology, disease resistance and stress responses (Guerreiro et al., 2017). As alternative to antibiotics, functional ingredients such as probiotics, prebiotics and synbiotics become to be used in aquaculture and other animal production industries to promote animal health and well-being (Ringø et al. 2010; Dimitroglou et al., 2011a; Carbone & Faggio, 2016; Dawood & Koshio 2016).

1.1.Objectives

After reading this article, readers will be able:

1. To develop an alternative method need to maintain a healthy microbial environment in aquaculture systems.

2. To highlight the roles of probiotic bacteria in the growth performances, digestion, immunology, reproductive performance, water quality management of fish.

3. To review the possible applications of probiotics in a prominent fast-growing aquaculture industry

CHAPTER 2

MATERIALS AND METHODS

This seminar paper is exclusively a review paper. All of the information has been collected from the secondary sources. It has been prepared by reviewing the various articles, books, journals, reports, publications etc. published in different available in the internet. I got suggestion and valuable information from my major professor and my course instructors. After collecting all the available information, I compiled and prepared this seminar manuscript systematically and chronologically to enrich this paper.

CHAPTER 3

REVIEW OF FINDINGS

3.1. Probiotic bacteria

A wide range of yeasts (Debaryomyces, Phaffia and Saccharomyces), microalgae (Tetraselmis), Gram-negative bacteria (Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas and Vibrio) and Gram-positive (Bacillus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Micrococcus, Streptococcus and Weissella) and has been evaluated as probiotic strains (Irianto and Austin, 2002). The recent identified probiotics that influence fish immune system, disease resistance and other performance indices include: *Bacillus subtillis*, *Bacillus circulans*, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Lactobacillus rhamnosus*, *Carnobacterium maltaromaticum*, *Carnobacterium divergens*, *Carnobacterium inhibens*, *Saccharomyces cerevisiae* and *Candida sake*.

3.1.1. Characteristics of Probiotics

Efficient probiotics must be (a) resistant to pH and bile acids, (b) have no pathogenicity, (c) be viable, (d) be stable in storage and in field, (e) survive and potentially colonize in the gut, (f) be cultivable on a large scale, (g) be able to adhere to the epithelial lining of the gut, and (h) affect host animals beneficially. All new strains used for probiotic development should possess all the aforementioned characteristics (De et al., 2009).

3.1. 2. Types of Probiotics

Probiotics are classified into following two groups:

3.1.2.1 Feed Probiotics

Some bacterial, fungal strains can be blended with feeding pellets or by encapsulating into live feed stock or administered orally to feed rearing animals to prevent disease and enhance essential microbial flora of the gut (Prasad et al., 2003; Nageswara and Babu, 2006).Viability of strains should be tested before feeding animals. Probiotics like lactic acid bacteria applied in the feed of fry of Atlantic cod, showed adequate growth, survival and immune response (Gildberg et al., 1997).

3.1.2.2 Water probiotics

According to Prasad *et al*. (2003) water probiotics are applied to reduce organic pollutants and various contaminants in water by directly applying to rearing medium. These convert organic matter to smaller units and improve water quality. Breakdown of organic matters evolve simpler substances like glucose and amino acids that are used as food for beneficial bacteria which reduce the accumulation of organic pressure and provide congenial environment to farmed stock. Probiotic bacteria such as *Bacillus* sp. can change organic matter to CO2 so that organic waste can be minimized in aquaculture system. By using nitrifying bacteria, the quantity of nitrate, nitrite, ammonia are reduced to a large extent. These lead to purify the water in the hatchery enhancing larval survival and growth (Moriarty et al, 1998; Lipton et al., 1997).

3.2. Significance of Probiotic in aquaculture system

Great impact on aquatic organisms can be seen by probiotics use in aquaculture. Probiotics decrease organic load and maintain water quality parameter in an efficient way. Probiotic organism can easily meet the hope of sustainable aquaculture development. Because two major key factors of it disease resistance and growth performance can heighten by it (Dawood, 2016). Prasad et al., (2003) reported that *Lactic Acid Bacteria*, a popular probiotic strain, can be applied to control bacterial pathogen. In addition, another well-known probiotic organism, *Bacillus* sp. is used to remove metabolic waste in aquatic system. Many strains of *Aeromomas* sp., *Pseudomonas* sp., *Vibrio* sp. act against infectious hematopoietic necrosis virus to show antiviral activity (Kamei, 1998). These probiotic organisms may be used singly or in combination such as incorporation of individual or combined supplementation of *Lactobacillus rhamnosus* and *Lactobacillus sporogenes* enhance health and disease resistance of common carp (Allameh , 2014; Chi, 2014; Faramazi, 2011; Harikrishnan, 2010). Probiotics does not pollute water because of their eco-friendly nature. So that it is more and more suitable for aquaculture system. They not only promote animal health but also maintain consumer health safety (Prasad, 2003). Uses of probiotics and their target aquatic organisms are displayed in Table -1.

Table-1: Uses of Probiotic in aquaculture system.

Uses of Probiotic	Probiotic Species	Target aquatic species	Reference
Water quality	*Lactobacillus acidophilus*	*Clarias gariepinus*	Dohail et al.(2009)
Control of diseases	*Enterococcus faecium* SF 68 *Pseudomon fluorescens* *Lactococcus lactis* *Pseudomonas* sp.	*Anguilla Anguilla* *Oncorhynchus mykiss* *Epinephelus coioides* *Oncorhynchus mykiss*	Chang and Liu (2002) Gram et al.(1999) Zhang et al. (2012) Spanggaard et al. (2001)
Growth promoter	*Lactobacillus lactis* AR21 *Bacillus* sp. *Streptococcus thermophiles* *Bacillus coagulans*	*Brachionus plicatilis* Catfish *Scophthalmus maximus* *Cyprinus carpio koi*	Harzeveli et al.(1998) Queiroz et al. (1998) Gatesoupe et al. (1999) Lin et al. (2012) .
Digestion	*Lactobacillus acidophilus* *Lactobacillus helveticus*	*Clarias gariepinus* *Scophthalmus maximus*	Dohail (2009) Gatesoupe (1999)
Improvement of immune response	*Clostridium butyricum* *L. casei* *L. acidophilus*	*Rainbow trout* *Poecilopsis gracilis* *Paralichthys olivaceus*	Sakai (1995) Hernandez (2001) Taoka (2006)

3.3. Fish responses to dietary supplementation with various probiotics

Prebiotic have been reported to have numerous beneficial effects in terrestrial animals such as increased disease resistance and improved nutrient availability.

Table-2: Summary of fish responses to dietary supplementation with various probiotics.

Probiotic	Species	Measured response	Reference
Live bacteriophage	Ayu	Resistance to *Pseudomonas plecoglossicida*	Park et al. (2000)
Aeromonas media strain A199	Eel	Resistance to *Saprolegnia parasitica*	Lategan et al. (2004)
Bacillus subtilis and *B. licheniformis*	Rainbow trout	Resistance to *Yersinia ruckeri*	Raida et al. (2003)
Bacillus circulans	Rui	Immune enhancer and Control *A. hydrophila*	Bandyophyay and Das Mohapatra (2009)
B. pumilus	Tilapia	Immunity enhencer and Better survival	Aly *et al*. (2008)
Bacilllus subtillis and *Lactobacillus delbriieckii*	Gilthead seabream	Cellular innate immune response	Salinas et al. (2005)
Carnobacterium divergens	Atlantic cod	Survival , Resistance to *Vibrio anguillarum*	Gildberg et al. (1997) Gildberg and Mikkelson (1 998)
Enterococcus faecium	European eel	Resistance to *Edwardsiella tarda*	Chang and Liu (2002)
L. acidophilus	African catfish	Better growth performance, haematological parameters and immunological profile	Al-Dohail *et al*., (2009)
Saccharomyces cerevisiae	Nile tilapia	Weight gain and feed efficiency	Lara-Flores et al. (2002)

3.4. Role of probiotics in carp aquaculture:

Researchers have demonstrated the use of probiotics have positive impact in carp species. Probiotic applications in carps refer to Table- 3.

Table-3: Studies using probiotics in carp aquaculture.

Species	Probiotic	Results	References
Catla	B. circulans PB7	Weight gain, feed conversion ratio, protein efficiency ratio increase	Bandyopadhyay and Mohapatra (2009)
Common carp	Streptococcus faecium, L. acidophilus and S. cerevisiae	Weight gain, specific growth rate, protein efficiency ratio increase	Faramarzi et al. (2011)
Rui	L. plantarum VSG3	Specific growth rate, feed conversion ratio increase	Giri et al. (2013)
Gibel carp	S. cerevisiae	Final weight, weight gain, specific growth rate, feed conversion ratio	He et al. (2011)
Koi carp	L. acidophilus and/or S. cerevisiae	Weight gain, specific growth rate, feed conversion ratio increase	Dhanaraj et al. (2010)
Grass carp	B. subtilis Ch9	Specific growth rate, feed conversion ratio increase	Wu et al. (2012)

3.5. Probiotics in immune system

Probiotics are beneficial bacteria capable of inhibiting not only pathogens, but also regulate the host immune system (Fig-1). Immunomodulation by probiotics are considered as a community effort of the introduced microorganism, host and commensals. The host can detect whether the organism is pathogenic or not through pathogen pattern recognition receptors (PRRs). To identify these recognition receptors, the microbial associated molecular patterns (MAMPs) which are present in both pathogenic and non-pathogenic microorganism. Some MAMPs are lipopolysaccharides (LPS), peptidoglycan, flagellin, and microbial nucleic acids. The binding of MAMPs to PRRs trigger intracellular signaling cascade, urging the release of specific cytokines and transmit signals to adjacent cells, or to exert anti-viral, pro- or anti-inflammatory exercise effects. The same mechanism of recognition regulates the homeostasis of the commensal of microbiota in the mucosa. Moreover, probiotics can also manipulate the richness and diversity of commensal microbiota (Nayak, 2010).

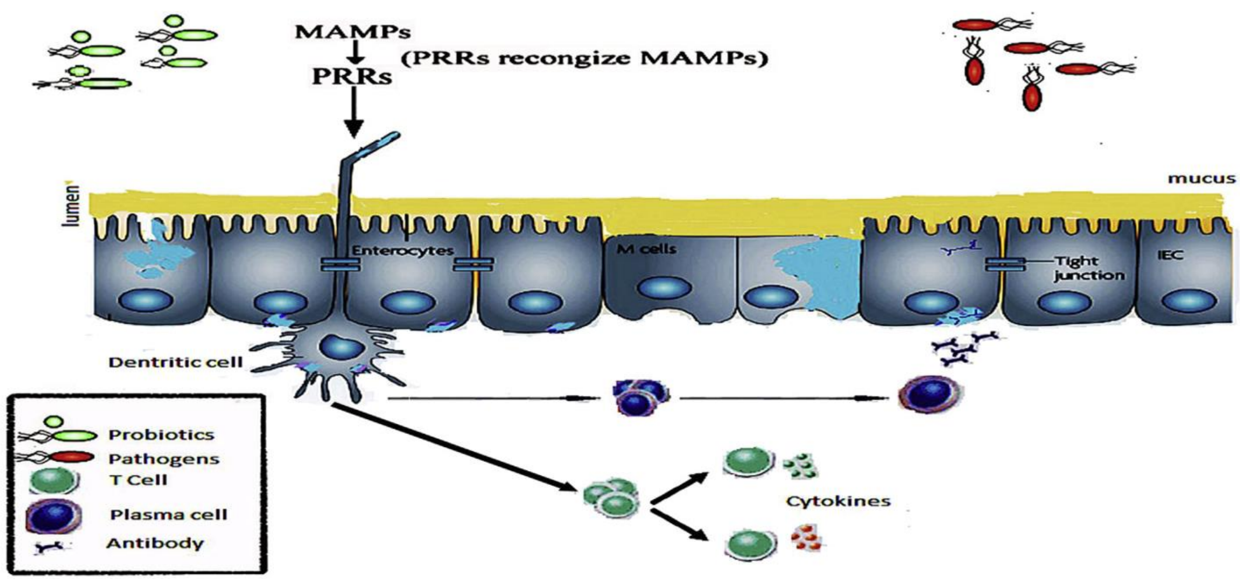

Fig.1. Probiotics showing the activity of host immunnomodulation. Abbreviations: MAMPs / Microbe associated molecular patters, PRRs / Pathogen pattern recognition receptors. (Source: Akhter et al., 2015)

Gupta et al. (2014) conduct a research on immune response and disease resistance of *Cyprinus carpio* fry. Laboratory maintained *B. coagulans*, *B. licheniformis* and *P. polymyxa* were used to study activity against fish pathogen by agar well diffusion assay. For determination of its safety they challenge healthy fish fry by this bacterium. They fed fish control basal diet (B0) and experimental diets containing *B. coagulans* (B1), *B. licheniformis* (B2) and *P. polymyxa* (B3) at 109 CFU/g diet for 80 days. Various disease resistance study and immune parameters were conducted at 80 days post-feeding. The antagonism study showed inhibition zone against *Aeromonas hydrophila* and *Vibrio harveyi*. No mortality or morbidities were observed of the challenge. So it can be said that the probiotic bacterial strains were harmless to fish fry. Different non-specific innate immunological parameters viz. lysozyme activity, respiratory burst assay and myeloperoxidase content showed significant higher values in fish fry fed B3 diet at 109 CFU/g (Table-4).

Lysozyme is an important component of the immune system. It acted as defensive factor sagainst invasive microorganisams in vertebrates (Ellis, 1990). Lysozyme disrupts bacterial cell walls by splitting glycosidic linkages in the peptidoglycan layers (Alexander, 1992). In the present study, fish fed diets supplemented with different probiotics showed significantly higher lysozyme activities after 80 days of feeding than the control group.

Respiratory bursts are produced by phagocytes to attack invasive pathogens during phagocytosis and have been widely used to evaluate host defense capabilities against pathogens (Dalmo, 1997). In this study showed that fish groups fed with different probiotic supplements had significantly higher respiratory bursts than the control group.

The myeloperoxidase is an important enzyme that utilizes oxidative radicals to produce hypochlorous acid to kill pathogens. During oxidative respiratory burst, it is mostly released by the azurophilic granules of neutrophils (Das, 2013; Dalmo, 1997). In the present study myeloperoxidase content of serum was significantly higher after 80 days of feeding with *Bacillus coagulans*, *B. licheniformis* and *P. polymyxa* supplemented diets.

Table-4: Non-specific immune response of *Cyprinus carpio* fry fed basal diet and diet supplemented with *Bacillus coagulans, B. licheniformes* & *Paenibacillus polymexa* as probiotics for 80 day. B0 (control): fish fed with basal diet. B1, B2 and B3: fish fed with basal diet supplemented with *Bacillus coagulans, B. licheniformis* and *Paenibacillus polymyxa* at 10^9 CFU/g, respectively.

Immune response	B0 (control)	B1	B2	B3
Lysozyme (U/ml)	31.16	42.3	42.53	44.39
Respiratory burst (OD 540 nm)	0.414	0.498	0.941	0.989
Myeloperoxidase (OD 450 nm)	0.115	0.138	0.157	0.193

(Source: Gupta et al., 2014)

The challenge test showed dietary supplementation of *B. coagulans*, *B. licheniformis* and *P. polymyxa* significantly enhanced the resistance of fish fry against bacterial challenge. This result indicate that *Paenibacillus polymyxa* is a potential probiotic species and can be used in aquaculture to improve growth performance, digestion, immune responses and disease resistance of fry common carp, *Cyprinus carpio*.

3.6. Dietary probiotics influence growth performance

A study by Munir et al., (2016) evaluate the effect of snakehead (*Channa striata*) fingerlings feeding with selected probiotics (Saccharomyces cerevisiae, Lactobacillus acidophilus)and β-glucan, galacto-oligosaccharide(GOS), mannanoligosaccharide (MOS) stimulants. Best performance was found in fish whish fed with L. acidophilus supplement. The growth trends were lower in all other compared to fish on the probiotics supplemented diets. The result obtained from this study showed that supplementation with *L. acidophilus* is best for growth.

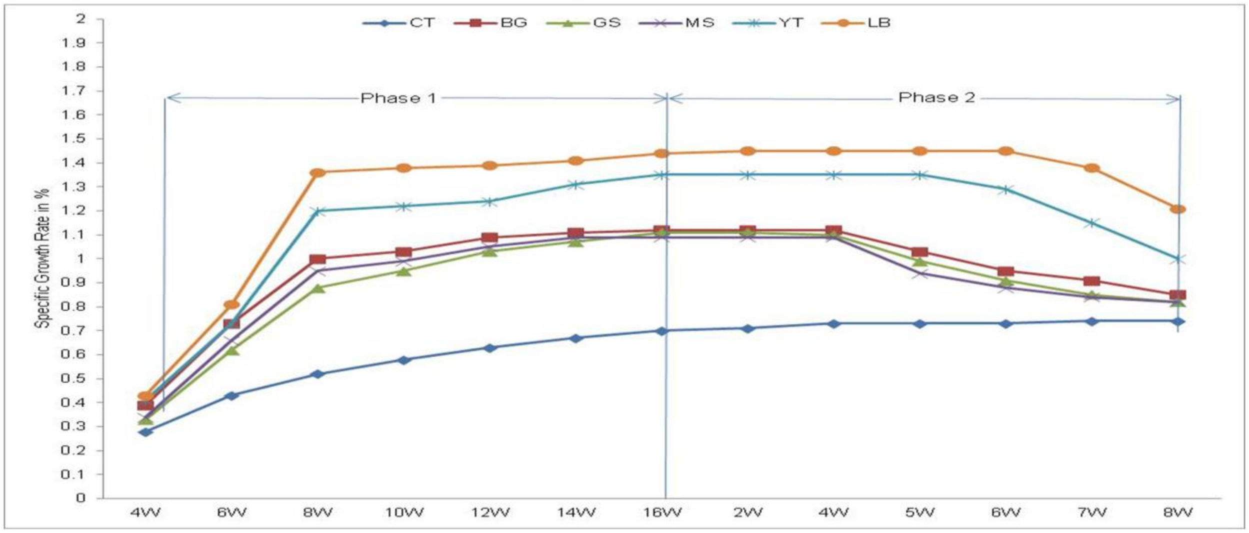

Fig.2. Comparison of specific growth rate of *Channa striata* fingerlings feeding with different diet. CT=control without any supplementation; BG=feed with β-glucan; GS=feed with glacto-oligosaccharides; MS=feed with mannan-oligosaccharides; YT = feed with live yeast; LB= feed with Lactobacillus acidophilus (Source: Munir et al., 2016)

Wang, Y. B., (2007) Conduct a research on Effect of probiotics on growth performance of the shrimp (*Penaeus vannamei)*. Photosynthetic bacteria and Bacillus sp. were added to shrimp basal diets as probiotics at three concentrations: T-1, 2gkg− 1 (1 g kg− 1 lyophilized photosynthetic bacteria cells (PSB) and 1 g kg− 1 lyophilized Bacillus sp. (BS); T-2, 10gkg− 1 (5gkg− 1PSB and 5gkg− 1 BS); and T-3, 20 g kg− 1 (10 g kg− 1 PSB and 10 g kg− 1 BS). After 28 days, shrimp receiving the diets supplemented with probiotics showed significantly better growth performance than those fed the basal diet (Table-5).

Table-5: Effect of basal diet supplemented with different concentrations of probiotics (T1–T3) on growth performance of shrimps.

	Control	**T-1 (2gkg− 1)**	**T-2 (10gkg− 1)**	**T-3 (20 g kg− 1)**
Initial Weight (g)	0.66	0.65	0.66	0.66
Final Weight (g)	1.57	1.69	1.73	1.73
DG (g d− 1)	0.0325	0.0369	0.0380	0.0384
RGR (%)	1.3784	1.5856	1.6190	1.6566

(Source: Wang Y. B., 2007)

3.7. Effects of probiotics on digestion

Wang, Y. B. (2007) conducted a research on Effect of probiotics on digestive enzyme activity of the shrimp (*Penaeus vannamei)*. Photosynthetic bacteria and Bacillus sp. were added to shrimp basal diets as probiotics at three concentrations: T-1, 2gkg− 1 (1 g kg− 1 lyophilized photosynthetic bacteria cells (PSB) and 1 g kg− 1 lyophilized Bacillus sp. (BS);T-2,10gkg− 1 (5gkg− 1 PSB and5gkg− 1 BS); and T-3, 20 g kg− 1 (10 g kg− 1 PSB and 10 g kg− 1 BS). The mean digestive enzyme activity of each treatment groups was significantly different from that of the Control. The protease activity of T-2 and T-3 was significantly higher compared with T-1 and the Control. However, there was no significant difference between T-2 and T-3 (Fig-3).

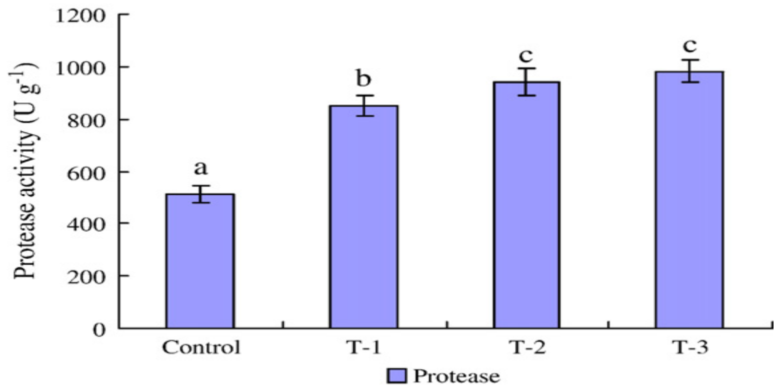

Fig.3. Specific activity of intestinal protease from shrimp fed a basal diet (Control) and three diets containing different concentrations of probiotics (T1–T3) at the end of 28 days culture.(Source: Wang, Y. B., 2007).

Amylase activity was significantly higher for T- compared to T-1 and the control. However, there was no significant difference between T-2 and T-3 (Fig-4).

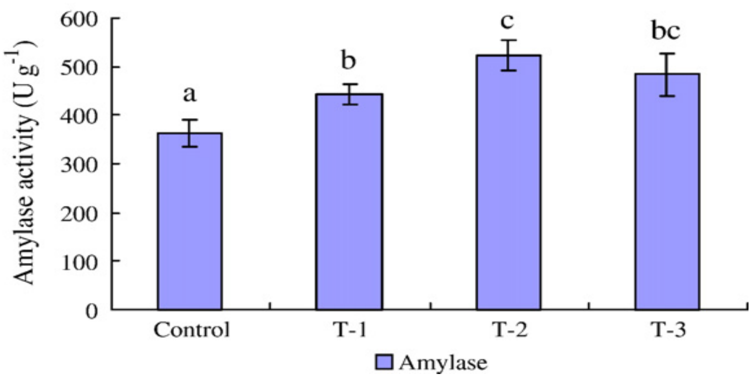

Fig.4. Specific activity of intestinal amylase from shrimp fed a basal diet (Control) and three diets containing different concentrations of probiotics (T1–T3) at the end of 28 days culture. (Source: Wang, Y. B., 2007).

The average values of lipase activity of shrimp intestines for all treatment groups differed with different concentrations of probiotics, and were significantly different from those of the Control (Fig-5).

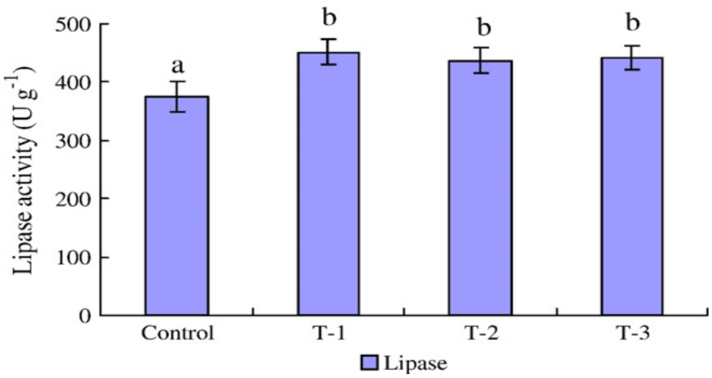

Fig.5. Specific activity of intestinal lipase from shrimp fed a basal diet (Control) and three diets containing different concentrations of probiotics (T1–T3) at the end of 28 days culture (Source: Wang, Y. B., 2007).

3.8. Effects on the reproduction

Rahman et al. (2018) conducted a research on probiotic enrich dietary effect of the reproduction of butter catfish (*ompok pabda).* 120 pairs of broodfish was used and fed with different levels of probiotic diets viz. 0 (served as a control), 0.6, 0.8 and 1.0g / kg diet under 4 treatments, i.e. PRO0, PRO1, PRO2 and PRO3. The experimental fish were observed for the success in reproductive performance like Gonado Somatic Index (GSI), percentage of fertilization, percentage of hatching. GSI, fertilization rate and percentage of hatching found increased in PRO2 treatment. Besides these, percentage of dead and deformed larvae was significantly lower in fish fed with the probiotic pro2 treatment.

3.8.1. Ganado-Somatic index

Gonado-somatic index give a clear indication about the gonadal development as well as breeding season of a fish. Data of gonado-somatic index is presented in Figure 1. In this case highest gonado-somatic index was found in PRO**2** (10.24±0.94) followed by PRO**1** (10.18±0.78), PRO**3** (9.94±0.77) and PRO**0** (9.62±1.71). The ANOVA test showed that there was a significant difference among treatments regarding gonado somatic index.

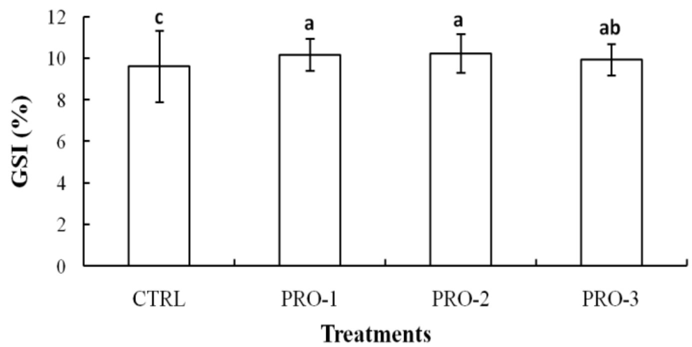

Fig.6. Comparison of gonado-somatic index of O. *pabda* under different levels of probiotic. (Source: Rahman et al., 2018)

3.8.2.Fertilization rate

Fertilization rate of *O. pabda* eggs were greatly influenced by probiotic feed although different probiotic concentration had no significant effect on it (Fig.-7). Highest fertilization rate (76.07±1.67) was recorded in PRO**2** followed byPRO**1 (**73.86±1.44) and PRO**3** (72.17±5.675) compared to the lowest fertilization rate in PRO**0** (59.24±2.853).

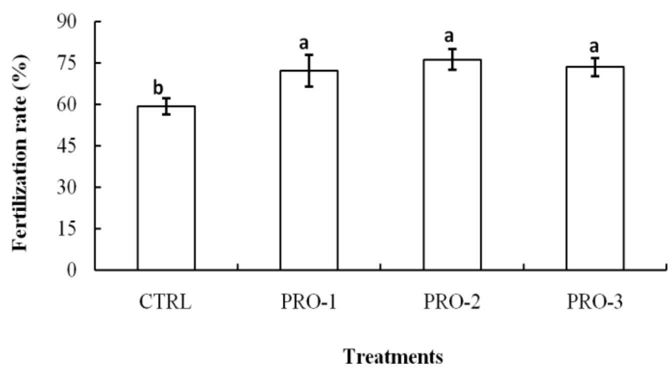

Fig.7. Comparison of fertilization rate of the eggs produced by the O. *pabda* Brood fish reared under different levels of probiotic (Source: Rahman et al., 2018)

3.8.3. Hatching rate: Minimum hatching rate was recorded in PRO**0** (48.56±0.80) where all probiotic treatments showed better result than that of non-probiotic treatment (Fig.-8).Maximum hatching rate was found in treatment PRO**2** (78.65±4.17). PRO**3** showed the second highest hatching rate (73.06±3.21) following PRO**1** (52.41±5.06).

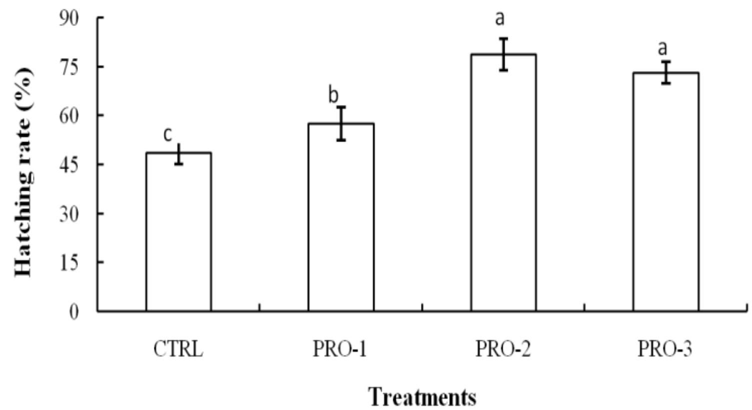

Fig.8. Comparison of hatching rate of eggs produced by female O. *pabda* reared under different levels of probiotic (Source: Rahman et al., 2018).

Overall, the results demonstrated the beneficial effects of probiotics on the reproductive performance of this indigenous species, as the GSI, fecundity and larval survival were significantly enhanced by probiotic administration. So, it could be concluded that the probiotic is useful for enhancing fish reproduction efficiency (especially for females) and development of fish gonad. So, probiotic can be used as brood stock feed additive in hatcheries which have a greater economic point of view.

Gioacchini et al. (2011) conduct a research on effects of probiotics on zebrafish reproduction. In this study, the effects of dietary probiotic *Lactobacillus rhamnosus* IMC 501 administration was investigated on female zebra fish (*D. rerio*) reproductive performance and ovarian development. After feeding the zebrafish experimental diets for 10 days, real-time PCR and culture based methods revealed alterations on gut microbiota, characterised by high levels of *L. rhamnosus* IMC 501 in the gut of probiotic treated zebrafish, which were absent in the control.

The microbial modulation in the gut was related with a significant increase of the gonadosomatic-index (GSI) associated, at the molecular level, to a significant increase of ovarian expression of cytochrome p 19 (cyp19a), hepatic vitellogenin (vtg) and the αisoform of the estradiol receptor (erα) genes evidencing a positive role of probiotic administration on the ovarian growth phase (Fig.-9).

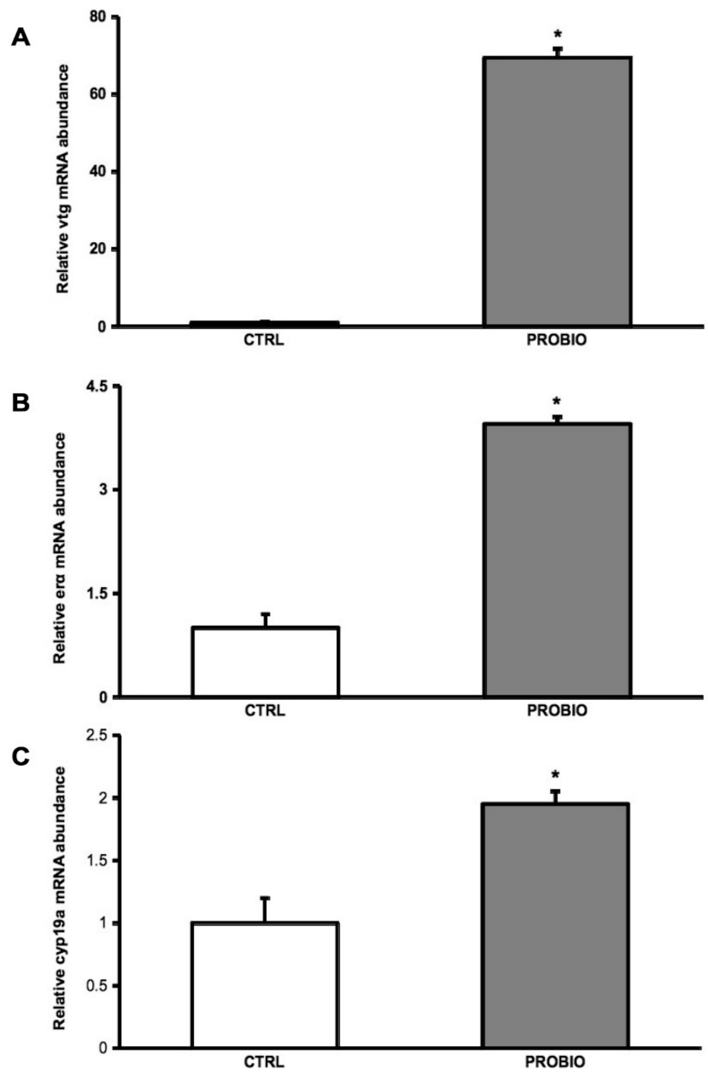

Figure.9. (A) vtg (B) erα mRNA levels in the liver and (C) cyp19a mRNA levels in the ovary, in zebrafish females fed on *L. rhamnosus* IMC 501 (PROBIO) and control diet (CTRL). (Source: Gioacchini et al., 2011)

The cyp19a gene codifies for the enzyme responsible for converting androgen into estrogen. The higher level of this gene indicates a possible increase in plasma estradiol and, in turn, in VTG. The involvement of probiotic on follicle growth phase was also supported by histological studies. These changes were associated with a higher GSI found in fish administered with *L. rhamnosus* IMC 501 (Fig.-10) and showed the involvement of *L. rhamnosus* IMC 501 in follicle growth.

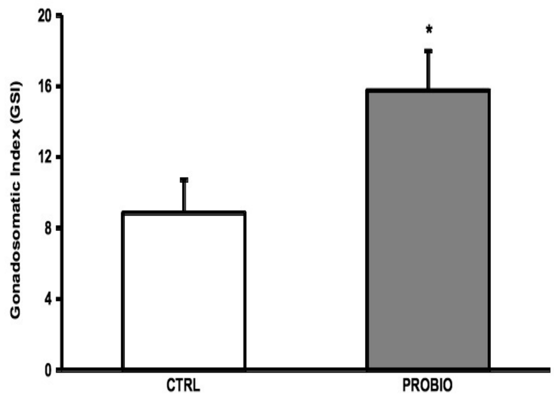

Figure.10. Gonadosomatic index (GSI) values of zebrafish females fed on *L. rhamnosus* IMC 501 (PROBIO) and control diet (CTRL). (Source: Gioacchini et al., 2011)

Finally, the significant increase of the total numbers of ovulated eggs (Figure 10A) observed, concomitantly with the elevation of cox2a (the enzyme in charge of prostaglandin biosynthesis which is the hormone responsible of ovulation) mRNA at the ovary level.

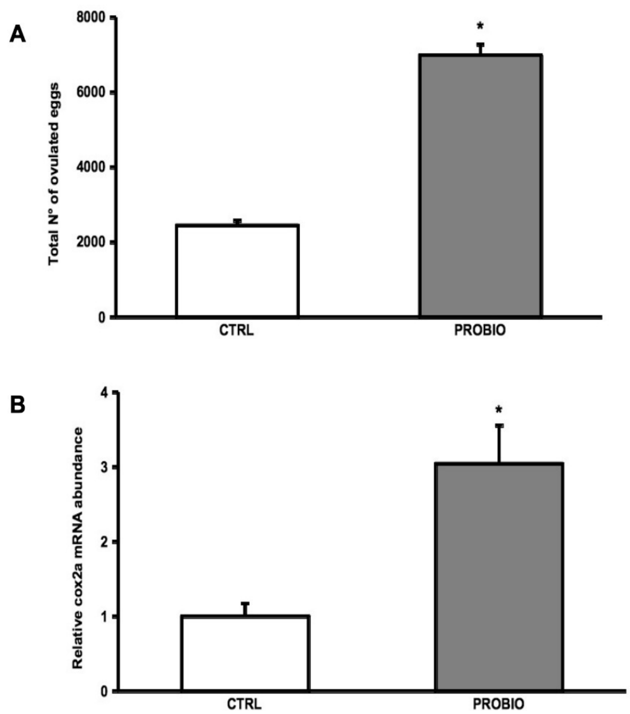

Fig.11. (A) Total number (ten days of treatment) of eggs spawned by zebraf- ish females fed on *L. rhamnosus* IMC 501 (PROBIO) and control diet (CTRL). (B) cox2a mRNA levels in the ovary, in zebrafish females fed on *L. rhamnosus* IMC 501(PROBIO) and control diet (CTRL). (Source: Gioacchini et al., 2011)

3.9. Effect of probiotic on water quality

An experimental study was conducted by Hura et al. (2018) to evaluate the effect of commercial probiotic *Bacillus megaterium* on water quality parameters. Two cemented ponds of uniform dimensions were selected in which composite culture of major carps *Cirrihinus nrigala, Labeo rohita and Catla catla* in the ratio of 30:40:30 respectively was done. The first pond (Pond A) was kept as control received only basal diet and second one (Pond B) received along with basal diet commercial probiotic *Bacillus megaterium*. During the culture period, pond water was analysed for various water parameters after every 15 days. The results of the research work showed that commercial probiotic *Bacillus megaterium* is having promising effects on water parameters. The parameters that showed significant effect were BOD, dissolved oxygen, COD and ammonia. The results also showed that less total dissolved solids were present in treated water than control one. This could be attributed to enhancement of better utilisation of feed and improvement of digestion and assimilation by probiotics in aquatic cultured organisms (Yanbo and Zirong, 2006, Ziaei-Nejad *et al*. 2006).

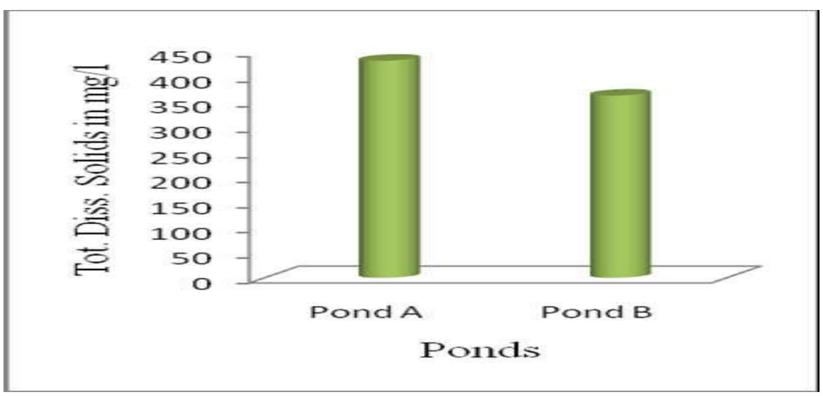

Fig.12. Total dissolve solid values of pond treated with only basal diet (Pond A) and second one (Pond B). (Source: Hura et al., 2018)

The biochemical oxygen demand values for treated and control ponds clearly indicate the effect of probiotic *Bacillus megaterium* on the water quality of treated ponds. Biochemical oxygen demand for treated ponds was less for treated ponds as compared to control one. This can again attributed to better feed utilisation and production of lesser wastes due to enhanced digestion as well as assimilation.

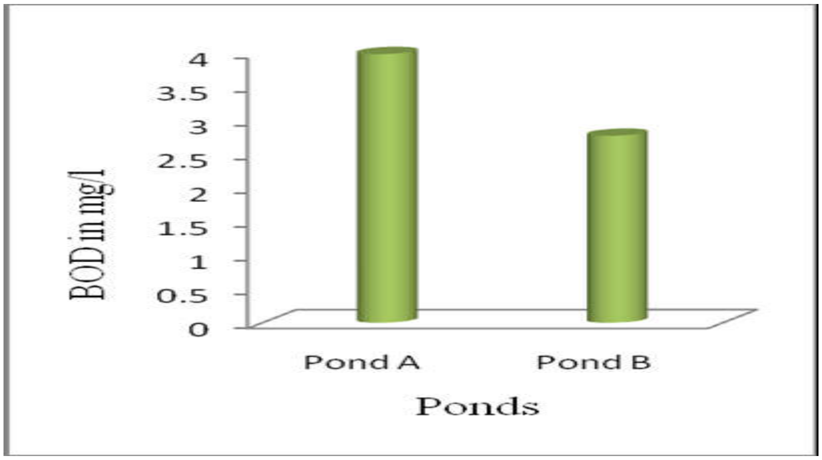

Fig.13. Biological oxygen demand of pond treated with only basal diet (Pond A) and pond treated with probiotic (Pond B). (Source: Hura et al., 2018)

Ammonia concentration beyond certain limits is harmful for culturing organisms. The result from the present work showed that probiotic *Bacillus megaterium* treated ponds have less ammonia concentration as compared to control one. This could be also attributed to lesser production of wastes by animals fed with probiotics.

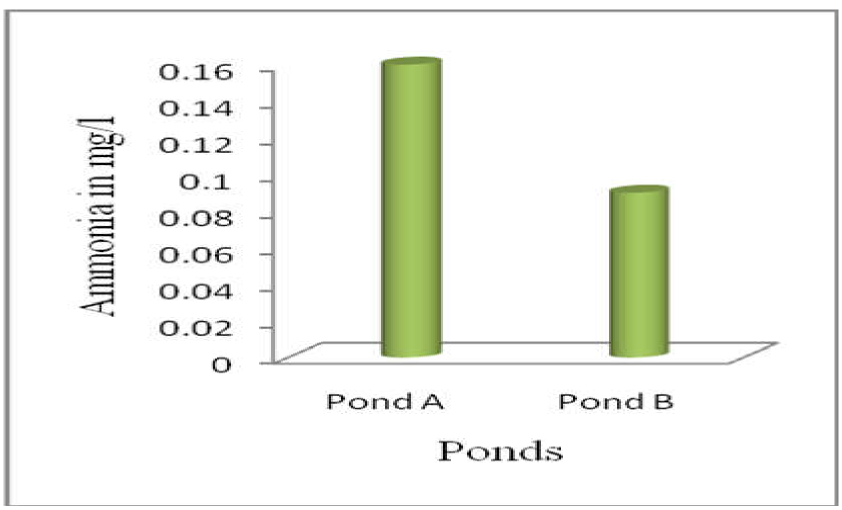

Fig.14. Ammonia concentration of pond treated with only basal diet (Pond A) and pond treated with probiotic (Pond B). (Source: Hura et al., 2018)

Dissolved oxygen values also were better for treated ponds than control one. These values clearly indicated that probiotic *Bacillus megaterium* is essential for fish culture especially major carps in a composite culture.

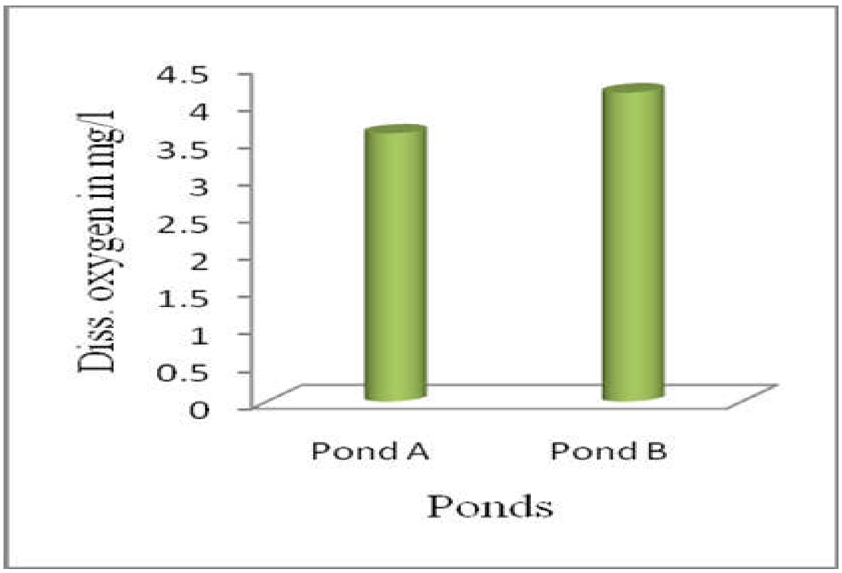

Fig.15. Dissolve oxygen concentration of pond treated with only basal diet (Pond A) and pond treated with probiotic (Pond B). (Source: Hura et al., 2018)

CHAPTER 4
CONCLUSION

Probiotics have become an essential parts of the aquaculture practices for improving the growth performance. Probiotics play an important role in feed conversion, growth rates, weight gain of fish. It increase growth performance of rui, catla, common carp, grass carp, shrimp. On the other hand, probiotics also have various beneficial effects mainly in disease resistance and nutrient availability of fish. Probiotics helps in fish and shellfish disease control strategies. Their use can help to replace some of the inhibitory chemicals currently used in aquaculture. Probiotic increase lysozyme, respiratory bursts, myeloperoxidase activity which increase immune response. It has been documented in a number of food animals that gastrointestinal bacteria play important roles in affecting the nutrition and health of the host organism. Protease, amylase and lipase enzyme activity increase on shrimp. When probiotic compounded with feed, improved the reproductive performance in terms of higher GSI, fecundity, fry survival of fry. It has positive effects on the water quality of ponds. It has significant effect were BOD, dissolved oxygen, ammonia, chemical oxygen demand. Probiotics have much potential to increase the efficiency and sustainability of fish production.

Although probiotics offer a promising alternative to the use of chemicals and antibiotics in aquatic animals, and to assist in the protection of cultured species from diseases, but the mechanism behind these effects is still a mystery. Various factors like source, dose and duration of supplementation of probiotics can affect the immune modulatory activity of probiotics. Therefore, appropriate administration methods help to provide favorable conditions for probiotics to perform well. Furthermore, understanding the modes of action along with appropriate application methods may be the key for the use of probiotics in aquatic systems.

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