A Seminar Paper

On

Effects of Probiotics on Growth and Immunity of Tilapia (*Oreochromis niloticus*)

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Effects of Probiotics on Growth and Immunity of Tilapia (*Oreochromis niloticus*)[1]

By

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ABSTRACT

Recently the world population is increasing at an alarming rate and the demand for food is increasing also. But natural calamities associated with alteration in climate pattern, higher production cost of food, high poverty etc. creates barriers to fulfill the demand of food to this ever growing population. For better and stable health everybody requires to take a minimum level of animal protein daily. To meet the demand of animal protein easily and cheaply there are no alternatives to aquaculture compared to other sector. The development of intensive farming techniques has led to a rise in Nile tilapia, *Oreochromis niloticus*, output over the past several years. Tilapia feeded with *Bacillus sp., Pediococcus sp., Enterococcus sp., Lactobacillus sp.* as a supplementary feed of a diet at three concentration 0 g/kg (A0: control), 3 g/kg(A1) and 6 g/kg(A2). After 8 weeks feeding growth and immunity of tilapia is better in treatment A1 and A2. However, the ongoing expansion of *O. niloticus* production must be based on environmentally friendly methods. One factor that affects the production, profitability, and productivity of the aquaculture sector is the way fish use their nutrients. However, research on probiotics in aquaculture, particularly in the culture of *O. niloticus*, is heavily focused on immunity and disease resistance while neglecting areas of equal importance such as nutrient utilization that correlates with excellent health and growth as well. The effects of probiotics on tilapia development, immunological function, and disease resistance are explained in the current review. Nutrient utilization includes the uptake of proteins, lipids, fatty acids, carbohydrates, vitamins, and minerals. This assessment also seeks to identify any gaps in the body of knowledge that call for additional research and development.

Key words: Aquaculture, Probiotics, bacteria, fish, antibiotics.

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CHAPTER I

INTRODUCTION

Fish is considered one of the most popular and edible diets throughout the world as well as in Bangladesh. In the glove, fish provides high-quality protein, essential macro and micro-nutrients, vitamins and minerals. Approximately, 3.0 billion people rely on fish for up to 20% of dietary animal protein (FAO, 2019). In Bangladesh 60% of the animal protein intake in the daily diet of the people comes from fish (DoF, 2022).

Bangladesh has a total inland water area of 6.7 million hectare (ha.) of which 94% is used for open water culture fishery and 6% for closed-water culture fishery (Hossain, 2014). The inland open water fishery playing a significant role in the economy, culture, tradition and food habit of the people of Bangladesh. It contributes 3.57% to national GDP and 25.30% to the agricultural GDP and 1.5% to foreign exchange earnings by exporting fish in 2017-18 (DoF, 2018). Bangladesh is very rich in freshwater fish diversity comprising 260 species of fish and 24 freshwater prawns (DoF, 2022). Bangladesh has ranked 3rd in the world in inland fish production, 5th in aquaculture production and 11th in marine fish production in 2018 (FAO, 2018). Bangladesh currently produces about 46.21 lakh metric ton (MT) of fish in 2020-21 (DoF, 2022).

Tilapia are the second most important farmed fish after carp; they are cultured in over 100 countries and are native to Africa (Menaga and Fitzsimmons 2017). Tilapia contributes 4.5 million metric tons yearly to the global food security by providing an inexpensive source of protein. Nile tilapia, Oreochromis niloticus, commonly referred to as tilapia is one of the most important aquaculture fish in the world (Senapin *et al*. 2018). The attributes that make tilapia an excellent aquaculture candidate are its ability to feed on a wide variety of food items, tolerance to a wide range of environmental conditions (pH, temperature, nitrogenous wastes), fast growth, and high economic value. (Abarike *et al*. 2013; Wang and Lu 2016).

Probiotics are microorganisms that act beneficial on host health, inhibit pathogens, enhance immunity and have a protective effect on the gut microflora (Fuller 1989). Previous researches showed that Probiotics can be used as growth promoters as well as fill the requirement of controlling potential pathogens, promoting the growth and improve stress tolerance (Aly *et al*., 2008; Balcázar *et al*., 2007; Eissa *et al*., 2014a; Eissa and Abou El-Ghiet, 2011; Eissa *et al*., 2010; Giri *et al*., 2013; Lauzon *et al*., 2014). The mode of action of the probiotics is a complex thing and rather 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 c).

Recently, capture fish production has declined to about 50%, with a negative trend of 1.2% per year (Hossain, 2014). Inland open water capture fisheries is decreasing due to various activities over exploitation, unplanned construction of flood protection embankments, irrigation canals, improper use of pesticides, habitat degradation, industrial waste, inorganic fertilizer and natural causes such as siltation, weather change, disease outbreak and so on. To keep these barriers in mind and to full fill the demand of fish as a source of dietary protein we need to produce more fish. For the larger production of fish maintain good health of fish and successful breeding is necessary.

Probiotics has found use in aquaculture as a means of disease control, supplementing or even in some cases replacing the use of antimicrobial compounds. Probiotics in aquaculture has significantly reduce the use of antimicrobial compounds (particularly antibiotics) in improved appetite and/or growth performance of the farmed species. Probiotics can improve fish in growth, in feed efficiency, immune status, digestive enzyme activities, gut morphology, disease resistance and stress responses (Guerreiro *et al*., 2018). Probiotics used in feed made the fish healthy that ultimately increased production. The former research also suggests that if the animals are healthy then there will not be any need to use antimicrobial compounds (Cao *et al*., 2020).

The demand for food fish is increasing day by day and the use of antibiotics for fish growth promotion has been banned. As well as public awareness has increased for healthy fish production. These led to think about potential functional feeds as health promoters. As alternative to antibiotics, functional ingredients such as probiotics become to be used in aquaculture and other animal production industries to promote animal health and well-being (Dimitroglou *et al*., 2011a; Dawood & Koshio, 2016; Carbone & Faggio 2016). This review paper contains core information to understand the effects of probiotics on fish growth, hematology and breeding activity of fish.

O**bjectives of the Study**

The specific objectives of this review paper are as follows:

1. To review the effects of probiotics on growth performances of tilapia
2. To highlight the effects of probiotics on immunity of tilapia

CHAPTER II

MATERIALS AND METHODS

This paper is absolutely a review paper. With a view to preparing this paper, information was collected from secondary sources. The topic related findings have been reviewed by studying extensively various articles and research papers published in varied journals, proceedings, internet browsing, dissertation available in online. Information and valuable suggestion were received from honorable major professor and course instructors. After collecting information, these were organized chronologically for preparing this seminar manuscript.

CHAPTER III

REVIEW AND FINDINGS

3.1 Probiotics

The term probiotics were first given by Lilly & Stillwell in 1965. Probiotic was described as the microbiological origin factor that can stimulate the growth of hosts. In 1989 Roy Fuller introduced the idea that probiotics generate a beneficial effect on the host. Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer benefit to the host's health, improving the balance of the microbiota in the intestine' by him. WHO provides the definition of probiotics as "live microbes when administered in sufficient amounts give an improved health of the host".

However, according to some authors, in aquaculture used probiotics are cells (live or dead) or components of cells of microbes are that, when incorporated to the water or feed, enhance health status of the host animal via flourishing the contents of microbes in the gut‟. It is often reported that a probiotic must eliminate pathogens, attach and colonize within the gut, replicate in high numbers, it must produce digestive enzyme, nutrients and antimicrobial substances, and it must remain in acidic environment of the gastro-intestinal-tract of fish (Kesarcodi-Watson A. *et al*., 2008).

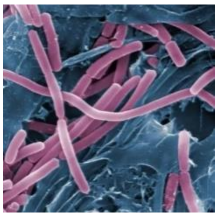
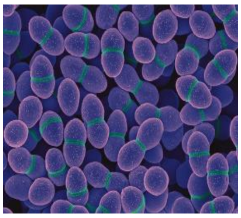
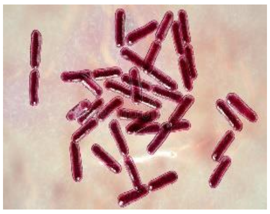
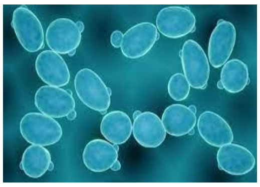
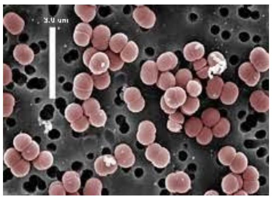
When probiotics are given into the body of fish regularly through probiotic supplemented feed, probiotics build a positive balance among beneficial microbial population in the gut. (Bermudez-Brito, M. *et al*., 2012, Soccol *et al*., 2010). Gram positive bacteria, gram-negative bacteria, and many other organisms as for example yeasts, bacteriophage, and single-celled algae may be used as probiotics (Mukherjee A. *et al*., 2016).

3.2 Characteristics of probiotics

Probiotics must be(a) resistant to pH and bile acids, (b) showed no pathogenicity, (c) be viable and be be stable in storage and in field, (d) survive and potentially (e) be largely cultivable (f) be able to adhere to the epithelial lining of the gut, and most importantly (g) beneficial for host animals. New strains used for probiotic development should possess all the mentioned characteristics (Zang *et al*., 2013).

3.3 Probiotics used in Aquaculture

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

 *Bacillus subtillis*	 *E. faecium*	 *E. gallinarum*	 *B. licheniformis*
 *Saccharomyces cerevisiae*	 *Pediococcus sp*	 *P. acidilactici*	 *L. rhamnosus*

(Source: Google)

Figure 01: Microscopic view of some Probiotic bacteria species.

3.4 How probiotics work on fish

Probiotic bacteria work on fish by the following ways: (Source: Balcazar *et al*., 2006 and Pandiyan *et al*., 2013)

Compete with pathogenic bacteria: Probiotic bacteria compete with pathogen for attachment sites on the mucosa, for nutrients and produce inhibitory substances which act against replication and reduce colonization of pathogen.

Antimicrobial compounds secretion: Probiotics bacteria produce antibacterial compounds as like as hydrogen peroxide, bacteriocins, lactic acid in the gut of the host to prevent colonization and elimination of pathogens.

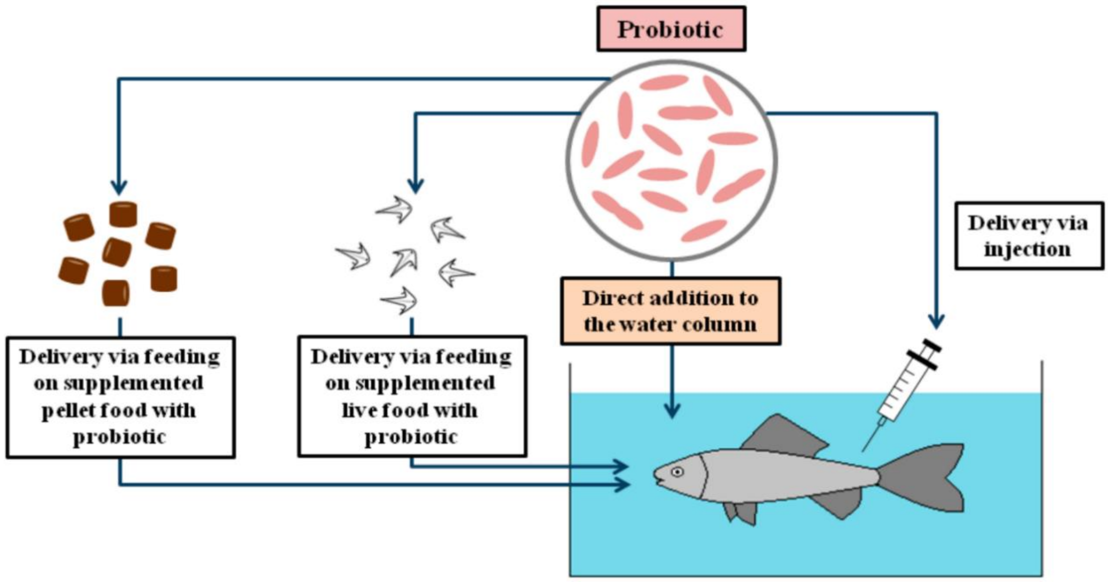
Maintain good aquatic environment: Yeast as probiotic can reduce dissolved and particulate organic carbon matter problems in cultural water and make water good for aquaculture, provide increased survival rate, growth rate and improved health status of fish a disease in fish.

Provide nutrients and secrets enzyme: Some probiotic yeast improve digestion through producing proteases, lipases in the gut of fish and enhances digestion of the host's nutrition through providing fatty acids and vitamins.

Act as immune-modulator: The nonspecific immune system is stimulated by probiotics. They activate several immune cells including macrophages, neutrophils, monocytes, natural killer cells etc.

3.5 **Methods of application of probiotics in fish**

There is huge difference between aquatic and terrestrial environments. For this reason, the physiology of the aquatic animal is much more different than terrestrial animal so the way of the administration of drugs greatly varies also. There are several problems with drug applications in aquatic environments (Park *et al*., 2012). The researchers create many protocols to introduce probiotics/drugs in fish with different criteria. Replacement of dietary feed (via live food such as *Artemia* and rotifers or pellet food) or addition to the water directly may be the way of administration of probiotic in organism (Moriarty and D. J. W. 1998, Skjermo and Vadstein 1999). Intraperitoneal injection of probiotic solution has also been reported (LaPatra *et al*., 2014). There have many studies which are performed to know the effect of probiotic incorporated feed in fish in aquaculture. Addition of probiotic directly in water to investigate the effects on fish is somehow until low (Jahangiri and Esteban 2018).



(Source: Google)

Figure 02: Different methods of probiotic administration in fish.

3.6. Effects of probiotic on fish

3.6.1. Growth

Probiotics are microorganisms that can be used in aquaculture in good purposes. Studies showed that optimum concentration of dietary probiotic improves growth of fish. Growth performance of fish subjected to feeding manipulation was determined at the end of the experiment. The following parameters is used to determine the following formula

- Weight gain (g) = W2 (g) - W1 (g)
- Specific growth rate (SGR) (%/day) = 100 (ln W2 - ln W1)/T.
- Feed conversion ratio (FCR) = feed intake (g) / weight gain (g).
- Survival rate (%SR) = (final amount of fish / initial amount of fish) ×100.

here,

W1 is the initial weight

W2 is the final weight and

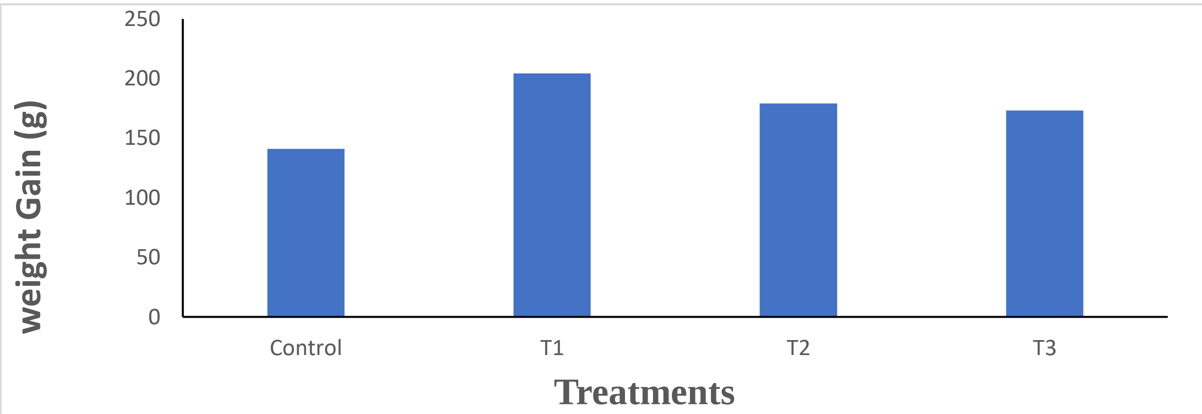
T is the number of days in the feeding period.

(Opiyo *et al*., 2019) used commercial feed which is composed of probiotic Bacillus subtilis (1 × 10^9 CFU g-1)) for conducting the feeding trial in Nile tilapia (*Oreochromis niloticus*). Feed was incorporated in the basal diet with probiotic at three different inclusion level 5 g kg-1, 10 g kg-1 and 15 g kg-1 namely T1, T2 and T3 respectively. The feeding experiment was 7-week long. Feed was given two times a day at ration of 3% body weight. Here it is evident that weight gain is highest at the T1 treatment. The FCR value is highest in control treatment followed by T1, T3 and T2 respectively.

Table 01: Growth performance parameters of *O. niloticus* fed on diets supplemented with probiotics at different levels in low input ponds

Index	Control	T1	T2	T3
Weight Gain (g)	140.92	204.17	179.07	173.03
Specific Growth Rate (%/day)	0.63	0.72	0.76	0.70
Feed Conversion Ratio	2.03	1.85	1.67	1.73

(Source: Opiyo *et al*., 2019)



(Source: Opiyo *et al*., 2019)

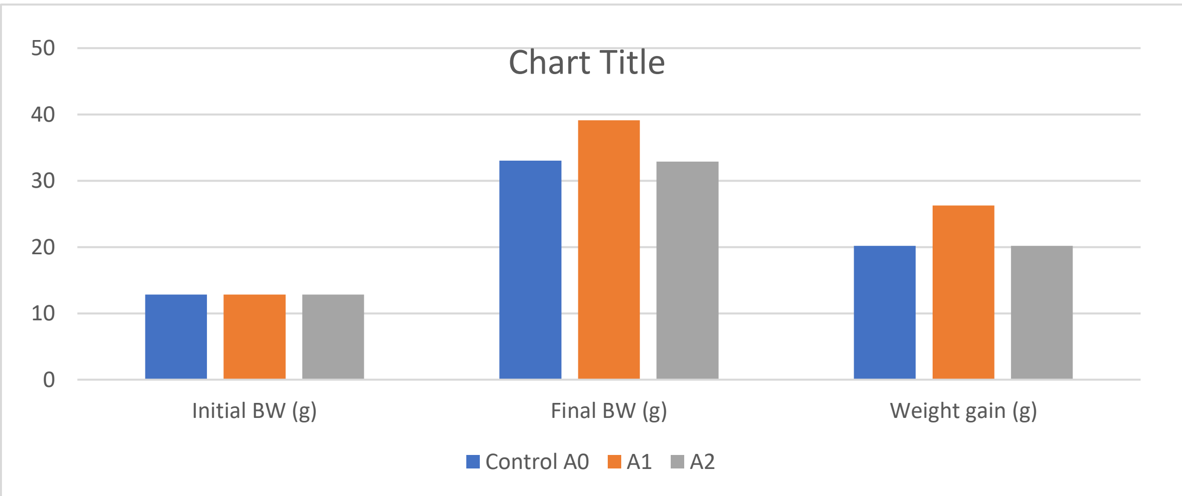
Figure 03: Effect of probiotic on growth of Nile tilapia treated with four different experimental diets.

Ramos at al. (2017) conducted research on probiotic effects on the growth of tilapia fish (*O. niloticus*). The goal of the current study was to determine how a probiotic blend (*Bacillus sp.*, *Pediococcus sp.*, *Enterococcus sp.*, *Lactobacillus sp.*) as a dietary supplement affected juvenile Nile tilapia's growth performance, feed utilization (*Oreochromis niloticus*). Using three different concentrations, the probiotic was added to a standard diet: 0 g/kg (A0: control), 3 g/kg, and 6 g/kg. After 8 weeks of probiotic feeding, weight and specific growth rate where significantly higher in fish-fed A1 diet than in fish-fed A0.

Table 02: Growth performance of *O. niloticus* fed on diets supplemented with probiotics (*Bacillus sp.*, *Pediococcus sp.*, *Enterococcus sp.*, *Lactobacillus sp.*) at different levels.

Parameter	Control	Probiotics	
	A_0(0g/kg)	A_1(3g/kg)	A_2(6g/kg)
Initial BW (g)	12.83	12.82	12.83
Final BW (g)	33.01	39.10	32.99
Weight gain (g)	20.18	26.28	20.16
SGR	1.69	1.98	1.69
FCR	1.55	1.41	1.60
PER	2.17	2.39	2.10
VFI	2.43	2.52	2.51
Mortality (%)	8.33	6.67	3.33

(Source: Ramos at al. 2017)



(Source: Ramos at al. 2017)

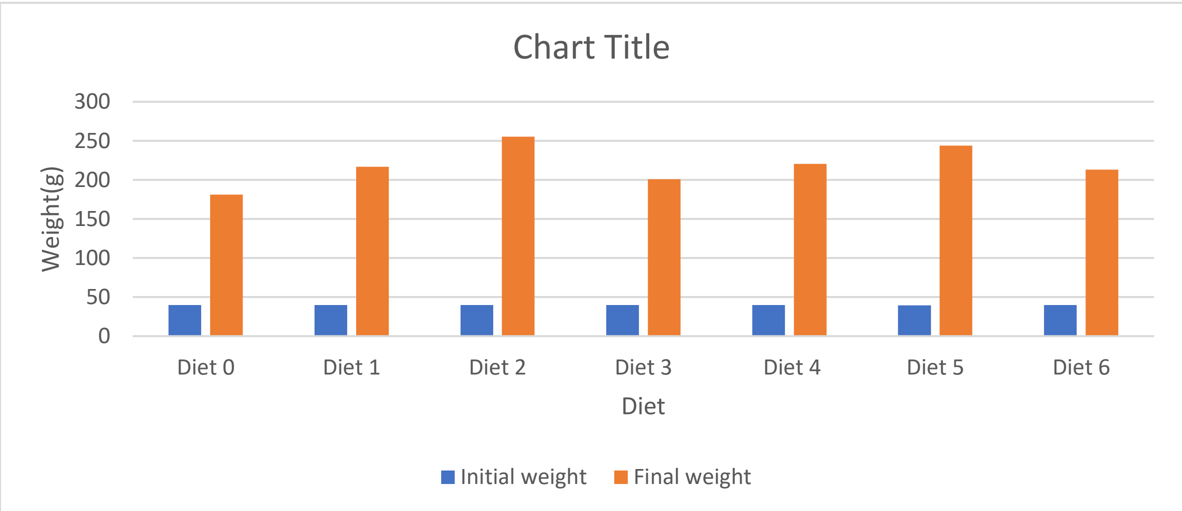
Figure 04: Effect of probiotic on growth of Nile tilapia treated with three different experimental diets.

Jumbe j et. al., (2019) used different levels of probiotics (baker's yeast (*Saccharomyces cerevisiae* and *Bacillus subtilis*) on Nile tilapia (*Oreochromis niloticus*) reared in low input ponds. Mono-sex male fingerlings (40 g) were randomly distributed into 28, 1.25 m3 net cages at 50 fish m-3 and fed twice daily at 3% body weight on seven isonitrogenous (28% crude protein) diets supplemented with either *Saccharomyces cerevisiae* or *Bacillus subtilis* at different levels: Diet 0 (control); Diet 1–3 were supplemented with *S. cerevisiae* at 2 g kg -1 (Diet 1); 4 g kg-1 (Diet 2) and 6 g kg -1 (Diet 3) whereas Diet 4–6 were supplemented with *B. subtilis* at 5 g kg-1 (Diet 4); 10 g kg-1 (Diet 5) and 15 g kg-1 (Diet 6).

Table 03: Growth performance parameters of *O. niloticus* fed on diets supplemented with probiotics baker's yeast (*Saccharomyces cerevisiae*) and *Bacillus subtilis* at different levels.

Parameter	Control	***Saccharomyces cerevisiae***			***Bacillus subtilis***		
	Diet 0	Diet 1 (2g/kg)	Diet 2 (4g/kg)	Diet 3 (6g/kg)	Diet 4 (5g/kg)	Diet 5 (10g/kg)	Diet 6 (15g/kg)
Initial length(cm)	13.24	13.20	13.26	13.35	13.26	13.28	13.22
Initial weight (g)	39.90	39.75	39.99	39.65	39.63	39.57	39.90
Final length (cm)	22.18	23.46	23.88	23.10	23.31	23.93	23.29
Final weight (g)	180.96	216.93	255.31	200.84	220.62	243.99	212.93
SGR (% day -1)	0.63	0.71	0.77	0.67	0.72	0.76	0.70
Weight gain (g)	140.92	177.18	215.32	161.29	204.17	179.07	173.03
FCR	2.00	1.87	1.61	1.95	1.85	1.67	1.73
Survival (%)	77.00	83.50	89.50	81.50	87.50	88.50	80.00

(Source: Jumbe j et. al., 2019)



(Source: Jumbe j et. al., 2019)

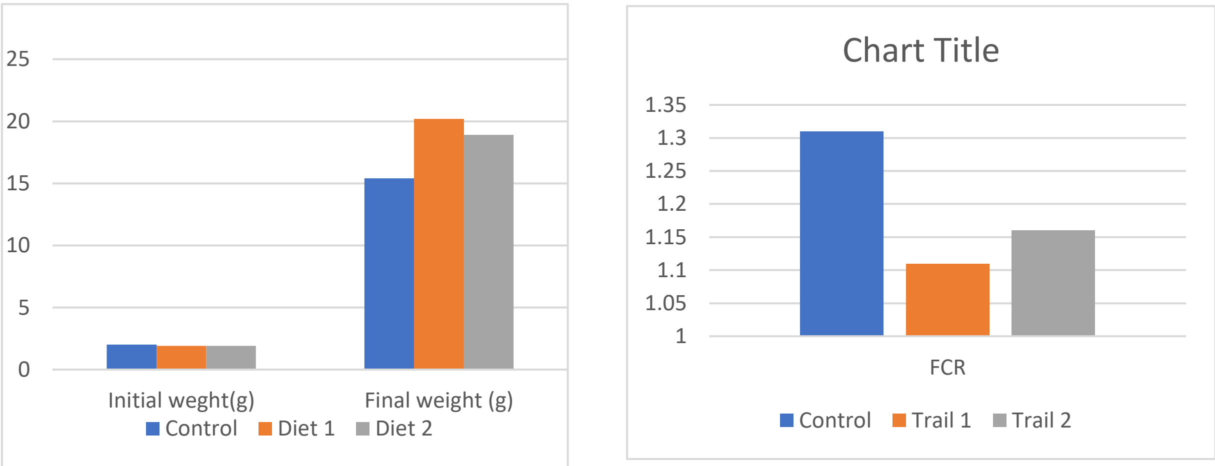
Figure 05: Effect of probiotic on growth of Nile tilapia treated with seven different experimental diets.

Tan H. U. *et al*. (2019) conducted research on effects of probiotics on growth performance of tilapia (*O. niloticus).* By measuring the intestinal digestive enzyme activities and the growth parameters of WG, FCR, and FE after 8 weeks of administration, the effectiveness of *R. stabekisii* in nutrient utilization and growth performance was assessed.

Table 04: Growth performance parameters of *O. niloticus* fed on diets supplemented with probiotics (*R. stabekisii*) at different levels.

Parameters	Control A_0	Probiotics	
		A_1(10^6 CFU/Fish)	A_2(10^7 CFU/Fish)
Initial weight (g)	2.01	1.97	1.90
Final weight (g)	15.4	20.2	18.9
Weight gain (WG) (g)	13.4	18.3	17.0
Survival rate (%)	92.2	93.3	94.4
Feed conversion ratio (FCR)	1.31	1.11	1.16

(Source: Tan H. U. *et al*. 2019)



(Source: Tan H. U. *et al*.2019)

Figure 06: Effect of probiotic on growth of Nile tilapia treated with three different experimental diets.

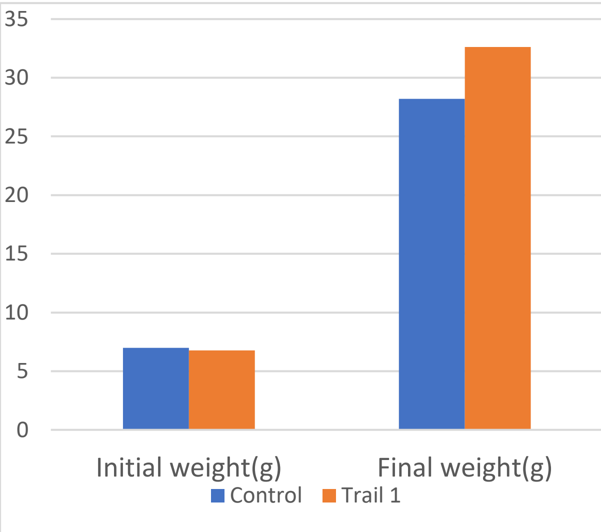
A study by Wang Y. B. *et al*. evaluates the effect of *Enterococcuss faecium* on growth performance in Tilapia (*O. niloticus*). The tilapias were treated with *E. faecium* ZJ4 at a final concentration of 1×10^7 cfu ml- 1 in aquaria water every 4 days.

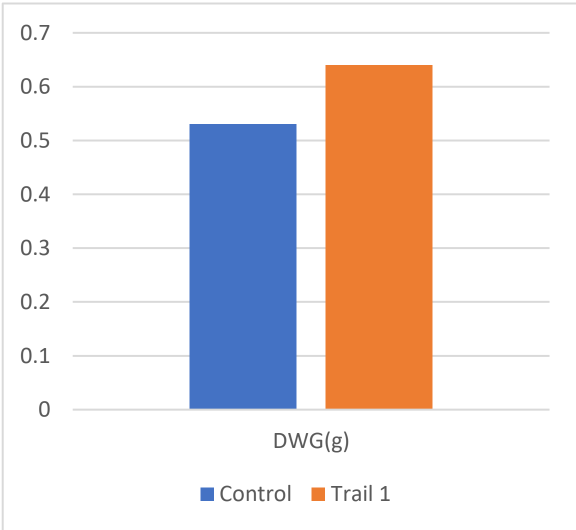
Table 05: Growth performance parameters of *O. niloticus* fed on diets supplemented with probiotics (*Enteroccus faecium*) at different levels.

Parameters	Control	Trail 1(1×10^7 cfu ml- 1)
Initial weight(g)	6.99	6.76
Final weight(g)	28.21	32.63
DWG(g)	0.53	0.64

(Source: Y. B. *et al*., 2019)

DWG=Daily Weight Gain.



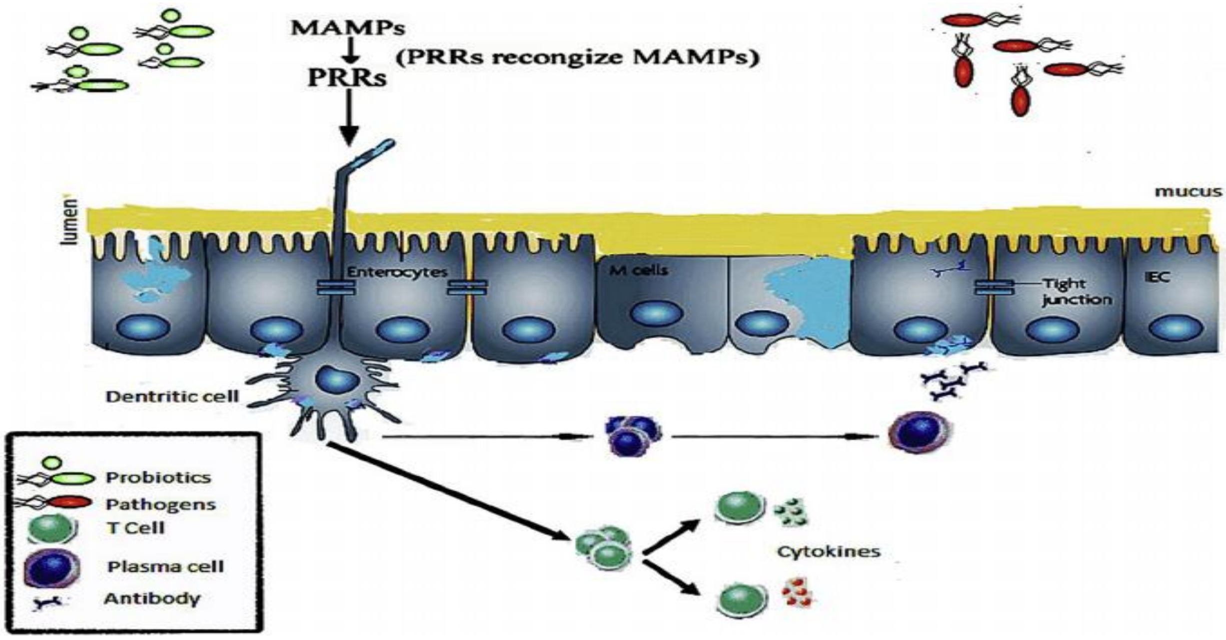


(Source: Y. B. *et al*., 2019)

Figure 07: Effect of probiotics on growth of Nile tilapia treated with two different experimental diets.

3.6.2. Immunity

Probiotics are healthy microorganisms that can prevent infections as well as control the host immune system. Probiotic immunomodulation is viewed as a collaborative effort of the introduced microbe, host, and commensals. Pathogen pattern recognition receptors can determine if an organism is pathogenic or not (PRRs). The microbial associated molecular patterns (MAMPs), which are present in both pathogenic and non-pathogenic microorganisms, are used to identify these recognition receptors. Lipopolysaccharides (LPS), peptidoglycan, flagellin, and microbial nucleic acids are a few examples of MAMPs. MAMPs' binding to PRRs initiates an intracellular signaling cascade that urges the release of particular cytokines, transmits messages to neighboring cells, or has antiviral, pro- or anti-inflammatory effects on exercise. The same recognition system controls the commensal's homeostasis.



(Source: Akhter *et al*., 2015)

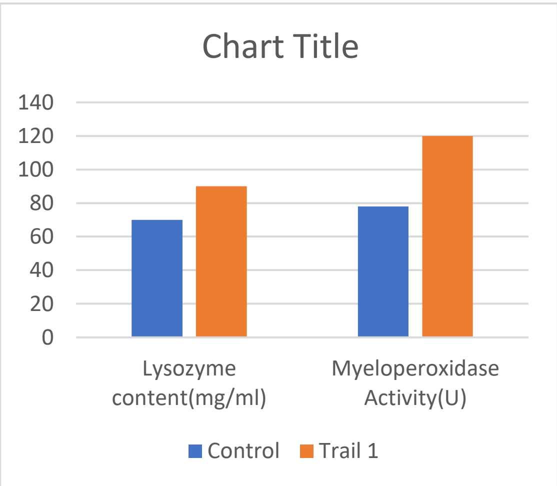
Figure 08: Probiotics showing the activity of host immunomodulation. Abbreviations: MAMPs / Microbe-associated molecular patters, PRRs / Pathogen pattern recognition receptors.

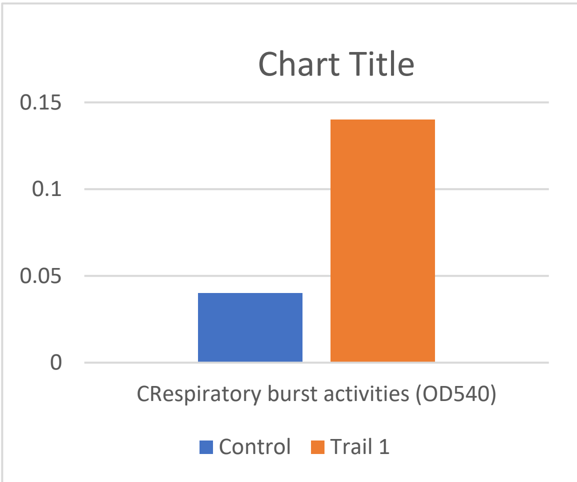
Wang Y. *et al*., 2008 conduct research on immunity performance of tilapia fish (*O. niloticus*) feeded with *Enterococcus faecium.*

Table 6: Effect of probiotic, *E. faecium* on serum lysozyme content, myeloperoxidase (MPO) activity and respiratory burst activities (OD_{540}) after 40 days.

Immune Response	Control	Trail 1(10^7 cfu/ml)
Lysozyme content(mg/ml)	70	90
Myeloperoxidase Activity(U)	78	120
Respiratory burst activities (OD540)	0.04	0.14

(Source: Wang Y. *et al*., 2008)





(Source: Wang Y. *et al*., 2019)

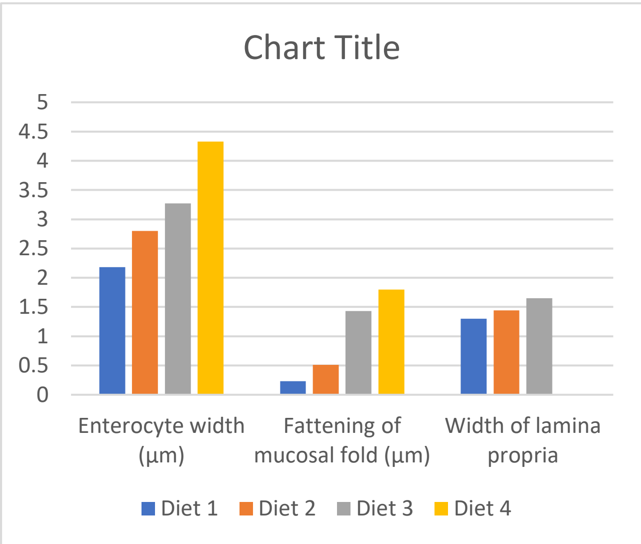
Figure 09: Effect of probiotic, *E. faecium* on serum lysozyme content, myeloperoxidase (MPO) activity,u and respiratory burst activities (OD_{540}) after 40 days

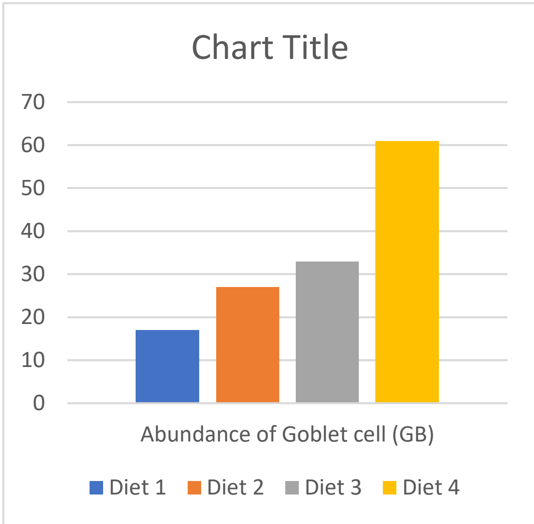
A study by Islam S.M.M. *et al.*, (2021) evaluated the effect of probiotics yeast on tilapia(*O.niloticus*). Nile tilapia fed with probiotic yeast supplemented diets 0 g kg- 1 diet; control and 1, 2 & 4 g kg- 1 diets for 60 days.

Table 07: Immune response parameters of histological gut of Nile tilapia fed with probiotic yeast supplemented diets for 60 days.

Parameters	Probiotic yeast supplementation in the feed (g kg- 1)			
	0	1	2	4
Enterocyte width (µm)	2.18	2.80	3.27	4.33
Fattening of mucosal fold (µm)	0.23	0.51	1.43	1.80
Abundance of Goblet cell (GB)	17	27	33	61
Width of lamina propria	1.30	1.44	1.65	1.96

(Source: S.M.M. *et al.*, 2021)





(Source: S.M.M. *et al.*, 2021)

Figure 10: Effect of probiotics on the immunity of Nile tilapia treated with four different experimental diets.

3.6.3 Effects of probiotics on the mucosal immune response

Lysozyme, peroxidase, superoxide dismutase (SOD), protease and antiprotease, and catalase (CAT) are serum immunological parameters that have strong bactericidal effect against microbial infections (Nayak 2010). In general, an increase in immunological parameter activity reflects an improvement in the host's immune response. (Alexander *et al*. 2010; Rauta *et al*. 2012). It has been established over time that the use of probiotics in fish culture dramatically changes the activity of these immunological indices. Probiotics have been shown to significantly increase the activities of serum immune parameters such as lysozyme, SOD, CAT, protease, and antiprotease in tilapia (Iwashita *et al*. 2015), Bacillus sp. (Abarike *et al*. 2018), and the combination of probiotic Bacillus spp. and herbs (Abarike *et al*. 2018). A single administration of B. subtilis (Telli *et al*. 2014), Pediococcus acidilactici (Standen *et al*. 2013), Enterococcus faecium (Wang *et al*. 2008), BS containing B. subtilis and B. licheniformis (Abarike *et al*. 2018), and a mixture of B. subtilis and B. licheniformis and Chinese herbs (Abarike *et al*. 2018) has been demonstrated to improve the serum immune parameters in tilapia. Not all probiotic applications, meanwhile, can considerably boost tilapia's immune response. The blood immunological characteristics of tilapia-fed L. lactis, for instance, were unaffected. (Zhou *et al*. 2010).

CHAPTER IV

SUMMARY & CONCLUSIONS

On the basis of the findings of this review paper, the following conclusions are drawn.

1. Probiotics have become an essential part of the aquaculture practices for improving the growth performance. After 8 weeks of feeding probiotic feed at different concentrations,5g/kg feed concentrations give better growth performance. Probiotic ensures specific growth(%SGR) rate as well as weight gain (WG) in tilapia. SGR and weight gain also higher at 5g/kg feed concentration. Lower feed conversion ratio (FCR) value in tilapia demonstrates that the inclusion of probiotic in feed decrease feed cost. Lower FCR value enables farmers to get highest growth performances of fish at considerably low cost thus consequently gives the higher profit. FCR value also lower at 5g/kg concentration.
2. One of the beneficial effects of probiotics is stimulation of immune system. Probiotic bacteria *Enterococcus faecium* and probiotic yeast made the significant difference in immune system of tilapia. Probiotics can enhance enterocyte width, fattening of mucosal fold, abundance of Goblet cell, width of lamina propria which indicate improve immunity. Enhance enterocyte width (4.33 µm) is obtain from feeding 4g/kg feed which is higher than other concentration feed, fattening of mucosal fold (1.80 µm) is obtain from feeding 4g/kg feed which is higher than other concentration feed, abundance of Goblet cell (61 GB) is obtain from feeding 4g/kg feed which is higher than other concentration feed, width of lamina propria 1.96 is obtain from feeding 4g/kg feed which is higher than other concentration feed.

REFERENCES

Abarike, E. D., Cai, J., Lu, Y., Yu, H., Chen, L., Jian, J., ... & Kuebutornye, F. K. (2018). Effects of a commercial probiotic BS containing Bacillus subtilis and Bacillus licheniformis on growth, immune response and disease resistance in Nile tilapia, Oreochromis niloticus. *Fish & Shellfish Immunology*, *82*, 229-238.

Abarike, E. D., Obodai, E. A., & Attipoe, F. Y. (2013). Growth and economic performance of fingerlings of Oreochromis niloticus fed on different non-conventional feeds in out-door hapas at Akosombo in Ghana. *African Journal of Agricultural Research*, *8*(26), 3384-3391.

Akhter, N., Wu, B., Memon, A. M., & Mohsin, M. (2015). Probiotics and prebiotics associated with aquaculture: a review. *Fish & shellfish immunology*, *45*(2), 733-741.

Alexander, R. D., Innocente, S. A., Barrass, J. D., & Beggs, J. D. (2010). Splicing-dependent RNA polymerase pausing in yeast. *Molecular cell*, *40*(4), 582-593.

Balcázar, J. L., De Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., & Múzquiz, J. L. (2006). The role of probiotics in aquaculture. *Veterinary microbiology*, *114*(3-4), 173-186.

Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. *Annals of Nutrition and Metabolism*, *61*(2), 160-174.

Cao, H., Yu, R., Zhang, Y., Hu, B., Jian, S., Wen, C., ... & Yang, G. (2019). Effects of dietary supplementation with β-glucan and Bacillus subtilis on growth, fillet quality, immune capacity, and antioxidant status of Pengze crucian carp (Carassius auratus var. Pengze). *Aquaculture*, *508*, 106-112.

Guerreiro, I., Oliva-Teles, A., & Enes, P. (2018). Prebiotics as functional ingredients: focus on Mediterranean fish aquaculture. *Reviews in Aquaculture*, *10*(4), 800-832.

Iwashita, M. K. P., Nakandakare, I. B., Terhune, J. S., Wood, T., & Ranzani-Paiva, M. J. T. (2015). Dietary supplementation with Bacillus subtilis, Saccharomyces cerevisiae and Aspergillus oryzae enhance immunity and disease resistance against Aeromonas hydrophila and Streptococcus iniae infection in juvenile tilapia Oreochromis niloticus. *Fish & shellfish immunology*, *43*(1), 60-66.

Jahangiri, L., & Esteban, M. Á. (2018). Administration of probiotics in the water in finfish aquaculture systems: a review. *Fishes*, *3*(3), 33.

Kesarcodi-Watson, A., Kaspar, H., Lategan, M. J., & Gibson, L. (2008). Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. *Aquaculture*, *274*(1), 1-14.

LaPatra, S. E., Fehringer, T. R., & Cain, K. D. (2014). A probiotic Enterobacter sp. provides significant protection against Flavobacterium psychrophilum in rainbow trout

(Oncorhynchus mykiss) after injection by two different routes. *Aquaculture*, *433*, 361-366.

Lauzon, H. L., Dimitroglou, A., Merrifield, D. L., Ringø, E., & Davies, S. J. (2014). Probiotics and prebiotics: concepts, definitions and history. *Aquaculture nutrition: Gut health, probiotics and prebiotics*, 169-184.

Lee, J., Park, I., Choi, Y., & Cho, J. (2012). Bacillus strains as feed additives: In vitro evaluation of its potential probiotic properties. *Revista Colombiana de Ciencias Pecuarias*, *25*(4), 577-585.

Menaga, M., & Fitzsimmons, K. (2017). Growth of the tilapia industry in India. *World Aquaculture*, *48*(3), 49-52.

Moriarty, D. J. (1999). Disease control in shrimp aquaculture with probiotic bacteria. In *Proceedings of the 8th international symposium on microbial ecology* (pp. 237-243). Halifax, Canada: Atlantic Canada Society for Microbial Ecology.

Mukherjee, A., & Ghosh, K. (2016). Antagonism against fish pathogens by cellular components and verification of probiotic properties in autochthonous bacteria isolated from the gut of an I ndian major carp, C atla catla (H amilton). *Aquaculture Research*, *47*(7), 2243-2255.

Nayak, S. K. (2010). Probiotics and immunity: a fish perspective. *Fish & shellfish immunology*, *29*(1), 2-14.

Opiyo, M. A., Jumbe, J., Ngugi, C. C., & Charo-Karisa, H. (2019). Different levels of probiotics affect growth, survival and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *Scientific African*, *4*, e00103. Opiyo, Mary A., et al

Opiyo, M. A., Jumbe, J., Ngugi, C. C., & Charo-Karisa, H. (2019). Different levels of probiotics affect growth, survival and body composition of Nile tilapia (Oreochromis niloticus) cultured in low input ponds. *Scientific African*, *4*, e00103.

Pandiyan, P., Balaraman, D., Thirunavukkarasu, R., George, E. G. J., Subaramaniyan, K., Manikkam, S., & Sadayappan, B. (2013). Probiotics in aquaculture. *Drug invention today*, *5*(1), 55-59.

Ramos, M. A., Batista, S., Pires, M. A., Silva, A. P., Pereira, L. F., Saavedra, M. J., ... & Rema, P. (2017). Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. *Animal*, *11*(8), 1259-1269.

Rauta, P. R., Nayak, B., & Das, S. (2012). Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms. *Immunology letters*, *148*(1), 23-33.

Senapin, S., Shyam, K. U., Meemetta, W., Rattanarojpong, T., & Dong, H. T. (2018). Inapparent infection cases of tilapia lake virus (TiLV) in farmed tilapia. *Aquaculture*, *487*, 51-55.

Skjermo, J., & Vadstein, O. (1999). Techniques for microbial control in the intensive rearing of marine larvae. *Aquaculture*, *177*(1-4), 333-343.

Soccol, C. R., de Souza Vandenberghe, L. P., Spier, M. R., Medeiros, A. P., Yamaguishi, C. T., De Dea Lindner, J., ... & Thomaz-Soccol, V. (2010). The potential of probiotics: a review. *Food Technology and Biotechnology*, *48*(4), 413-434.

Tan, H. Y., Chen, S. W., & Hu, S. Y. (2019). Improvements in the growth performance, immunity, disease resistance, and gut microbiota by the probiotic Rummeliibacillus stabekisii in Nile tilapia (Oreochromis niloticus). *Fish & shellfish immunology*, *92*, 265-275.

Telli, G. S., Ranzani-Paiva, M. J. T., de Carla Dias, D., Sussel, F. R., Ishikawa, C. M., & Tachibana, L. (2014). Dietary administration of Bacillus subtilis on hematology and non-specific immunity of Nile tilapia Oreochromis niloticus raised at different stocking densities. *Fish & shellfish immunology*, *39*(2), 305-311.

Wang, M., & Lu, M. (2016). Tilapia polyculture: a global review. *Aquaculture research*, *47*(8), 2363-2374.

Wang, Y. B., Tian, Z. Q., Yao, J. T., & Li, W. F. (2008). Effect of probiotics, Enteroccus faecium, on tilapia (Oreochromis niloticus) growth performance and immune response. *Aquaculture*, *277*(3-4), 203-207.

Zhang, W., Liu, M., & Dai, X. (2013). Biological characteristics and probiotic effect of Leuconostoc lactis strain isolated from the intestine of black porgy fish. *Brazilian journal of microbiology*, *44*, 685-691.