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Seyed Hossein Hoseinifar
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Novel Approaches Toward Sustainable Tilapia Aquaculture

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Editors

Novel Approaches Toward Sustainable Tilapia Aquaculture

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Dedicated to Zahra, Tik, and our lovely kids
Sina
Nima
Gerda
Sheryn

Preface

First of all, we would like to thank the ten coauthors for donating their time and knowledge to this book. We hope that it will advance sustainable aquaculture, enhance public perception of tilapia culture, and contribute to the preservation of the ecosystem, upon which we all rely.

Sustainable agriculture has been broadly defined in the 1990 US Farm Bill as "a system that meets basic human food and fiber needs, is economically viable, improves environmental quality and the resource base on which agriculture depends over the long term, makes the most efficient use of nonrenewable resources and on-farm resources, and integrates biological cycles and controls where appropriate." In the EU's current Blue Economy for a Sustainable Future, Green Deal, and F2F policies, aquaculture and sustainable aquaculture have emerged as crucial components. This method of food production has a lot of potential because it offers easily absorbed protein, has a small carbon footprint, and, with the appropriate strategy, favors short supply chains. The conviction behind this book, which was substantiated by study and practice, is that health management, feed additives, gut microbiota, probiotics, prebiotics, synbiotics, medicinal plants, alternative protein sources, and culture systems contribute to sustainable aquaculture. These novel approaches have played an important role in sustainable aquaculture development in the past few decades.

Our book was divided into nine chapters with approaches that contribute to sustainable Nile tilapia production. Chapter 1 covers the current status of world tilapia farming and highlights the advantages and challenges of commercial tilapia production including the economic aspects and the improvement of effluent quality to minimize the impact on the environment, which were covered in a different section. Chapter 2 discusses the health management of tilapia with a particular focus on various diseases and treatments. Chapter 3 emphasizes the effects of different feed additives on tilapia reproduction. A detailed reproductive physiology and breeding behavior of tilapia is discussed in this chapter with a special emphasis on the use of feed additives to control tilapia reproduction, sexual maturation, and sex determination. Chapter 4 covers the recent findings on the gut microbiota of tilapia with a particular focus on structure and modulation using environment-friendly approaches such as pro-, pre-, and synbiotics. Chapter 5 provides updated information regarding definitions, sources of bacterial probiotics, probiotic use in fish diets against pathogenic bacteria, mechanisms of action, beneficial aspects, and potential applications of probiotics in fish. It is anticipated that these will be of significant value for nutritionists, agricultural engineers, researchers, pharmacists, scientists, pharmaceutical industries, and veterinarians. Chapter 6 addresses the use of prebiotics and synbiotics as growth promotors and as alternatives to antibiotics in tilapia aquaculture, along with their possible mechanism of action. Moreover, the positive effects of pre- and synbiotics on tilapia health are also highlighted with emphases on growth, immune modulation, and alteration in gut microflora. Chapter 7 focuses on the use of different medicinal plants in tilapia aquaculture, their routes of administration, and their effects on fish physiology. Moreover, various modes of action of these medicinal plants are also discussed. Chapter 8 reviews the advances, novel ingredients, and new techniques that serve the mission for the future of aquafeed research on alternatives for fishmeal to achieve global aquaculture sustainability, with a focus on partial or full replacement of fishmeal especially in the feed of tilapia. Chapter 9 provides insights into the application of modern technology for sustainable tilapia production, which focuses on their effects on growth performance, immune response, and disease resistance.

Gorgan, Iran Seyed Hossein Hoseinifar
Chian Mai, Thailand Hien Van Doan

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Chapter 1
Tilapia Fish for Future Sustainable Aquaculture

Ghasem Ashouri, Seyed Hossein Hoseinifar, Ehab El-Haroun, Roberta Imperatore, and Marina Paolucci

Abstract Lately, aquaculture has been recognized as the fast-growing industry in the food production sector, and it requires maintainable development to cover the world population's demand for aquatic and seafood products. Among the 400 farmed fish species, warm-water fish species such as tilapia need a little quantity of fishmeal in their diets compared to other species. Tilapia is classified as the second most widespread species whose production is increasing every year; Nile tilapia (*Oreochromis niloticus*) is easily adaptable to a large variety of environments, is capable of reproducing in cavities, has an excellent market position in Asia and Africa, is highly resistant to diseases, has good fillet quality, shows moderate feed conversion ratio and excellent growth rate on many natural and artificial diets. Nile tilapia (*O. niloticus*) is known in the market as "aquatic chicken" due to its high tolerance to adverse physical and environmental conditions and overcrowding, its capability to survive at low oxygen levels, and a wide range of salinity concentrations. Tilapia adapts easily to natural and artificial feeds, has good feed conversion value, grows moderately fast, has a final high yield potential, and is accepted by customers worldwide. In addition, tilapia can grow in different aquaculture systems, ranging from extensive, semi-intensive, and intensive; also it can be grown in monoculture or polyculture techniques. Since tilapia grows well in adverse environmental conditions, tolerates stress factors as handling, and is resilient to disease agents of pathogen infections and infectious diseases, it has become the most

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Ghasem Ashouri, Seyed Hossein Hoseinifar, Ehab El-Haroun, Roberta Imperatore, and Marina Paolucci contributed equally with all other contributors.

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common species among farmers. Nile tilapia (*O. niloticus*) could be cultured in both fresh and saltwater; and in tropical, subtropical, and temperate climates. The authors in this chapter will cover the environmental and nutritional requirements of tilapia, defined as key factors in profit production. As mentioned above, tilapia has a wide tolerance for high stocking densities and environmental conditions. In addition to these advantages, tilapia grows very well in integrated culture systems with aquatic species such as carp and shrimps, as well as with crops like tomato and lettuce. However, the global extension of tilapia farming growing at a remarkably high rate is likely to pose environmental and socioeconomic threats. This chapter highlights the advantages and challenges of commercial tilapia production including the economic aspects, living behind the improvement of effluent quality to minimize the impact on the environment, which will be covered in a different section.

Keywords Tilapia · Farming · Sustainable · Production

1.1 Introduction

In the last few decades, aquaculture has become the fastest-growing sector in animal livestock production, securing a global food supply that reached 2018 ~115 million tons representing 263,400 million dollars (USD$) (FAO 2020). Feed formulation of warm-water fish species requires less fishmeal compared to other species. Tilapia production represents 40% of cultured fish (Prabu et al. 2019). Annual worldwide production of cultured tilapia was 3.4 million tons in 2011 and reached 6.2 million tons in 2019 (FAO 2020) (Fig. 1.1). Tilapia and catfish are considered to be native to the Middle East and Africa. Tilapia culture, though ancient (probably firstborn in Egypt simultaneously with Chinese polyculture), has experienced a recent commercial development. Today, tilapia has become one of the most attractive fish species in aquaculture due to several advantages such as i) massive adaptability to numerous environmental conditions, ii) easy reproduction in captivity, iii) resistance to environmental stress, diseases, and microbe pathogen infections, iv) good quality of flesh, v) feed on a low trophic level and excellent growth rate on a variety of diets (Welker and Lim 2011; Prabu et al. 2019). In 1980 tilapia was considered an ideal candidate for aquaculture in different regions of the world. Consequently, tilapia culture is currently growing commercially in at least 120 countries (Yue et al. 2016) all around the globe. Asian countries (e.g. China, Egypt, Indonesia, Philippines, and Thailand) are the major producers as well as consumers of tilapia (Chen et al. 2018). The most common cultured genus of tilapia is *Oreochromis*, and around 89% of these farmed fish are Nile tilapia (*Oreochromis niloticus*), due to their good growth performance in ponds (Ng and Hanim 2007).

Egypt and China are considered the main producers of Nile tilapia (*O. niloticus*) and represent one-third of the total global production (FAO 2020). The reasons for such a rapid expansion of the Nile tilapia (*O. niloticus*) culture could be attributed to

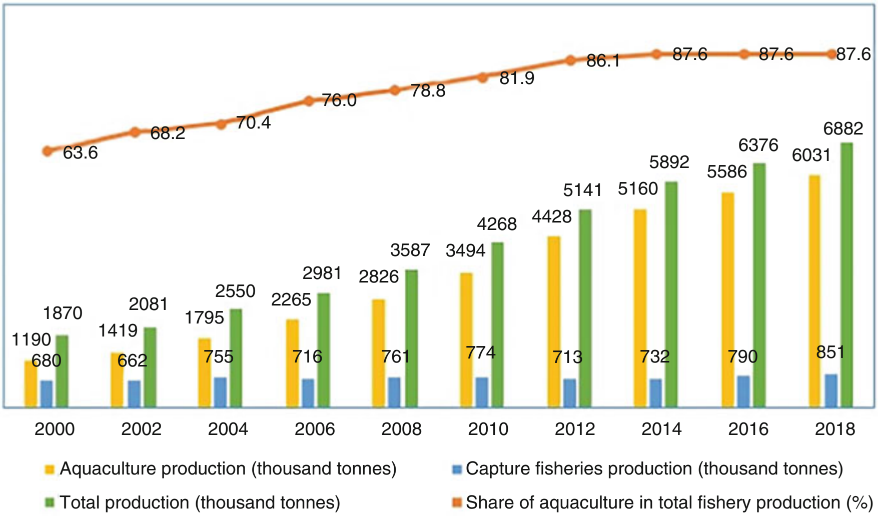

Fig. 1.1 Contribution of aquaculture to world tilapia production, 2000–2018. Data source: FAO (2020). FAO Global Fishery and Aquaculture Production Statistics (FishStat J; March 2020; www.fao.org/fishery/statistics/software/fishstatj/en)

technological advances associated with the intensification of cultural practices (Bhujel 2014b; Watanabe et al. 2002) including, i) development of novel strains and hybrids, ii) possibility to set up monosex male culture, iii) formulated diets, iv) use of a variety of production techniques such as semi-intensive and intensive systems (Ng and Romano 2013), and vi) marketing programmes aiming at enhancing the demand for tilapia on national and international markets (Wang and Lu 2016). Based on the increasing commercialization and continuing growth of the tilapia industry, the product is not only the second most important farmed fish globally (Fitzsimmons 2000), next to carp, but it is also described as the most important of all cultured fishes in the twenty-first century (Celik 2012).

The importance of tilapia among aqua farmers can be summarized as follows: (i) tilapia have fast growth and survival rate, (ii) reproduce easily in captivity, (iii) tolerate low water quality conditions and environmental variables such as temperature, salinity, low dissolved oxygen, etc., (iv) is easily adaptable to mono and polyculture techniques in intensive fish farming, (v) feed on low-quality diets and easily adapt to artificial diets, vi) show high profitability and low production costs, (vii) is highly resistant to stress and disease (lower risks for aqua farmers), and (viii) is highly accepted by consumers, with a good market request (Prabu et al. 2019; El-Sayed 2006b).

Although tilapia's culture is promising for aquaculture, in light of the many advantages above-mentioned, enhancing the production efficiency of tilapia has some challenges and research issues that are of the biggest concern to tilapia culturists (Yuan et al. 2017). For instance, these involve growth performance, unwanted reproduction (Gupta and Acosta 2004; Ng and Romano 2013; Chen

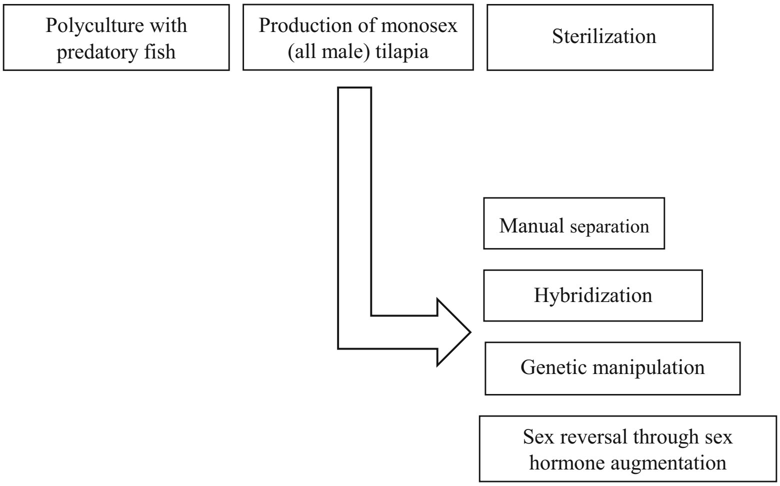

Fig. 1.2 Methods used for inhibiting reproduction or controlling the overpopulation of tilapias

et al. 2018), environmental tolerance (e.g. low temperatures and high salinity), disease resistance (Wang and Lu 2016; Li et al. 2016), quality of fillet yield (Yue et al. 2016), and increased production costs. Other issues of tilapia aquaculture are related to its negative effects on the environment and global biodiversity. Different protocols have been adopted to control and limit unwanted reproduction (Fig. 1.2). A brief overview of these methods is provided in one subsection of this chapter. However, none of these methods is considered 100% effective, and thus a combination of methods is suggested (Fuentes-Silva et al. 2013).

The poor aptitude of tilapia to tolerate low temperatures (<15°C) affects the geographical expansion of tilapia culture (El-Sayed 2006b; Lim and Webster 2006). The most cold-resistant species is blue tilapia, *Oreochromis aureus*, which is suitable for culture in regions with seasonal temperature changes and is usually used in the hybridization for the production of monosex. Moreover, most tilapia species are not tolerant to high salinity, although some (e.g., Mozambique tilapia, *Oreochromis mossambicus*; *Oreochromis spilurus*, redbelly tilapia, *Tilapia zillii*, and red tilapia hybrids) can grow in seawater. In comparison with other cultured fish (e.g. salmon), tilapia shows off-flavours and also minor levels of HUFAS, especially beneficial omega-3 fatty acids such as 20:5 n3 (EPA) and 22:6 n3 (DHA) which cause low market acceptance (Weaver et al. 2008). Finally, today, the disease resistance of tilapia has not received enough attention because rearing programmes have been focused only on growth efficiency and skin colour selection.

In brief, tilapia yield can be affected by several causes, so this chapter provides an overview of some aspects of tilapia culture. In particular, it focuses on some crucial factors for a successful production, such as management and nutritional requirements, evaluation of technological advances and different tilapia farming practices, environmental effects, and some constraints resulting from intensification practices.

1.2 Nutritional and Environmental Requirements

Tilapias possess highly desirable characteristics that make them good candidates for fish production under different production approaches as extensive, semi-intensive, and intensive, such as their ability to tolerate a wide range of environmental conditions (Chervinski 1982), high survival rate, and feed on a low trophic level which makes them attractive species to aquaculture investors. However, dietary requirements under different production techniques and the association with culture conditions are still not yet clear.

1.2.1 Nutritional Requirements

Fish feed generally accounts for as much as 60% or more of production costs in both semi-intensive and intensive aquaculture production systems (Montoya-Camacho et al. 2019). The nutritional requirements of tilapia have been comprehensively reviewed by Ng and Romano (2013), and Chavan et al. (2015). An obstacle to tilapia intensive culture is the rising cost and unpredictable reliability of fishmeal and fish oil global supplies (Ng and Romano 2013). Consequently, several alternative ingredients, in particular of plant origin, have been investigated, and some of them are currently used in tilapia feeds to reduce the fishmeal amount (Montoya-Camacho et al. 2019). Fish meal (FM) and fish oil (FO) are the main sources of, respectively, amino acids and fatty acids for many different species. The investigation of alternative ingredients of FM and FO requires a thorough understanding of the quantity and quality requirements of different nutrients of tilapia, besides the comprehension of the factors that may influence the nutrient utilization efficiency and consequently affect the production (Ng and Romano 2013).

1.2.1.1 Protein and Amino Acid Requirements of Tilapia

Proteins represent the most expensive components in aquafeeds (El-Sayed 1999). Several factors affect protein requirements such as protein source and amino acid (AA) profile, fish species, age, size, and life stage. Tilapia larvae, fry and fingerling require a high level of protein (30–40%) compared to tilapia broodstock (20–25%). Male tilapia requires a higher level of protein than females to reach optimal growth performance (Ng and Romano 2013; Abdel-Tawwab et al. 2010; Sweilum et al. 2005). In terms of protein, it is not just the quantity, but the quality and the essential amino acid (EAA) profile that will tremendously impact the total protein requirement. The ideal proteins to be introduced to the diet are represented by those whose amino acid composition is similar to the ratio required by the animal (Nguyen and Davis 2009). Furthermore, this will also decrease nitrogenous waste related to amino acids being underutilized or deaminated as an energy source (Abdel-Tawwab et al.

Table 1.1 The quantitative essential amino acid requirements of warm-water fish.[a] Table modified from Jauncey (2000)

	Common carp[b]	Channel cattfish[c]	Nile tilapia[d]	Mozambique tilapia[e]
Arginine	3.3	4.3	4.2	2.8
Histidine	2.1	1.5	1.7	1.1
Isoleucine	2.5	2.6	3.1	2.0
Leucine	3.3	3.5	3.4	3.4
Lysine	5.7	5.1	5.1	3.8
Methionine	2.1	2.3	2.7	1.0
Phenylalanine	3.4	5.0	3.8	2.5
Threonine	3.9	2.0	3.8	2.9
Tryptophan	0.8	0.5	1.0	0.4
Valine	3.6	3.0	2.5	2.2

[a] All values as % of dietary protein

[b] Experimentally determined data for common carp (*Cyprinus carpio*) from the review of Tacon (1987). Requirement estimated by the dose-response method

[c] Experimentally determined data for channel catfish (*Ictalurus punctatus*) from the review of Wilson (1991). Requirement estimated by the dose-response method

[d] Experimentally determined data for Nile tilapia (*Oreochronis niloticus*) from Santiago and Lovell (1988). Requirement estimated by the dose-response method

[e] Experimentally determined data for Mozambique tilapia (*O. Mossambicus*) from Jauncey (1983). Requirement estimated by the whole body and muscle amino acid composition method

2010). Prediction of EAA requirement of different fish species could be measured by either dose-response protocol or by measuring the amino acid profile of the whole body of fish. As known, warm-water species including the Nile tilapia (*O. niloticus*) require 10 essential amino acids (Table 1.1). EAA requirements could be covered using a mixture of plant and animal proteins (Montoya-Camacho et al. 2019), or by the inclusion of free amino acids (Nguyen and Davis 2009).

1.2.1.2 Lipid and Fatty Acid Requirements of Tilapia

Lipids and oils are considered to be the main source of digestible energy and essential fatty acids (EFAs) for the normal growth and development of fish. In addition, phospholipids play main functions in cell membrane structure and integrity, facilitate and control the absorption of fat-soluble vitamins, act as forerunners for sex hormones, and improve the texture and flavour of the diet.

Tilapia requirements of lipids rely on several factors including fish species, age, size, source of lipids, protein, and energy content (El-Sayed 2006b). For example, it was noticed that the level of protein decreased in the Nile tilapia (*O. niloticus*) diets from 33.1% to 25.6% by elevating lipid content from 5.2% to 9.1% and carbohydrates (CHO) from 31.7% to 36.7% (Li et al. 1991). The role of increasing lipid content could be described as a sparing protein effect, confirmed by Jauncey (2000) in hybrid tilapia (*O. niloticus* × *O. aureus*). Though increasing lipid levels up to 12% harm the growth of juvenile *O. aureus* × *O. niloticus* hybrids and augments the

Table 1.2 The essential fatty acid requirements of tilapia[a]

Species	Requirement	Reference
Tilapia zillii	1% 18:2n-6 or 1% 20:4n-6	Kanazawa et al. (1980)
Oreochromis niloticus	0.5–1% 18:2n-6 or 1% 20:4n-6	Teshima et al. (1982)
O. niloticus	0.5% 18:2n-6	Takeuchi et al. (1983)
O. aureus	18:2n-6 or 18:3n-3 $\leq$1%	Stickney and McGeachin (1983)

[a]Table adapted from Jauncey (2000)

accumulation of lipid in the carcass of the fish (Jauncey 2000), also it has a negative impact on the pelleting processing of the diets. However, an extruded feed where fat is added after the pelleting process solved the problem. In general, tilapia require about 10–15% dietary lipids (El-Sayed 2006b); however, the oil inclusion of commercial tilapia feed is typically about 4–5% (Orachunwong et al. 2001). The required EFAs cannot be synthesized by fish and must be provided by the diet (Jauncey 2000). Research on fatty acid requirements revealed that cold-water fish and marine fish require *w*-3 polyunsaturated fatty acids (n-3 PUFA), while freshwater and warm warm-water species require n-6 PUFA. Thus, warm-water species, including tilapia, utilize plant oil as a source of n-6 fatty acids more efficiently than FO and lipids as a source of n-3 fatty acids (El-Sayed 2006b). Several studies have indicated that tilapia requires n-6 EFA rather than n-3 EFA (Table 1.2).

The findings of previous research summarized that EFAs are considered a source of the fatty acid content in tilapia fillets and support the growth of fish. It has been suggested that diets for farmed tilapia should contain 0.5–1.0% of both n-3 and n-6 PUFA (Lim et al. 2011a; Ng 2005). Tilapia-fed diets containing high levels of n-3 PUFA have positive effects on the health of consumers, such as its positive impacts on the cardiovascular system (Lecerf 2009; Russo 2009), immune system (Ruxton et al. 2004) and inflammatory disorders (Calder 2006). In the last few decades, research conducted to find novel ingredients to substitute FO with vegetable oil in tilapia feed has been successfully carried out; however, high interest remains in using palm oil because of the low price compared to other vegetable oils and its easy availability on the market (Ng et al. 2001; Ng et al. 2006; Ng and Gibon 2010; Bahurmiz and Ng 2007). Fortunately, the use of palm oil in the diet does not reduce ($P \leq 0.05$) the performance of the Nile tilapia (Ng et al. 2001). However, the high inclusion of plant oil raises an important question to be addressed by scientists, that is, the plant oil's role in the fish diets and the impact on the fatty acid composition of the final product and, thus, the impact on human health (Huang et al. 1998; Young 2009; Bahurmiz and Ng 2007).

Recently, there has been an interest in conducting research using finishing diets rich in n-3 PUFA to investigate their effect on adjusting the final fatty acid profile of tilapia fillets to enhance their nutritional value before harvest (Ng and Chong 2004; Visentainer et al. 2005; Tonial et al. 2009; Trushenski et al. 2009; Dos Santos et al. 2011; Luo et al. 2012). In this context, Tonial et al. (2009) found that Nile tilapia (*O. niloticus*) fed diets containing soybean oil showed a decrease in the n-6/n-3 ratio in the fillet from 7.4 to 1.0 when reverted to a flaxseed-based diet which is rich in n-3

PUFA. In addition, Teoh et al. (2011) examined the FAs metabolism of both Genetically Improved Farmed Tilapia (GIFT) strain and red hybrid tilapia-fed purified diets with vegetable oil blends, and they found that FAs digestibility was not different among the tilapia strains.

1.2.1.3 Carbohydrates

Previous research carried out on tilapia requirement of carbohydrates (CHO) declared that tilapia does not have CHO specific requirements. Though, Wilson (1994) reported that warm-water fish species such as tilapia utilize CHO more efficiently than cold-water species. The main purpose of CHO inclusion in the diet is to act as an effective source of energy that can spare the protein as a source of energy to support fish growth. In addition, CHO act as a binder, facilitating the pelleting process, also CHO acts as a precursor of different metabolic components (NRC 1993). Fish species can utilize up to 35 to 40% digestible CHO (Anderson et al. 1984; El-Sayed and Garling Jr 1988). Several factors affect CHO digestion and assimilation, including the source of CHO since fish utilize complex carbohydrates (polysaccharides) more efficiently than mono and disaccharides (Shiau and Chuang 1995). It has been shown that increasing the dietary CHO/lipid ratio leads to increased glycolysis and lipogenesis but reduces gluconeogenesis and amino acid degradation in the liver of the Nile tilapia, *O. niloticus* (Shimeno et al. 1993). Moreover, it has been reported that CHO metabolism is influenced by their fibre content (Shiau and Yu 1999) and is affected by the dietary protein source (Shiau and Suen (1992). In this context, El-Sayed (1991) found that sugarcane bagasse could be included in *T. zillii* diets without a negative impact on both growth and feed digestibility, while the inclusion of sugarcane in Nile tilapia (*O. niloticus*) diets resulted in poor performance. Also, larger fish of the hybrid of *O. niloticus* × *O. aureus* utilized CHO better than smaller ones (Tung and Shiau 1993). Finally, previous research concluded that increasing feed frequency from 2 to 6 times/day enhanced growth, CHO utilization, and protein sparing effect (Tung and Shiau (1991); Shiau and Lei (1999); (Jauncey 2000).

1.2.1.4 Vitamin and Mineral Requirements of Tilapia

Micronutrients such as vitamins and minerals are essential cofactors in several metabolic mechanisms involved in different physiological processes in fish health and welfare. Different factors affect vitamin and mineral requirements, such as culture conditions and chemical dietary composition (Celik 2012). For example, in both extensive and semi-intensive fish production system, the inclusion of vitamins and minerals are not necessary since the fish consume natural food such as phytoplankton and zooplankton that contain enough amounts of vitamins and minerals that fulfil the fish requirements (El-Sayed 2006b) On the contrary, in intensive systems the presence and availability of natural food are limited or absent. Thus vitamins and minerals must be incorporated into the diets to support growth, health, and survival rate (Ng and Romano 2013). Fish feed manufacturers usually over-supplement feed with vitamins and minerals to counteract losses due to processing, storage, and leaching. It is well known that the nutritional requirements of vitamins and minerals depend on the life stage of tilapia. Tables 1.3 and 1.4 refer to the water-soluble and lipid-soluble vitamin requirements of tilapia fry and fingerlings stages, respectively. Shiau and Lung (1993) indicated that vitamin B12 is not required for tilapia hybrid (*O. niloticus* × *O. aureus*), likely due to the ability of gut bacteria to synthesize it (Shiau and Lung 1993; Shiau and Huang 2001; Barros et al. 2009). In terms of vitamin A requirement, Guo et al. (2010) reported that Nile tilapia (*O. niloticus*) does not require supplementation of vitamin A since cod liver oil, with its high content of vitamin A, is used as a source of lipids in the diet. In addition, Hu et al. (2006) reported that tilapia hybrids (*O. niloticus* × *O. aureus*) can synthesize vitamin A from β-carotene.

Mineral requirements of tilapia have been comprehensively reviewed by Ng and Romano (2013), and also Makwinja and Geremew (2020). Vital minerals are involved in many physiological processes, such as

- build skeletal structures
- osmoregulation (e.g., Na^+/K^+-ATPase)
- nerve and muscle contraction
- regulation of the pH of the blood and other body liquids
- metabolism-related enzyme activity (lipase, alkaline phosphatase) as cofactors
- key components of many enzymes, vitamins, hormones, and respiratory pigments

Nile tilapia (*O. niloticus*) usually require minerals from two major sources: water and feed. Brackish/marine environments are considered as main sources of minerals. However, since tilapias are mostly farmed in freshwater/low salinity waters, supplementing the diets with minerals is important to fulfil their needs to achieve optimal health and productivity. Previous research conducted to measure the mineral requirements of hybrid tilapia stated that the requirement of dietary NaCl or KCl ranged between 1.5 and 2–3 g kg^{-1} of diet, respectively (Shiau and Lu 2004); (Shiau and Hsieh 2001). Correct mineral requirement fulfilment is considered essential since the deficiency or excess of minerals leads to depressed growth performance.

Previous research confirmed the function of minerals as essential to support growth performance and the health status of different fish species. Robinson et al. (1987) reported that the inclusion of a 7 g Ca kg^{-1} purified diet was important to maintain the optimum growth of blue tilapia. Also, Shiau and Tseng (2007) found that hybrid tilapia-formulated diets should be supplemented with 2.7–3.3 g L^{-1} Ca and purified diets supplemented with 3.5–4.3 g Ca kg^{-1} to achieve optimum growth and feed efficiency. Research carried out on magnesium showed that 0.59 to 0.77 g and 0.50 to 0.65 g kg^{-1} diet were required for optimum performance of the Nile tilapia, *O. niloticus,* and blue tilapia, *O. aureus* (Dabrowska et al. 1989: Reigh et al. 1991), respectively. Moreover, trials conducted on phosphorus showed that a 5 g P kg^{-1} diet of phosphorus is required for the best growth and bone mineralization of *O. aureus* (Robinson et al. 1987).

Table 1.3 Dietary water-soluble vitamin requirements of tilapia species. Units are mg kg^{-1} of diet unless otherwise stated

Vitamin	Species and size (g)	Recommended level	Deficiency signs	Reference
Choline	*O. niloticus* (3.0)	3000		Kasper et al. (2000)
	O. niloticus × *O. aureus* (0.6)	1000	poor growth, reduced survival, reduced blood triglyceride and phospholipids concentrations	Shiau and Lo (2000)
Thiamin (B1)	*O. mossambicus*	2.5	anorexia, light coloration, nervous disorder, poor growth and poor feed efficiency, low hematicrit and red blood cell count, and increased serum pyruvate	Lim et al. (1991)
	O. niloticus	4.0		Lim et al. (2000)
	O. niloticus (4–5)	3.5		Lim et al. (2011b)
Riboflavin (B2)	*O. aureus* (0.7)	6.0	lethargy, anorexia, poor growth, high mortality, loss of colour, fin erosion, short body dwarfism, and cataracts	Soliman and Wilson (1992b)
	O. mossambicus × *O. niloticus*	5.0		Lim et al. (1993)
Niacin (B3)	*O. niloticus* × *O. aureus* (2.2)	26 (fed glucose) 121 (fed dextrin)	haemorrhage, deformed snout, gill and skin oedema, fin and mouth lesions	Shiau and Suen (1992)
Pantothenic acid (B5)	*O. aureus* (0.7)	10	poor growth, haemorrhage, sluggishness, high mortality, anaemia, hyperplasia of gill lamellae, and increased mortality	Soliman and Wilson (1992a)
	O. niloticus (13.7)	10		Ayyat et al. (2011)
Pyridoxine (B6)	*O. mossambicus* × *O. niloticus*	3		Lim et al. (1995)
	O. niloticus × *O. aureus* (0.7)	1.7–9.5 (28% CP) 15.0–16.5 (36% CP)	poor growth, high mortality, abnormal neurological signs, caudal fin erosion, mouth lesion, and convulsions	Shiau and Hsieh (1997)
Biotin (B7)	*O. niloticus* × *O. aureus* (1)	0.06	poor growth reduced hepatic pyruvate carboxylase and acetyl CoA carboxylase activities	Shiau and Chin (1999)
Folic acid (B9)	*O. niloticus* ()	0.5	anorexia, reduced growth, lethargy, increased mortality, low haematocrit	Lim and Klesius (2001)
	O. niloticus × *O. aureus* (0.4)	0.8		Shiau and Huang (2001)

	O. niloticus (14.5)	0.5–1.0		Barros et al. (2009)
Vitamin B12	*O. niloticus* × *O. aureus* (1.0)	Not required		Shiau and Lung (1993)
Vitamin C[a]	*O. niloticus* × *O. aureus* (1.5)	80 (Ascorbic acid) (41–48) C2S (37–42) C2MP-Mg	lordosis, scoliosis, poor growth, feed efficiency, poor wound healing, haemorrhage, anaemia, exophthalmia and gill, and operculum deformity	Shiau and Hsu (1999)
Inositol	*O. niloticus* (5.8)	Not required		Peres et al. (2004)
	O. niloticus × *O. aureus* (0.5)	400	poor growth, higher muscle/liver lipid	Shiau and Su (2005)

O. Oreochromis sp., *CP* crude protein

[a] Since ascorbic acid is unstable, storage, more stable forms of ascorbic acid such as L-Ascorbyl-2-sulphate (C2S) and L-ascorbyl-2-monophosphate-magnesium (C2MP-Mg) should be used

Table 1.4 Dietary lipid-soluble vitamin requirements of tilapia species. Units are mg kg^{-1} of diet unless otherwise stated

Vitamin	Species and size (g)	Recommended level	Deficiency signs	Reference
Vitamin A	*O. niloticus* (11.4)	5000 IU kg^{-1}	poor growth, abnormal swimming, restlessness, exophthalmia, high mortality, haemorrhage, and reduce the mucus secretion	Saleh et al. (1995)
	O. niloticus × *O. aureus* (1.6)	6000–7000 IU kg^{-1}		Hu et al. (2006)
	O. niloticus (7.7)	Not required		Guo et al. (2010)
β-carotene	*O. niloticus* × *O. aureus* (0.5)	29.0–44.0		Hu et al. (2006)
Vitamin D	*O. niloticus* × *O. aureus* (0.8)	375 IU kg^{-1}	poor growth, low haemoglobin, reduced hepatosomatic index, and alkaline phosphatase	Shiau and Hwang (1993)
	O. aureus (2.3)	Not required		O'Connell and Gatlin (1994)
Vitamin E	*O. niloticus* (7.5)	20–100		Eleraky et al. (1995)
	O. niloticus × *O. aureus*	63–206 (12% CL)	poor growth, anorexia, haemorrlage, impaired erythropoiesis, muscle degeneration, ceroid deposition in liver and spleen and lack of skin colour	Shiau and Shiau (2001)
	O. niloticus × *O. aureus* (0.7)	42–44 (5% CL)		Huang et al. (2003)
Vitamin K	*O. niloticus* × *O. aureus*	5.2	poor growth and low plasma prothrombin	Lee (2003)

O. Oreochromis sp., *CL* crud lipid

Chromium (Cr), zinc (Zn), copper (Cu), selenium (Se), and Iron (Fe) are considered trace elements, and research on these elements showed their importance in improving the growth and health status of tilapia. Shiau and Shy (1998) found that the inclusion of 140 mg Cr kg^{-1} improved the growth of hybrid tilapia due to its role as a cofactor in the activity of insulin and enhancing carbohydrate utilization. Furthermore, (Eid and Ghonim 1994) and do Carmo Sá et al. (2004) stated that the inclusion of 0.03 g Zn kg^{-1} for fry and 0.08 Zn kg^{-1} for fingerling diet is required for optimum growth. More research conducted by Watanabe et al. (1988) found that Nile tilapia (*O. niloticus*) required 2 to 3 mg Cu kg^{-1}. Shiau and Su (2003) found that hybrid tilapia (*O. niloticus* × *O. aureus*) required 150 to 160 mg kg^{-1} Fe.

1.2.1.5 Nutrition and Immunity

The production of the Nile tilapia (*O. niloticus*) under a semi-intensive and intensive production system exposes the fish to pathogen infections and disease outbreaks. The main strategy to maintain fish health in aquaculture is the provision of a balanced diet supplemented with immune stimulants that help boost the immune system and keep it under control of disease outbreaks. Functional feed additives such as prebiotics, bioactive compounds derived from medicinal plants, and probiotics could avoid the use of antibiotics and chemotherapy and aid in limiting disease outbreaks by controlling fish mortality in intensive aquatic farms (Merrifield et al. 2010; Hoseinifar et al. 2016: Dawood and Koshio 2016). Functional feed additives modify the gut microbiome, increase the activity of beneficial bacteria, increase the secretion of digestive enzymes, and decrease harmful bacteria. In addition, these feed additives upregulate gene expression related to immunity and inflammatory cytokines such as *IL-1*, *IL-8*, and *Lyz*, oxidative enzymes such as catalyse, superoxide dismutase, and glutathione, and growth genes such as *GH* and *IGF-1*. Such compounds act as health factors capable of modulating the immune responses in tilapia (Table 1.5 included as supplementary data), and other cultivated fish species.

Furthermore, functional feed additives have a positive impact on the immune system defence system by (i) stimulating the production of plasma proteins (globulin and albumin), which play a vital role in the synthesis of antibodies (immunoglobulins), (ii) enhancing the activity of lysozyme, (iii) increasing the production of defence cells such as leukocytes and lymphocytes that produce antibodies), (iv) stimulating the production of macrophages, which are responsible for the phagocytosis, and (v) modulating the composition of the gut flora and improving gut health via the increase in villi length, width goblet cells, improving mucus secretion, and reducing gut inflammation.

Probiotics, generally defined as live microorganisms, are provided via the diet or rearing water (when supplied in an adequate amount). They possess different beneficial characteristics leading to the exclusion of pathogenic bacteria and the modulation of the immune system of the host, by improving the microbial balance of the host (Merrifield et al. 2010). The most common probiotics used in aqua feeds are

Table 1.5 Tilapia studies to evaluate the effects of functional feed additives on immunity*

Feed additives	Tilapia species	Immune responses	Immune-related genes	Stress-regulating genes	Anti-oxidative capacity	Haematological and biochemical parameters	Disease challenge	Disease resistance and survival rate	Reference
Probiotics									
L. rhamnosus	Nile tilapia (*O. niloticus*)	LZM↑, PA↑, ACH50↑	–	–	–	–	*E. tarda*	↑	Pirarat et al. (2006)
Commercial probiotics[a]		LZM↑, BA↑, NBT→	–	–	–	Neutrophil↑, Hb↑, Plasma protein↑	*E. tarda*	↑	Taoka et al. (2006)
S. cerevisiae		BA↑	–	–	–	Neutrophil↑, RBCs↑, Hb↑, Ht↑, Albumin↑, Globulin↑	*A. hydrophila*	↑	Abdel-Tawwab et al. (2008)
B. subtilis + *L. acidophilus*		NBT↑, LZM↑, BA↑	–	–	–	Ht↑	Mixed species pathogen[b]	↑	Aly et al. (2008)
E. faecium		LZM↑, C3↑, RBA↑	–	–	MPO↑	Serum protein→, Albumin→, Globulin→	–	–	Wang et al. (2008)
P. acidilactici		LZM↑, RBA→	–	–	–	WBCs↑, RBCs→, Serum protein→, Albumin→, Globulin→	–	–	Ferguson et al. (2010)
B. coagulans		LZM→, RBA↑	–	–	SOD↑, CAT↑, MPO↑	Serum protein↑, Albumin→, Globulin↑	–	–	Zhou et al. (2010a)
Lactococcus lactis		LZM↑, RBA↑	–	–	SOD↑, MPO↑	–	*A. hydrophila*	↑	Zhou et al. (2010b)

L. rhamnosus		LZM→, PA↑, ACH50↑, RBA→, BA↓	*TNF-α*↑, *IL-1*↑	–	–	–	–	–	Pirarat et al. (2011)
P. acidilactici		LZM→, IEL↑, GC↑	*TNFα*↑	–	–	WBCs↑, Neutrophils↑, Monocytes↑,	–	–	Standen et al. (2013)
L. acidophilus		BA↑	*IL-1β*	–	–	–	*A. hydrophila*	↑	Villamil et al. (2014)
B. licheniformis		LZM↑, C3↑	–	–	SOD↑	–	*S. iniae*	↑	Han et al. (2015)
B. subtilis + *S. cerevisiae*		RBA↑	–	–	–	WBCs↑, Lymphocyte↑, Monocyte↑, RBCs↑, Ht↑, Hb→,	*A. hydrophila* *S. iniae*	↑	Iwashita et al. (2015)
L. plantarum		Total Ig↑, PA↑, LZM↑	*IL-4*↑, *IL-12*↑, *IFN-γ*↑,	–	–	WBCs↑, RBCs↑, Hb↑, Serum protein↑, Albumin↑, Globulin↑	*A. hydrophila*	↑	Hamdan et al. (2016)
AquaStar® Growout[c]		IEL↑, GC↑	*TLR2*↑, *TNF-α*↑, *IL-1β*↑, *IL-10*↑, *TGF-β*↑	*Caspase-3*↑, *PCNA*↑, *HSP70*↑	–	–	–	–	Standen et al. (2016)
AquaStar® Growout[c]	Nile tilapia (*O. niloticus*)	ACH50↑, RBA→, LZM→	–	–	CAT↑, GPx↑, GR↑, GSH↑	WBCs→, RBCs→, HT→	–	–	Ramos et al. (2017)
L. plantarum/ *B. velezensis*		Serum ACH50↑, RBA↑, PA↑; Serum and mucus LZM↑,	–	–	Serum and mucus Peroxidase activity↑	–	*S. agalactiae*	↑	Van Doan et al. (2018c)

(continued)

Table 1.5 (continued)

Feed additives	Tilapia species	Immune responses	Immune-related genes	Stress-regulating genes	Anti-oxidative capacity	Haematological and biochemical parameters	Disease challenge	Disease resistance and survival rate	Reference
		Peroxidase activity↑,							
L. rhamnosus / *Lactococcus lactis*		–	*TNF-α*↑, *IFN-γ*↑, *Lyzc*↑, *IL-1β*↑	*HSP70*↑	–	–	*S. agalactiae*	↑	Xia et al. (2018)
B. subtilis/ *S. cerevisiae*		LZM↑	–	–	–	WBCs↑, RBCs↑, Hb↑, Serum protein↑, Albumin↑, Globulin↑	–	–	Opiyo et al. (2019)
R. stabekisii		RBA↑, PA↑; serum LZM↑,	*TNF-α*↑, *IL-1β*↑, *TGF-β*↑	*HSP70*↑	SOD↑	–	*A. hydrophila*	↑	Tan et al. (2019)
Bacillus sp.		LZM↑, IgM↑, GC↑	–	–	SOD↑, CAT↑	ALP↑	*A. hydrophila*	↑	Kuebutornye et al. (2020)
B. subtilis / *B. cereus*		–	*Lyzc*↑	–	–	–	*S. agalactiae*	↑	Xia et al. (2020)
S. cerevisiae	Hybrid tilapia (*O. niloticus* ♀ × *O. aureus* ♂)	LZM↑, PA↑, RBA↑, C3↑, C4↑	–	–	–	–	–	–	He et al. (2009)
B. subtilis		–	*TNF-α*↑, *IL-1β*↑, *TGF-β*↑	*HSP70*↓	–	–	–	–	He et al. (2013)
L. brevis/ *L. acidophilus*		–		*HSP70*↑	–	–	*A. hydrophila*	↑	Liu et al. (2013)

			TNF-α↑, *IL-1β*↑, *TGF-β*↑						
L. plantarum			*TNF-α*↑, *IL-1β*↑	*HSP70*↑			*A. hydrophila*	↑	Ren et al. (2013)
Lycogen™^{d}	Red tilapia (*O. mossambicus* × *O. niloticus*)	LZM↑, ACH50↑	–	–	–	–	–	–	Chiu and Liu (2014)
Bacillus spp.		–	*IFN-γ*↑, *IL-8*↑, *IRF-3*↑, *MX*↑, *RSAD-2*↑	–	–	–	TiLV^{e}	↑	Waiyamitra et al. (2020)
Prebiotic									
β-glucan	Nile tilapia (*O. niloticus*)	LZM↑	–	–	–	–	*S. iniae*	↑	Whittington et al. (2005)
Sangrovit®^{f}		LZM→	–	–	–	WBCs↑, RBCs→, Hb→, ALT→	–	–	Rawling et al. (2009)
Inulin		LZM↑, NBT↑	–	–	–	Ht↑	*A. hydrophila*	↑	Ibrahem et al. (2010)
GroBiotic®-A^{g}	Nile tilapia (*O. niloticus*)	LZM↑, NBT↑	–	–	SOD↓, CAT↓	–	*A. hydrophila*	↑	Zheng et al. (2011)
GroBiotic®-A^{g}		LZM→, ACH50→, total Ig→	–	–	–	Serum total Protein→	*S. iniae*	→	Vechklang et al. (2012)
β-glucan		Serum LZM→, ACH50→, RBA↑, BA→	–	–	–	WBCs→, RBCs→, Hb→, Ht→	*S. iniae*	→	Welker et al. (2012)
Inulin or JA^{h}		LZM↑, ACH50↑, total Ig↑, GC↑	–	–	–	Albumin↑, Total Protein↑	–	–	Tiengtam et al. (2015)

(continued)

Table 1.5 (continued)

Feed additives	Tilapia species	Immune responses	Immune-related genes	Stress-regulating genes	Anti-oxidative capacity	Haematological and biochemical parameters	Disease challenge	Disease resistance and survival rate	Reference
MOS		LZM→, RBA→	–	–	–	Serum total Protein→	–	–	Yuji-Sado et al. (2015)
FOS		LZM↑, serum IgM↑, NO↑	–	–	SOD↑CAT↑, GPx↑, MDA↑	–	–	–	Abd El-Gawad et al. (2016)
LMWSA		LZM↑, Serum ACH50↑, RBA↑, PA↑	–	–	–	–	*S. agalactiae*	↑	Van Doan et al. (2016b)
XOS		Serum and mucus LZM↑, Serum ACH50↑, RBA↑, PA↑	–	–	Serum and mucus Peroxidase activity↑	–	*S. agalactiae*	↑	Van Doan et al. (2018b)
COS	Hybrid tilapia (*O. niloticus* ♀ × *O. aureus* ♂)	–	*TNF-α*↓, *TGF-β*↑	*HSP70*↓	–	–	*A. hydrophila*	↑	Qin et al. (2014)
GOS	Red hybrid tilapia red tilapia (*O. niloticus* × *O. mossambicus*)	–	–	–	–	WBCs↑, RBCs↑, Ht→	*S. iniae*	↑	Plongbunjong et al. (2011)
Synbiotic									
S. cerevisiae + β-glucan	Nile tilapia (*O. niloticus*)	LZM→, total Ig→, ACH50→	–	–	–	Serum total protein→	–	–	Shelby et al. (2009)
L. plantarum + LMWSA		Serum LZM↑, ACH50↑, RBA↑, PA↑	–	–	–	–	*S. agalactiae*	↑	Van Doan et al. (2016a)

B. subtilis + Malic acid		–	–	–	–	WBCs↑, RBCs↑, Ht↑, Hb↑, total Protein↑, Albumin↑, globulin↑, ALT↓, AST↓	–	↑	Hassaan et al. (2018)
L. plantarum + CCM[i]	Nile tilapia (*O. niloticus*)	Mucus LZM↑, Serum LZM↑, ACH50↑, RBA↑, PA↑	–	–	Serum and mucus Peroxidase activity↑	–	–	–	Van Doan et al. (2017a)
Kefir[j] + LMWSA		Serum LZM↑, ACH50↑, RBA↑, PA↑	–	–	–	–	*S. agalactiae*	↑	Van Doan et al. (2017b)
ASP[k] + β-glucan		NBT↑, LZM↑, IgM, BA↑, PA↑	–	–	SOD↑, CAT↑, GPx↑, MDA↓	WBCs↑, RBCs↑, Ht↑, Hb↑, total Protein↑	–	–	Dawood et al. (2020)
L. plantarum + XOS		Mucus LZM↑, Serum LZM↑, ACH50↑, RBA↑, PA↑	–	–	Serum and mucus Peroxidase activity↑	–	*S. agalactiae*	↑	Van Doan et al. (2020b)
Lactobacillus spp. + FOS	Hybrid tilapia (*O. niloticus* ♀ × *O. aureus* ♂)	–	–	–	–	–	*A. hydrophila*	↑	Liu et al. (2017)
Plant compounds									
Garlic (*Allium sativum*)	Nile tilapia (*O. niloticus*)	–	–	–	–	WBCs↑, RBCs↑, Ht↑, Hb↑, ALT↓, AST↓	*A. hydrophila*	↑	Shalaby et al. (2006)
		Serum LZM↑, RBA↑, PA↑	–	–	–	–	–	–	Yin et al. (2006)

(continued)

Table 1.5 (continued)

Feed additives	Tilapia species	Immune responses	Immune-related genes	Stress-regulating genes	Anti-oxidative capacity	Haematological and biochemical parameters	Disease challenge	Disease resistance and survival rate	Reference
Chinese herbs (*Astragalus radix*)	Nile tilapia (*O. niloticus*)								
Garlic (*Allium sativum*)		NBT↑	–	–	–	–	*P. fluorescens*	↑	Diab et al. (2008)
Ginseng Herb (Ginsana® G115)		–	–	–	–	WBCs↑, RBCs↑, Ht↑, Hb↑	–	–	Goda (2008)
Psidium guajava		–	–	–	–	–	*A. hydrophila*	↑	Pachanawan et al. (2008)
Garlic (*Allium sativum*)		–	–	–	SOD↑, CAT↑, GPx↑, MDA↓	Serum total Protein↑, ALT↓, AST↓	–	–	Metwally (2009)
Echnicacea and Garlic[1]		–	–	–	–	WBCs↑, Ht↑, Neutrophil↑, Lymphocyte↑	*A. hydrophila*	↑	Aly and Mohamed (2010)
Rosmarinus officinalis		–	–	–	–	–	*S. iniae*	↑	Zilberg et al. (2010)
Ginger (*Zingiber officinale*)		LZM↑, IgM↑	–	–	–	–	*A. hydrophila*	↑	El-Sayed et al. (2014)
Aloe vera		LZM→	–	–	–	WBCs↑, RBCs↑, Ht↑, Hb↑, Neutrophil↑, monocytes↑	*S. iniae*	↑	Gabriel et al. (2015)

Moringa oleifera		–	–	–	–	ALT↓, AST↓	*A. hydrophila*	↑	Gbadamosi et al. (2016)
Ginger (*Z. officinale*)		–	–	–	SOD↑, CAT↑, MDA↓	WBCs↑, RBCs↑, Ht↑, Hb↑, Neutrophil↑, monocytes↑	*A. hydrophila*	↑	Şahan et al. (2016)
Orange pectin		Mucus LZM↑, Serum LZM↑, ACH50, PA↑↑, RBA→	–	–	Serum and mucus Peroxidase activity↑	–	*S. agalactiae*	↑	Van Doan et al. (2018a)
Withania sominefera		NBT↑	–	–	Liver SOD↑, CAT↑, GST↑, GSH↑, GPx↑, MDA↓, serum TAC↑	–	*A. hydrophila*	↑	Zahran et al. (2018)
Ocimum basilicum		Serum LZM↑	–	–	–	Ht→, Hb↑, Plasma total Protein↑, ALT↓,	–	–	de Souza et al. (2019)
Thai ginseng (*Boesenbergia rotunda*)		Mucus LZM↑, Serum LZM↑, ACH50↑, RBA↑, PA↑	–	–	Serum and mucus Peroxidase activity↑	–	*S. agalactiae*	↑	Van Doan et al. (2019)
Berberine powder		Mucus LZM↑, Serum LZM↑, ACH50↑, RBA↑, PA↑	–	–	Serum and mucus Peroxidase activity↑	–	*S. agalactiae*	↑	Van Doan et al. (2020a)
Organic acids									
Alginic acid	Nile tilapia (*O. niloticus*)	–	–	–	–	–	–	–	Merrifield et al. (2011)
calcium lactate		–	–	–	–		–	–	

(continued)

Table 1.5 (continued)

Feed additives	Tilapia species	Immune responses	Immune-related genes	Stress-regulating genes	Anti-oxidative capacity	Haematological and biochemical parameters	Disease challenge	Disease resistance and survival rate	Reference
						Ht↑, Hb↑, Plasma total Protein↑, ALT↓, AST↓			Hassaan et al. (2014)
L-malic acid		LZM↑	–	–	SOD↑, MDA↑	–	–	–	Chen et al. (2016)
OAB[m]	Red hybrid tilapia red tilapia (*O. niloticus* × *O. mossambicus*)	–	–	–	–	–	*S. agalactiae*	↑	Ng et al. (2009)

Arrows indicate an increase (↑), decrease (↓), or no change (→) in the response

Probiotic genera abbreviations: *B.*: *Bacillus*, *E.*: *Entercoccus*, *L.*: *Lactobacillus*, *P.*: *Pediococcus*, *R.*: *Rummeliibacillus*

Pathogens genera abbreviations: *A.*: *Aeromonas*, *E.*: *Edwardsiella*, *P.*: *Pseudomonas*, *S.*: *Streptococcus*

Prebiotic abbreviations: *COS:* Chito-oligosaccharides, *FOS:* Fructooligosaccharide, *GOS:* Galactooligosaccharide, *MOS:* Mannan oligosaccharide, *XOS:* Xylooligosaccharides, *LMWSA:* Low molecular weight sodium alginate

*Parameters investigated abbreviations: *ACH50:* alternative complement haemolytic 50 activity, *BA:* Bactericidal activity, *C3 & C4:* Complement component 3 & 4, *Ig:* Immunoglobulins, *LZM:* Lysozyme activity, *PA:* Phagocytic activity, *RBA:* Respiratory burst activity, *NBT:* Nitroblue tetrazolium, *NO:* Nitric oxide activity, *RBCs:* Red blood cells, *WBCs:* Leucocytes, *Ht:* Haematocrit, *Hb:* Haemoglobin, *ALP:* Alkaline phosphatase, *ALT:* Alanine aminotransferase activity, *AST:* Aspartate aminotransferase activity, *SOD:* Superoxide dismutase, *CAT:* Catalase, *GPx:* Glutathione peroxidase, *GR:* Glutathione reductase, *GSH:* Glutathione, *GST:* Glutathione S-transfrase, *TSA:* Total antioxidant capacity, *MPO:* Myeloperoxidase, *MDA:* Malondialdehyde activity, *IEL:* Intraepithelial leucocyte levels in the intestine, *GC:* Gablet cells, *IL:* Interleukin, *TNFα:* Tumour necrosis factor-α, *TLR:* Toll-like receptors, *IFN-γ:* Interferon gama, *TGF-β:* Transforming growth factor beta, *IRF-3:* Interferon regulatory factor, *Mx:* Transcription of *mx*, *RSAD-2:* Radical S-Adenosyl Methionine Domain Containing 2 gene (*VIPERIN* gen), *PCNA:* Proliferating cell nuclear antigen, *HSP70:* Heat shock 70 kDa proteins, *Lyzc:* C-type lysozyme

[a] The commercial probiotics contained *Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum,* and *Saccharomyces cerevisiae*.

[b] The mixed pathogens include *A. hydrophila*, *P. fluorescens,* and *S. iniae*

[c] A commercial probiotic product contains *Bacillus* spp., *Pediococcus* spp., *Enterococcus* spp., and *Lactobacillus* spp.

[d] A commercial carotenoid product from the extract of probiotic Rhodobacter sphaeroides mutant strain WLAPD911 (Lycogen™) contains neurosporene, β-carotene, spheroidenone and methoxyneurosporene rather than lycopene.

[e] Tilapia lake virus (*Tilapia tilapinevirus*).

[f] A commercial product containing the isoquinoline alkaloid sanguinarine.

[g] GroBiotic®-A is a mixture of partially autolyzed brewer's yeast Saccharomyces cerevisiae, dairy components, and fermentation products such as β-glucan and oligosaccharides.

[h] Jerusalem artichoke (*Helianthus tuberosus*).

[i] Mushroom (*Cordyceps militaris*) substrate

[j] Kefir is a complex community of yeasts (*Kluyveromyces, Saccharomyces,* and *Torula*), lactobacilli (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus* spp.), and acetic acid bacteria (*Acetobacter*).

[k] *Aspergillus oryzae.*

[l] *Echinacea purpurea* and *Allium sativum.*

[m] OAB, organic acids blend (Sunzen Corporation, Malaysia); PDF, potassium diformate (FORMIs, ADDCON, Norway).

Bacillus spp., lactic acid bacteria (LAB), certain Gram-negative spp., and yeast. They are incorporated into fish diets and lead to high levels of colonization and moderate gut microbial ecology populations (Merrifield et al. 2010).

A prebiotic is defined as a feed additive derived from vegetables and fruit that enhances the fish's performance and/or modifies the community of gastrointestinal beneficial bacteria, which consequent improvement of the host's well-being and health (Gibson and Roberfroid 1995). The criteria used to identify and recognize compounds as prebiotics are: (i) compounds that are neither hydrolysed nor assimilated by the gut; (ii) these compounds should be fermented by the gastrointestinal microbe community, (iii) they should be beneficial for bacteria to the colon through the enhancement of their growth and/or metabolism activation; finally, (iv) they should be able to transfer the colonic flora into healthier compounds to the host (Gibson and Roberfroid 1995; Lauzon et al. 2014).

Ringø et al. (2014) reported that the most common prebiotics used in aquafeeds are fructooligosaccharides (FOS), mannanoligosaccharides (MOS), galactooligosaccharides (GOS), and many commercial products that could be fermented by the gut flora and recognized as non-digestible compounds. Bioactive compounds and oils derived from medicinal plants have a positive impact on the host, including enhancement of performance, immune system response, modification of the gut flora, reduction of free radicals from the metabolic process, and enhancement of the host health and welfare against pathogen microbes (Alemayehu et al. 2018; Jeney et al. 2015).

Organic acids are also employed as a functional feed additive to enhance gut health and performance in fish (Lim et al. 2015). Organic acids have three different mechanisms of action in the gastrointestinal tract of fish: (i) the pH-reducing action of organic acids in the gastrointestinal tract leads to enhanced activity of the digestive enzymes, (ii) the reduction of pH inhibits the growth of pathogens bacteria in the gastrointestinal tract, and (iii) the organic acids integrated into aquafeeds decrease the potential risk of microbial contamination including pathogenic bacteria and moulds or fungi during feed storage (Lim et al. 2015).

1.2.2 Environmental Requirements

The tilapia genus is recognized as the most widely cultured group of species since they are raised in different regions of the world and are highly adaptable to a wide range of environmental conditions (Table 1.6).

Tilapia is tolerant to a wide range of rearing conditions such as salinity, ammonia, pH, dissolved oxygen (DO), and temperature. Among tilapia species, the least adaptable species is Nile tilapia (*O. niloticus*), while the most tolerant species to saltwater are Mozambique tilapia (*T. mossambicus*), blue tilapia (*O. aureus*), and red belly tilapia (*T. zilli*) (El-Sayed 2006b, c).

El-Sayed (2006c) reported that the best salinity level to attain optimum growth in different tilapia species is up to 19 ‰. The optimal growing temperatures range

Table 1.6 Limits and optima of water quality parameters for tilapia

Parameter	Range	Optimum for growth	Reference
Salinity (‰)	Up to 36	<19	El-Sayed (2006c)
DO (mg L^{-1})	Down to 0.1	>3	Ross (2000)
Temperature (°C)	8–42	22–29	Mjoun et al. (2010)
pH	3.7–11	7–9	Ross (2000)
Ammonia (mg L^{-1})	Up to 7.1	<0.05	El-Sherif and El-Feky (2008)

between 22 and 29 °C, while the best temperature for spawning is higher than 22 °C. (El-Sayed 2006c) stated that tilapia can survive below 10° C and Nile tilapia (*O. niloticus*) is the least tolerant to cold water while *O. aureus* is the most tolerant to cold water. (Ross 2000) found that tilapia can tolerate a wide range of dissolved oxygen (DO); however, the optimum level of DO to achieve better growth performance is higher than 3 mg $L^{-1,}$ and the best DO for optimum feed utilization efficiency is 7 mg L^{-1} (Bergheim 2007). (Shelton and Popma 2006) found that Tilapia requires a pH of ~7 or slightly higher than 7 to achieve the best growth. In general, tilapia tolerate a wide range of pH between 3.7 and 11, but the optimum growth rate is achieved between pH 7.5 to 9.5 (Ross 2000).

In terms of ammonia, a concentration higher than 2 mg L^{-1} is considered toxic (Shelton and Popma 2006). While ammonia at a concentration of 1.2 mg L^{-1} causes low appetite and reduces growth (Celik 2012; El-Sherif and El-Feky 2008). (El-Sherif and El-Feky 2008) reported that the optimum ammonia concentration for Nile tilapia (*O. niloticus*) is estimated to be below 0.05 mg L^{-1}.

1.3 Comparative Assessment of Tilapia Culture Systems

The selection of the culture technique is principally affected by the purpose of the aquaculturists or determined by the geographical conditions which involve site selection, substructure, environmental and physical conditions (especially climate), socioeconomic aspects, technical knowledge, and marketing potential. Different types of tilapia production are well known all over the world as monoculture or polyculture in different rearing units such as cages, ponds, and tanks. Also new production systems are practised in different regions of the world as aquaponics, hydroponics, and biofloc. Tilapia production is divided into three categories: (i) extensive: where the stocking density is low and organic and inorganic fertilizers used to promote the natural food, (ii) Semi-intensive: where the source of food combined between fertilization to promote natural food includes phytoplankton and zooplankton plus supplemented diets; (iii) intensive: based on high stocking density, high water exchange rate, and balanced complete feeds are provided. The approximate annual yields of polyculture systems including tilapia with carps reach or exceed 5 tonnes/ha (Prabu et al. 2019).

The major challenges of tilapia production are deprived growth, pathogen infections, and disease outbreaks. However, there have been several solutions that could help to expand tilapia production outside their tropical and sub-tropical regions. Some of these include compiled intensive-extensive systems, closed-cycle that aids in controlling the environmental variables, and intensive system indoor using RAS system with advanced water treatment methods such as biofloc. The common production approach of Nile tilapia (*O. niloticus*) in Asia and Latin America in lakes and reservoirs in different countries is represented by the intensive system in floating cages. The success of intensive technique using cage/tank systems relies on several factors such as (i) cage/tank size and shape, (ii) stocking density and iii) water exchange/water flow rate. Cage culture offers several advantages as follows: (i) minimizes fertilization, (ii) allows the recovery of eggs by spawning fish, (iii) allows the fish to grow in a real environment. The pros of using cage production techniques could be summarized in the following points as stated by (Bhujel 2014a):

- Use of water bodies that cannot be drained or seined and would otherwise not be suitable for aquaculture.
- Flexible and convenient for management.
- No accumulation of ammonia, nitrite, nitrate, and other waste products that are quickly flushed out with water flow from the cage.
- High feeding rates are feasible, and a higher fish growth rate could be achieved.
- Predators (e.g., birds, otters, etc.) can be easily controlled.
- Ease and low cost of harvesting.
- Easy monitoring of fish feeding and health status.
- Low capital investment compared to other production techniques.

Nevertheless, some cons of cage culture involve: (i) poaching risk, (ii) failure of ability to prevent poor water quality conditions because of pollution by industries or factories, iii) difficulties in treating disease and parasites, (iv) need to frequently scrub the cages, (v) risk of fish escape from the cage, (vi) inability to provide natural foods and dependence on manufactured fish diets (complete in nutritional composition), and finally (vii) there may be criticism raised by environmentalists (Bhujel 2014a).

Thereafter, tanks and raceways can be another option to replace pond or cage culture if the conditions are not suitable for the cage technique. (Liao and Chen 2001) stated that in Asia, the intensive tilapia production system in tanks is commonly practised in Taiwan, Malaysia, and Philippines. Taiwan is considered a pioneer for tilapia-intensive culture in concrete tanks (small to medium-level operation), with a production of over 55,000 tons/year. In comparison to the pond production system, tanks and raceways are easy to monitor and handle the stocks and possess a high degree of control of the environmental conditions, specifically water quality parameters. On the contrary, the disadvantages of using tank and raceway culture are given by the high cost of construction, operation, and production and the requirement of proficient attention due to the higher risk of major fish mortality caused by disease outbreaks.

1.3.1 Biofloc Technology

Wastewater with high ammonia, nitrite, and nitrate concentrations damages the fish culture system and pollutes other natural water bodies, so there is a need to look for alternative culture techniques to decrease the environmental damages caused by aquaculture (Gutierrez-Wing and Malone 2006). “Biofloc” Technology (BFT) is considered a promising alternative technique to avoid the harmful environmental impact caused by aquaculture. Biofloc technology through aeration and the addition of extra carbon to the aquaculture system increases the nitrifying bacterial colonies that maintain water quality and, together with phytoplankton and zooplankton are considered additional foods in the aquaculture farming system (Gutierrez-Wing and Malone 2006). The basic concept and function of biofloc are to stimulate the growth of heterotrophic microbial bacteria that convert a toxic source of nitrogen “NH_3” to a safe source of nitrogen, in addition to converting nitrogenous waste to a microbial protein that could be used as another source of protein to the fish (De Schryver et al. 2008). To date, BFT technology has achieved worthwhile attention due to its higher production efficiency, protein recycling from food leftover through nitrifying bacteria, water quality improvement, and a novel approach to infectious disease prevention (Ekasari et al. 2015a). In addition, Ekasari et al. (2015a, b) reported that BFT technique employment could boost the reproductive performance of Nile tilapia (*O. niloticus*) broodstock by enhancing fecundity and larval survival rate and also improving the immune system against infectious diseases. Therefore, all the above advantages of biofloc technology attract the attention of scientists to conduct their research in BFT systems to guarantee and secure the factors within the recommended levels to achieve aquaculture sustainability and food security.

1.4 Genetic Improvement

Research for genetic improvement, particularly quantitative genetic approaches, can have a tremendous impact on aquaculture and can be responsible for increased production efficiency and improved productivity in aquatic animals. Over the past decades, the importance of tilapia in global aquaculture has increased, as well as the intensity and diversity of research for genetic improvement (Gupta and Acosta 2004). In addition, aquatic animals give the opportunity to scientists to implement different approaches for improving fish genetics, including hybridization, selective breeding, sex control, and crossbreeding. Recently research in fish genetics proved that using and implementing new approaches help discover new strains with high growth rate, feed efficiency, survival rate, tolerance to a wide range of environmental conditions, and disease resistance. Moreover, the adoption and implementation of new genetic approaches could help discover new strains of fish and shrimp that could grow in either freshwater or/and brackish water systems (Nguyen et al. 2010; Ninh et al. 2014). Nowadays, most tilapia genetic research has focused on hybridization and monosex male fry production. The most relevant findings of this research indicate that tilapia males grow faster than females, and tilapia shows early maturation, which leads to consecutive spawning during the growing period and thus inhibits and limits growth. Thereafter, female tilapia shows difficulty growing uniformly, so male fry is preferred (Chen et al. 2018; Martínez et al. 2014). This shows the importance of searching for novel techniques and modification and improvement of the existing ones such as manual sexing, interspecific hybridization, androgenesis, triploid, transgenesis, hormonal sex reversal, and YY male technology to produce monosex tilapia for successful and intensive tilapia production (Beardmore et al. 2001; Ponzoni et al. 2011). The current approaches to producing 'all male' tilapia have limitations that make them expensive, unmaintainable, or not acceptable. For example, manual sexing is labour-consuming, and attention to maintaining broodstock for interspecific hybridization is needed. Although the most widespread approach to producing monosex offspring is tilapia masculinization with hormone therapies or adrenal malfunction, hormone therapies have posed concerns because they may influence consumer acceptance and marketability of the fish, and hormone residues may have irreversible effects on biodiversity and water quality. In the case of the YY approach, the production of YY males requires at least three generations of breeding. Moreover, the employment of YY technology depends on the contribution of an outstanding laboratory with advanced facilities for the creation of YY males (Baroiller et al. 1995; Abucay et al. 1999; Baroiller and D'Cotta 2001; Tessema et al. 2006). Therefore, there is a need to look for alternative techniques to create 'all male' tilapia. Some research indicates an effect of temperature on genotype interaction, such as increasing the male: female ratio in response to thermal treatment. For example, thermal treatment of about 38 °C for 10 days after post-hatching succeeded in producing above 80% of males (Ponzoni et al. 2011). It should be mentioned that of all the genetic techniques, just selective breeding presents the chance of permanent genetic achievements because the achievements can be transferred from generation to generation. Finally, a combination of classic selective breeding using marker-assisted selection (MAS) and polygenic selection could considerably promote the male-to-female ratio as a response to thermosensitivity.

1.5 Environmental Impacts

Currently, tilapias have been introduced as exotic species in most countries worldwide, with successful growth and reproduction in new habitats. The traditional tilapia culture in semi-intensive, small-scale systems with minimum negative effects on the environment is now being replaced with intensive, large-scale farming systems. Since the use of manufactured inputs such as artificial feed, chemotherapeutic agents, and hormones will become inevitable in intensive culture systems. The worldwide expansion of tilapia rearing at an extremely high rate is very presumably to cause environmental and socioeconomic risks. In the last few decades, Nile tilapia (*O. niloticus*) production expanded to increase the seafood supply and fulfil the global demand for animal protein and food security. Nevertheless, although tilapia produces substantial economic growth, its fast expansion has caused numerous environmental threats like the destruction of wild habitats, the interaction between alien and endemic species, disturbance of wildlife, use of artificial culture inputs (e.g. chemotherapeutic agents, antibiotics, hormones, and fuels), and eutrophication because of the aquaculture wastewater (El-Sayed 2006a). Thus, the use of advanced and efficient management approaches is necessary. In this regard, some innovative methods have been recommended to boost responsible aquaculture activities including the amalgamation of aquaculture practices with livestock farming and agriculture (e.g. aquaponic, hydroponic, etc.), and also using the BFT and RAS systems that could help to control or manage infectious diseases outbreak and discharges of aquaculture farms (Wang and Lu 2016; Forio and Goethals 2020). These approaches can be the basis for effective long-term solutions for eco-friendly and green aquaculture in the future. Therefore, novel approaches are required if sustainable and green aquaculture is to be meaningfully understood and implemented (Montoya-Camacho et al. 2019). Since eutrophication, a process that is caused by the excessive input of nutrients (e.g., phosphorous and nitrogen), is largely recognized as a serious threat to the environment (Nakano et al. 2016), in the past few decades, researchers have been investigating techniques to reduce aquaculture waste outputs, mainly phosphorus and nitrogen, from aquaculture operations to obtain satisfaction of environmentalists (Azim and Little 2008; Pinho et al. 2017; Boyd 2019). The well-known techniques to achieve eco-friendly aquaculture are the BFT technique and the integrated multi-trophic approach. The biofloc approach has been accomplishing acceptance as an efficient alternative water quality management system (Emerenciano et al. 2013; Dauda 2020). This technique presents the elimination of nutrients from water with the production of microbial communities, which can be consumed by the culture species in situ as natural foods (De Schryver et al. 2008). The other approach is the integrated multi-trophic aquaculture which is defined as a unique self-cleaning approach for aquaculture ponds since the waste from one species (including uneaten feed, faeces, and metabolic excretion) is the source of feed to support the growth of other species (Sampantamit et al. 2020).

Another factor that could affect the tilapia aquaculture community environment is the introduction of alien species destroying the ecosystem compositions and posing risks the global biodiversity (Brown et al. 2018; Anton et al. 2019). Although transgenic tilapia provide several advantages for tilapia farming, the rate of genetic alteration in transgenic tilapia is such that their phenotypic and behavioural attributes cannot be easily predicted (Mair 2002). Furthermore, transgenic tilapias are a new tool that introduces new strains into the community of wild tilapia and may have negative effects on the environment and other native species. The negative impact of transgenic is the replacement of native populations with novel strains that could become a part of the gene pool and also change the hierarchy of the natural populations (Dunham 1999).

Unfortunately, despite the negative effects that the extension of tilapia rearing may have on the environment, most introductions have not been preceded by any environmental impact evaluation. Instead, in most cases, the evaluation was performed after the introductions took place. In such a case, modulating and managing the impacts of introduced tilapia in their new environments will be very challenging, or even unfeasible. Therefore, cautious and thorough assessment, as also proper management plans and programmes must be adopted to be carried out before any introductions or transfer of tilapia.

1.6 Some Constraints and Suggestions for Solutions to Tilapia Farming

Although tilapia farming holds great promise, there are some constraints to its development. Some constraints and their possible solutions are reported as follows:

1. Training resource allocation related to tilapia farming. In general, aquaculture needs education about new technologies and farm management, because education can play a substantial role in enhancing the skills and experiences of farmers and also resolving the restrictions and challenges facing aquaculture (Olaoye et al. 2013). Previous research expressed the importance of the aquaculturists' education level to select reasonable technologies and manage the facilities efficiently (Ogunmefun and Achike (2017); Uddin et al. (2021). Therefore, resource allocation for training courses and workshops for tilapia farmers has to be considered.
2. Insufficient supply of tilapia fry. The lack of larvae production to respond to the growing world demand is one of the major bottleneck restrictions to the development of the tilapia-intensive culture (El-Sayed 2002). One of the most important obstacles to high-quality tilapia fry production is the poor reproductive performance of bloodstock due to asynchronous spawning cycles and low fecundity rate.

   Bhujel (2000) stated that the monitoring and management of the environmental and nutritional status of brood stocks can improve their efficiency. Moreover, the selective breeding of superior brood stocks and strains with preferable size and age for breeding objectives could remarkably improve larvae production. Among the environmental factors, high salinity and low temperature might be helpful for the control and synchronization of broodstock reproduction when fry demand is low (Bhujel 2000).
3. Environmental tolerance. Environmental tolerance is the major factor in controlling the success of tilapia production. Although some tilapia like Mozambique tilapia can grow in seawater, most tilapia species are categorized as freshwater fish and not tolerant to high salinity (El-Sayed 2006b; Shelton and Popma 2006). One of the promising techniques to improve salinity tolerance is the crossbreeding between Nile tilapia (*O. niloticus*) and Mozambique tilapia

(*O. mossambicus*). Furthermore, the limited ability of tilapias to tolerate low temperatures (<15 °C) (El-Sayed 2006b; Shelton and Popma 2006) restricts the expansion of tilapia culture in a different geographic zone. Using warm water such as cooling water of some industries, thermal effluents, and/or warm springs and also maintaining tilapia in a greenhouse or indoor ponds because of their non-resistance to cold water can help them to overwinter in subtropical scopes of tilapia culture.

4. Early maturation. Tilapia's first maturity occurs at an early age (2–3 months old) and a short length (10–12 cm length). The most preferable and cost-effective technique is to create 'all male' tilapia because males grow faster than females and also have a more standard size (see Sect. 1.4).
5. Genetic deterioration. In some conditions, there is evidence of genetic deterioration. Genetic deterioration of introduced stocks is widely attributed to poor broodstock management resulting in inbreeding and introgression of less favourable genes. With the rapid advance of next-generation sequencing techniques (Metzker 2010), marker-assisted selection and genomic selection will significantly accelerate the genetic improvement of tilapias (Sonesson 2011; Yue 2014). In general, the desirable characteristics of improved tilapia have been focused on higher production efficiency, better appearance, tolerance to certain environmental conditions, and, especially, control of unwanted breeding.

   Remarkable interests in improved growth rate and performance of tilapia under farm conditions have been shown from breeding programmes for selection and sex control. The achievements of the implementation of such breeding strategies have been introduced to aquaculture through technically and economically sustainable programmes (Mair 2002). For example, farming the Genetically Improved Farmed Tilapia (GIFT) strain rather than the not-improved local strain of the Nile tilapia (*O. niloticus*) can enhance the growth rate and production efficiency of tilapia. Since feed accounts for over 50% of the cost of production, the higher feed conversion ratio of the GIFT strain would decrease production costs. The GIFT strain has a remarkably higher growth performance, better feed conversion ratio, and higher production efficiency than the local strain (Ridha and Cruz 2002).

   The other important aspect is the genetically improved strains dissemination to achieve the targeted beneficiaries effectively, including monitoring of the impact and adoption of improved breeds. On the other hand, genetic improvement programmes will require the development of production stocks that are acceptable to each environment. Therefore, success in genetic improvement programmes will require long-term support and collaboration between partners from the government, university, and industry.
6. Disease resistance. Since tilapia, cultural practices have been intensified, and the densities of tilapia have increased in different systems and culture has expanded into the colder climatic zones, where suitable environmental factors are more difficult to maintain, infectious diseases have emerged (Watanabe et al. 2002). Developing approaches for fish health stability through genetic improvement,

water quality management, stress reduction, and the use of preventive immunostimulants are required to control infectious diseases.

7. Lack of access to freshwater. Tilapias are freshwater fish; however, access to freshwater resources is one of the most critical environmental issues in developed and industrialized countries (Hankins 2002). Therefore, it is suggested to use water supplies that are not suitable for human consumption or agriculture such as brackish water or seawater. Moreover, the use of recirculating systems is another fit approach for water quality and quantity management.
8. Negative impacts on the environment and global biodiversity. It is clear from the previous section (see Sect. 1.5) that mismanaged transfers and/or introductions of tilapia can lead to destructive environmental impacts. If tilapias are established in their new environment, it would be approximately infeasible to control and reduce their catastrophic impacts. It is hence necessary that strict regulations be established to control the introduction of tilapias in a new habitat and also precise monitoring programmes and certification of tilapia farms are mandatory to protect the environment and aquatic biodiversity (Bush et al. 2013).

   Yue et al. (2016) expressed that the recirculating aquaculture systems and cage culture can mitigate the adverse effects of tilapia culture on the environment and aquatic biodiversity. Therefore, they would probably be developed in the production and technological advances of tilapia culture. These systems facilitate aquaculture and hence will be the key parts of next-generation aquaculture.
9. Flesh quality problems of tilapias. Tilapia, flesh quality issues, are as follows: (i) odour and flavour, which are attributed to culture conditions, (ii) high percentage of bone if the harvest has occurred in small-size fish, and (iii) farmed tilapia species contain low levels of omega-3 fatty acids compared with other fish, especially salmon (Weaver et al. 2008). Likely, selective breeding (Gjedrem and Baranski 2010) and supplementing tilapia feeds with marine microalgae containing a high level of omega-3 (Tadesse et al. 2003) can increase the essential fatty acid content of tilapias. Moreover, finishing diets and also the GIFT breeds (reviewed by Eknath and Hulata 2009) and transgenic tilapia can moderate this problem. Although, before the commercialization of transgenic fish, food safety issues should be addressed.
10. Failed marketing of products. The lack of attention given to marketing and business has also been recognized as one of the restrictions to the achievement of commercial tilapia production. The assessment of the tilapia market is rarely undertaken by aquaculturists due to time and expense and difficulties in attracting the cooperation of wholesalers and retailers, which should be considered (Watanabe et al. 1997).

1.7 Conclusions and Recommendations

This chapter may be considered as a short preface to tilapia rearing needs. Tilapia culture has gained significance increasingly in the world. Tilapia has a lot of positive characteristics that make it proper for culture. Amongst these are its general resilience, high tolerance to unfavourable environmental conditions and adaptability to high stocking densities, its potential ability to tolerate low levels of dissolved oxygen and a wide range of salinity concentrations, and its infectious diseases resistance. Tilapia can utilize and grow in a wide variety of natural and artificial feeds, has a high survival rate, acceptable feed conversion ratio, fast growth rate, and high yield potential, and is accepted by a wide range of farmers and consumers. Moreover, tilapia can grow well in different aquaculture systems, ranging from extensive systems with simple substructures to more intensive systems with complex infrastructure. With the increasing demand for tilapia products, tilapia farming will continue to be a source of different business benefits, since it is a cheap and easy source of affordable and inexpensive animal protein and provides several job opportunities to the community in developing countries. Finally, suitably designed fish farms, precisely selective breeding of tilapia strains, selection of a proper tilapia production system by the aquaculturists, government support on supply seed, feed, and instruments, training, extension services, and advice to the aquaculturists regarding tilapia culture, and the development of an organized marketing agenda would increase the commercial profitability and sustainability of tilapia production in many countries around the world.

Therefore, it can be unavoidable to conduct research studies on resolving the issues met in tilapia culture because tilapia culture will guarantee the socioeconomic advantages and food security of developing countries. For example, although tilapia feed on a wide range of natural and artificial feeds, specific dietary requirements are yet lacking, and the interactions among nutrients and with cultured conditions and tilapia health and welfare are not completely known. Nowadays, research and interest in dietary feed additives such as immunostimulants and growth stimulants, especially phytobioactive compounds to improve fish health and growth performance are likely to continue, which will fill existing research gaps. Moreover, more research work and resource and management development are required to improve breeds that are more cold-tolerant, salt-tolerant, and disease resistant.

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Chapter 2
Health Management for Sustainable Tilapia Farming

Farid Firouzbakhsh, Seyed Hossein Hoseinifar, and Mehwish Faheem

Abstract Aquatic animals, especially fish, tend to react more quickly to changes in the environment than terrestrial farm species. In an aquatic environment, there is a profound and inverse relationship between environmental quality and the disease status of fish. Health maintenance is a concept in which animals are reared under conditions that optimize the growth rate, feed conversion efficiency, reproduction, and survival while minimizing problems related to infectious, nutritional, and environmental diseases, all within an economic situation. Health management is, therefore, controlling the environmental fluctuations to reduce the magnitude of change and produce a more economical, healthier, and better-quality product.

Keywords Viral Diseases · Farming · Health · Parasites · Treatments

2.1 Principles of Health Maintenance

Health maintenance involves a series of principles that apply to most farm-raised animals. However, fish tend to react more quickly to environmental change than terrestrial farm animals. Because of their homeothermic nature, most terrestrial farm animals respond comparatively slowly to poor environmental conditions. In contrast, fish—being poikilothermic—respond quickly and often fatally to handling, temperature change, excessive or inadequate dissolved gases in the water, metabolites, or chemical additives, and so forth, to which they are unable to adapt. These elements

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also increase fish susceptibility to infectious agents and compromise their immune response (Plumb and Hanson 2011).

The objective of health maintenance is to help control environmental fluctuations through management practices, thus reducing the magnitude of change and producing a more economical, healthier, and better-quality product. The final goals of health management are disease prevention, reduction of infectious disease incidence, and reduction of disease severity when it occurs. Successful health maintenance and disease prevention or control do not depend on any single procedure but are the culmination of the application of integrated concepts and exercising management options (Plumb and Hanson 2011).

Health maintenance is a concept in which animals are reared under conditions that optimize the growth rate, feed conversion efficiency, reproduction, and survival while minimizing problems related to infectious, nutritional, and environmental diseases, all within an economic situation. As culture becomes more intensive, need for intervention increases accordingly, and principles of health maintenance become of greater importance.

In an aquatic environment, there is a profound and inverse relationship between environmental quality and the disease status of fish. As environmental conditions deteriorate, the severity of infectious diseases increases; therefore, sound health maintenance practices can play a major role in maintaining a suitable environment where healthy fish can be grown.

Health maintenance does not simply target infectious diseases but emphasizes proper utilization of physical facilities; use of genetically improved fish and certified “specific pathogen-free” (SPF) stocks whenever available and/or feasible; environmental control; prophylactic therapy; feed quality and quantity, pond, cage, raceway, tank, or recirculating system management; control of vegetation; aeration and use of other water quality maintenance practices; and a management commitment to provide an optimum habitat in terms of water quality for fish being cultured.

Stress generally is the reaction of an animal to a physical, physiological, or chemical insult. Experience has shown that a wide variety of viral, bacterial, parasitic, and other fish diseases will cause mortality if cultured fish are held in unfavourable environmental conditions (Wedemeyer 1996).

Multiple stressful parameters have a compounding synergistic effect on the fish, and even though the fish may be able to handle the stressors individually, the combined effect can be lethal (Boyd 1990).

A theory of host/pathogen/environment relationship was applied to fish with regard to the development of infectious diseases by Snieszko (1973). This theory is based on the premise that to have an infectious disease, a host and pathogen are required but an unfavourable environmental condition often acts as a trigger for the disease to improve. The interaction of these factors is expressed in the equation: $H (A + S^2) = D$,

where:

H = Species or strain of host (natural resistance).
A = Etiological agent.

S = Environmental stressors.
D = Disease.

Environmental stressors are squared because as fish approach adaptation limits, stressors increase accumulatively rather than additively. Also, when more than one stressor is involved (oxygen, ammonia, CO_2, temperature, etc.), detrimental factors act synergistically (Plumb and Hanson 2011). Intensive fish culture causes unique but manageable environmental problems for the aquaculturist (Boyd 1990; Wedemeyer 1996). All fish require adequate water maintained at a suitable temperature and oxygen concentration level for proper growth and reproduction.

2.2 Diseases of Tilapia

Tilapias are the most extensively cultured fish in the world, especially in China, Indonesia, and Egypt (FAO 2017) and they are recognized as one of the most important species in tropical freshwater aquaculture. Tilapia are hardy fish with good resistance to pathogens as long as they are kept under good water quality conditions, in the proper temperature range, and using proper husbandry practices. As culture systems intensify (cages, raceways, or recirculating systems), stress due to improper handling, exposure to poor water quality, high fish density requiring high feeding rates, or low water temperatures can exacerbate the impact of some pathogens on tilapia (Plumb and Hanson 2011).

2.2.1 Bacterial Diseases

The outbreak of bacterial diseases is the main limiting factor that threatens tilapia production, specifically when cultured under intensive conditions (Abdel-Latif et al. 2020). Fish diseases often occur following an initial stressor. By some accounts, bacterial diseases may be the number one threat to the future of the tilapia aquaculture industry. The most serious bacterial diseases in cultured tilapia are arranged in Table 2.1.

2.2.1.1 Streptococcosis

The first reports of *Streptococcus* spp. infections in fish date back to the mid-1950s (Hoshina et al. 1958), but the first case involving tilapia was a decade later (Wu 1970). Streptococcosis may be sub-acute but is often chronic in tilapia. Although multiple species of *Streptococcus* have at various times been reported from fish, *S. iniae* and *S. agalactiae* are the two that most frequently cause serious disease in tilapia. *Streptococcus* spp. infections have been reported in tilapia from

Table 2.1 The most bacterial diseases of tilapia

Disease	Agent	Clinical sings
Streptococcosis	*Streptococcus iniae, Enterococcus* sp.	Exophthalmia, haemorrhages in eyes, pale liver, spiral swimming
Francisellosis	*Francisella* spp.	Ulcer, ascites, exophthalmia, loss of appetite
Motile Aeromonas septicaemia (MAS)	*Aeromonas hydrophila* and related species	Swim lethargically, gasping
Edwardsiellosis	*Edwardsiella tarda*	Ascites, pale liver, swollen spleen and kidney, swollen abdomen
Columnaris	*Flavobacterium columnare*	Frayed fin, grey patches on the skin, pale gills, and necrotic
Phothbacterium infection	*Photobacterium damselae*	Dark skin, swollen kidney and spleen, ascites
Vibriosis	*Vibrio* spp.	Similar to MAS
Pseudomonas infections	*Pseudomonas* spp.	Similar to MAS

North, South, and Central America, Australia, Asia, South Africa, and other African countries, Great Britain, Norway, and Middle Eastern countries. Tilapia-associated streptococci are essentially worldwide in distribution (Klesius et al. 2008).

Clinical signs of streptococcosis in tilapia are not always specific, but in most species of fish, eye disease and meningoencephalitis are common. Affected fish generally are lethargic, and exhibit spiral swimming, abdominal distention, and exophthalmia with haemorrhage and diffuse haemorrhage in the skin and base of fins. Internal findings in affected fish include exudate in the abdominal cavity, pale livers, and a greatly enlarged, black spleen.

Streptococcus spp. infections occur in both fresh- and saltwater-grown tilapia. *S. iniae* is more common in freshwater systems, and *S. agalactiae is* more common in brackish water. Tilapia in water with 15–30 ppt salinity at 25 and 30 °C are more susceptible to *Streptococcus* isolated from fish in saltwater than when in freshwater at the same temperature (Chang and Plumb 1996). Mortality of tilapia infected with *Streptococcus* varies from low to high, depending on other circumstances. Under culture conditions, mortality is as high as 75% in naturally infected tilapia. In experimental infections with *S. agalactiae* in tilapia, mortality can reach as high as 90%, but mortality in natural infections is generally lower (Evans et al. 2002). Tilapia susceptibility to *Streptococcus* is usually associated with environmental stress, skin injury, scale loss, and other factors associated with intensive aquaculture (Chang and Plumb 1996).

2.2.1.2 Francisellosis

The bacterium belonged to the genus *Francisella* (Hsieh et al. 2006) and is an intracellular bacterium from tilapia and a limited number of other warm-water fish species were initially referred to as a piscirickettsia like organism (PLO) or

Francisella-like organism (FLO) and was believed to be non-culturable on cell-free media. The organism from hybrid striped bass and tilapia was identified by isolation of bacterial DNA from the tissue of diseased fish and amplification of the 16S rRNA gene and comparing the sequences to a comparative database (Mauel et al. 2007). The bacterium was later cultured on agar media and subjected to additional molecular studies where it was determined that warm-water strains of *Francisella* from tilapia were distinct from cold-water isolates from cod (Soto et al. 2009). Members of the genus *Francisella* affect a wide range of animals including humans (*F. tularenses*) and francisellosis has become a serious health problem for cultured tilapia in many geographical regions. The species of *Francisella* associated with disease in warm-water fish, *F. asiatica,* has been found in Taiwan, the United States, and Latin America (Mauel et al. 2007; Soto et al. 2009), where it affects both freshwater and saltwater fish species. The pathogen has been implicated in the disease of several species of tilapia (*O. mossambicus, O. niloticus,* and *Sarotherodon melanotheron*) (Mauel et al. 2003). *F. noatunensis* affects cold-water fish species such as the Atlantic cod. These reports indicate a wide geographical range and fish species susceptibility to *Francisella* spp.

Francisellosis may be either an acute, sub-acute or chronic disease, depending on culture conditions and water temperatures. Affected fish are dark, swim lethargically, and have a loss of appetite; they display skin haemorrhages and ulcers; exophthalmia, and ascites; the spleen and kidneys are enlarged and contain distinct white nodules of varying sizes (Mauel et al. 2007; Soto et al. 2009). The gills exhibit primary and secondary lamellar fusion because of epithelial hyperplasia. Also, multiple white granulomas occur in the gills, spleen, kidney, and testes but seldom in the liver; occasionally black granulomas are seen internally. Special stains such as Giemsa revealed small, pleomorphic coccobacilli inside and outside the cells. It should be pointed out that some fish may show no clinical signs (Mauel et al. 2003).

Francisellosis affects all ages and sizes of tilapia, from small fingerlings to adults. Mortality of tilapia infected with *Francisella* spp. ranged from 5% to 80%, with an average of 50% in Nile tilapia in Latin America (Mauel et al. 2007). The disease has the propensity to occur during cooler months of the year, and infections are stimulated by cold stress on farms in which it is endemic (Mauel et al. 2003). In temperature studies, tilapia maintained between 21.5 and 26.5 °C, initial mortalities occurred on day 15, and mortality doubled almost daily thereafter (Mauel et al. 2003). Tilapia maintained between 26.5 and 29.5 °C and showed no sign of disease or mortality.

2.2.1.3 Motile Aeromonas Septicaemia

In tilapia, motile Aeromonas septicaemia (MAS) is associated with several different species of bacteria, the most common of which is *Aeromonas hydrophila.* Although MAS is not uncommon in cultured tilapia, there are few published “case reports” of its occurrence, but when it occurs, it is often a secondary problem. Tilapia with MAS lose their equilibrium, swim lethargically, gasp at the surface, and generally display the same clinical signs as other fish species.

2.2.1.4 Pseudomonas

Pseudomonas fluorescens occasionally produces clinical signs and pathology similar to *A. hydrophila* and can cause significant mortality in tilapia. The infection occurred during winter and spring when water temperatures were 15–20 °C resulting in mortalities of 0.2–0.3% per day.

2.2.1.5 Vibriosis

Vibriosis in tilapia is often mild to chronic, and clinical signs do not differ significantly from those for MAS. Mortality of infected tilapia is usually chronic, with relatively low daily losses, but cumulative mortality can be significant. Epizootiology of vibriosis in tilapia is similar to that of MAS in the respect that both diseases are usually secondary infections (Sakata 1988). In saltwater, *V. anguillarum* or *V. vulnificus* are involved, while in freshwater, *V. mimicus*or *V. cholerae* is found, and *V. parahaemolyticus* can occur in either environment. Sakata (1988) reported that Nile tilapia suffered 10–20% mortality due to a vibriosis infection following transfer from freshwater to saltwater pens at 18–20 °C. Decreasing water temperatures, coupled with high salinity, are considered compounding stressors on tilapia populations.

2.2.1.6 Columnaris

When columnaris occur in tilapia, pale areas form on the body and frayed fins are the most frequently observed clinical sign of disease, and infected fish will swim lethargically or float at the surface. Nile tilapia are more susceptible to columnaris infections and have higher mortality when the pH is either very acidic or alkaline (Marzouk and Bakeer 1991). So physical injury and/or environmental stress can precipitate these infections.

2.2.1.7 Edwardsiellosis

Edwardsiella tarda affects tilapia cultured under high density and other stressful conditions in either freshwater or marine environments. The sign of Tilapia infected with *E. tarda is* swim lethargy, enlarged abdominal area, and swollen, opaque, and haemorrhaged eyes. Internally, focal areas of necrosis are seen; the liver is often pale and mottled, the spleen is dark red and swollen, the kidney is swollen and soft, and the intestine is inflamed and usually void of food.

2.2.1.8 Photobacterium

Photobacterium damselae subsp. *Damselae* was shown to cause disease in freshwater wild and cultured Nile tilapia in Egypt (Khalil and Aly 2008). Infected tilapia swam lethargically and had dark skin. Internally they had swollen kidney, spleen, and liver with bloody fluid in the abdominal cavity. Also, pinhead nodules were present on the liver, spleen, and kidney. The mortality of experimentally infected fish was 20–40%.

2.2.2 *Viral Diseases*

Compared with the research history of bacterial and parasitic diseases of aquatic animals, the study of fish viral diseases is relatively new. Infectious pancreatic necrosis virus (IPNV) of salmonids was the first proven viral fish disease. The infectious pancreatic necrosis virus was described by Wood et al. (1955). Several viruses have been isolated and identified in association with mortality cases of tilapia species (Machimbirike et al. 2019). The most important viral diseases in cultured tilapia are arranged in Table 2.2.

Tilapia lake virus (TiLV) is one of the most devastating viral infections negatively affecting tilapia culture worldwide. It has been associated with mass kills of

Table 2.2 Summary of tilapia viral diseases (Machimbirike et al. 2019)

Agent	Family/ genus	Host	Clinical signs	Distribution
Tilapia lake virus (TiLV)	Tilapinevirus (Orthomyxovirus-like)	*Oreochromis niloticus*, hybrid tilapia, red tilapia, red hybrid tilapia, wild black tilapia, *Oreochromis aureus*	Lethargy, skin erosions, darkening of the skin, loss of appetite, exophthalmia	In 13 countries in Asia, Africa, and South America
Viral nervous necrosis (VNN)	Nodaviridae/ Betanodavirus	*Oreochromis niloticus*	Erratic swimming, loss of balance	Indonesia, France, and Thailand
Infectious spleen and kidney necrosis virus (ISKNV)	Iridoviridae/ unknown	*Oreochromis niloticus*	Lethargy, ascites, pale gills	Canada
Infectious pancreatic necrosis virus (IPNV)	Birnaviridae/ aquabirnavirus	*Tilapia mossambica*, *Oreochromis niloticus*	Darkening of skin, ascites, pale gills, abnormal swimming	Taiwan, Kenya
Lymphocystis disease virus (LCDV)	Iridoviridae/ lymphocystivirus	*Tilapia amphimelas*, *T. esculenta*, *T. variabilis*, *Haplochromis* sp.	Wart-like growths on the tails of fish	North Tanzania

tilapia species in Ecuador, Colombia, Egypt, and Malaysia (Abdel-Latif et al. 2020). Tilapia lake virus can cause disease in various species by natural or experimental infection (Surachetpong et al. 2020). Similar to natural infection, tilapia lake virus disease could be reproduced in different species of tilapia and giant gourami under laboratory-challenged conditions (Behera et al. 2018). However, most warm-water fish are resistant to the virus as determined by experimental studies (Jaemwimol et al. 2018); the labeorohita also appear to be resistant to TiLV since experimentally challenged fish displayed no clinical signs or mortality, and no viral RNA was detected (Pradhan et al. 2020). The reason behind the insusceptibility of these fish species to TiLV could be due to the absence of viral receptors of mechanisms that allow the virus to replicate in these non-natural species. Therefore these fish are unlikely to be the carrier of TiLV.

Furthermore, *Iridovirus* is another important virus that has been reported to induce heavy mortalities in Nile and Mozambique (*O. mossambicus*) tilapias (Ariel and Owens 1997). In addition, infectious spleen and kidney necrosis virus (ISKNV), first reported in Africa, has been linked with mass mortalities in farmed tilapia (Ramírez-Paredes et al. 2019).

2.2.3 Parasitic Diseases

Parasites are important components of host biology and can be found in any fish species. Numerous parasites, especially monogenetic and digenetic trematodes, and ciliated protozoans have been identified in tilapia. Moreover, ectoparasite infestation with ciliated protozoans, such as *Trichodina* sp. and *Ichthyophthirius multifiliis*, has been investigated and described in tilapia (Pantoja et al. 2012). The monogenetic trematode *Cichlidogyrus* species have been described from tilapia such as *C. sclerosus* and *C. tilapiae* (Le Roux and Avenant-Oldewage 2010). In addition, gyrodactylid parasites such as *Gyrodactylus cichlidarum* are one of the main *Monogenean* species infecting juvenile Nile tilapia and could lead to severe mortalities of intensively farmed fish worldwide (García-Vásquez et al. 2010).

Among the digenetic trematodes that infect tilapia is *Clinostomum* and *Euclinostomum*. *Clinostomum tilapiae* and *C. complanatum* have been identified in the intestine of Nile tilapia and *Sarotherodon galilaeus* (Ukoli 1966). These digenetic metacercariae are encysted in tilapia tissues such as the sub-mucosa of the mouth cavity, the gill chamber, the operculum, the muscles, the abdominal cavity, mesentery, and viscera (Chung et al. 1995). The encysted metacercariae of *Euclinostomum heterostomum* were also recorded in tilapia in Egypt (Eissa et al. 2011).

A particularly common instance of concurrent infections may be attributed to colonization by ectoparasites, which act as a portal of entry for the second co-infecting pathogen. Suppression of the host immune response resulting in an enhanced effect of the secondary invaders can also occur, and both co-infecting pathogens act synergistically to induce disease with greater economic losses for the affected tilapia (Abdel-Latif et al. 2020).

2.3 Control and Treatment Approach of Diseases

As tilapia culture has become more intensive, the need for environmental control, water quality stability through management, stress reduction, and other sound health maintenance procedures are more essential.

2.3.1 Management

Most management procedures should be applied to regularly eliminate the accumulation of detritus, waste, and dead fish. Water quality should be maintained at the highest possible level, and the reduction of environmentally induced stress is a top urgency. Prudent stocking densities, adequate water exchange in intensive culture units, removal of metabolites and faecal waste from recirculating water, supplemental aeration, and feeding high-quality diets are a means to that end. Water treatment with ultraviolet (UV) light and ozone will help reduce bacterial populations in recirculating water or open water supplies. Disinfection is accomplished by dipping utensils and boots into 200 mg/L of chlorine, 100 mg/L of a quaternary ammonium compound (Roccal), or Iodine at 1000 mg/L.

2.3.2 Chemotherapy

Although not FDA-approved, some drugs and chemicals have been successfully used prophylactically or in chemotherapy for bacterial infections of tilapia. Prophylaxis includes salt baths (NaCl or $CaCl_2$ at 0.5–3%) for a dip or prolonged treatments and/or potassium permanganate (5–10 mg/L) for 1 h or 2–5 mg/L indefinitely. Erythromycin is effective against Gram-positive bacteria, and its use is currently under FDA consideration for use in *Streptococcus* spp.-infected tilapia. If approved, the medication level would probably be 50 or 100 mg/kg of fish per day for 10 days.

2.3.3 Vaccination

Few immunological or vaccination experiments involved tilapia until the last decade, when significant strides were made in vaccinating them against *S. iniae* and *S. agalactiae* (Klesius et al. 2008). The first vaccines used experimentally to prevent *Streptococcus* infections in tilapia were in Japan (Sakata 1988), but since then, numerous vaccination studies have been carried out. A formalin-killed vaccine of two *S. iniae* isolates (ARS-10 and ARS-60), injected IM and IP individually and in combination, protected against the respective antigens (Klesius et al. 2008). These data as a whole indicate that vaccines against *S. iniae, S. agalactiae*, or *E. tarda* provide a feasible and effective proactive approach to prevent serious infections in tilapia by these pathogenic bacteria.

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Chapter 3
Effects of Feed Additives on Tilapia Reproduction

Giorgia Gioacchini, Ike Olivotto, Ghasem Ashouri, and Oliana Carnevali

Abstract Reproduction in fish is an energy-demanding process. The successful development of gonads requires adequate resources to sustain the high-energy demand. Nutrition can affect gametogenesis, fecundity, and consequently, gamete and offspring quality in gravid females. Therefore, an artificial diet for brood stock is formulated to improve nutritional status during this important stage of their life cycle. A detailed reproductive physiology and breeding behaviour of tilapia is discussed in this chapter, with special emphasis on the use of feed additives to control tilapia reproduction, sexual maturation, and sexual determination.

Keywords Reproduction · Physiology · Gametogenesis · Feed additives

3.1 Introduction

Nutrition is known to have a considerable effect on the reproductive performance and gonadal development of fish (Izquierdo et al. 2001; Volkoff and London 2018; Saborido-Rey and Kjesbu 2005). Successful reproduction requires adequate resources to sustain the high-energy demand for gametes production and reproductive behaviours, and, in most cases, negative energy balance and low-food consumption adversely affect reproductive success. In particular, in reproductive females, nutrition can affect gametogenesis, fecundity, and, consequently, gamete and offspring quality (Izquierdo et al. 2001; Lubzens et al. 2017; Bombardelli et al. 2017). Consequently, over the last decades, for most cultured fish species, several commercial broodfish diets have been formulated to improve their nutritional status during this important stage of their life cycle.

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Nile tilapia, *Oreochromis niloticus,* is one of the most cultured fish species worldwide. Thanks to its rapid growth rate and great adaptation to intensive culture conditions, its farming has been intensified; however, there is a strong need for improving the rearing techniques which are essential to guarantee a more effective and sustainable production. Despite the great potential of tilapia culture, there are still problems related to their reproduction, causing an imbalance between seed production and increased global demand representing the main limitation to its further intensive culture expansion (Bhujel 2000). These limitations are related to the low number of eggs released per spawn, the asynchronous spawning behaviour of tilapia, the early sexual maturation in farming conditions which cause overcrowding and fish stunting, and finally, the lower growth rate of females with respect to males, which induce to move toward a mono-sex (only males) culture (Tsadik Getinet 2008; El-Sayed 2019). Therefore, the ability to control sexual determination, sexual maturation, reproduction, and spawning to produce high-quality fry is essential for successful tilapia production (El-Sayed and Kawanna 2008a, b; Migaud et al. 2013). In this light, the optimisation of tilapia reproductive performances using feed additives has been exploited to maximise hatchery production efficiency, sustainability of seed production, mono-sex culture and in turn, profitability.

In this chapter, a general overview of fish reproduction and a focus on the reproductive behaviour and physiology of tilapia, besides a description of the most common systems for seed production, will be done. Particular emphasis will be done on the use of feed additives to control tilapia reproduction, sexual maturation, and sexual determination.

3.2 Fish Reproduction

3.2.1 Reproductive Strategies

The great diversification in reproductive strategies of fish is a key issue supporting their success during evolution (reviewed by Murua and Saborido-Rey (2003), Lowerre-Barbieri et al. (2011), Serrat et al. (2019)). Reproductive strategies could differ in several characteristics. For example, gonochorism, in which sexes are separated, is the most common condition in fish, although hermaphroditism, which could be either simultaneous or sequential, is also observed. Another diversifying factor consists in the choice of breeding dynamics: the most common situation is represented by iteroparity, i.e. fish have multiple reproductive cycles during their lifetime. However, some cases of semelparity, in which the spawning is followed by death, are observed. Reproductive strategy diversification can also depend on fertilisation type and embryo development. External fertilisation is the widespread strategy in fish, in which haploid gametes are released in the aquatic environment where fertilisation occurs. Anyway, several species of fish show internal fertilisation. Regarding embryo development, in oviparous species, it occurs within the eggshell outdoor the mother's body. On the contrary, in the case of viviparity, embryo development occurs within the uterus or the ovary. Finally, parental care can be considered a diversifying factor for reproductive strategies. Many fish species take care of their offspring by applying a high diversity of strategies, ranging from hiding to guarding eggs. They may include nest construction (e.g. *tilapia zillii*), mouthbrooding (e.g. *Oreochromis* spp.), or carrying embryos in specialised body structures (Murua and Saborido-Rey 2003; Lowerre-Barbieri et al. 2011; Serrat et al. 2019).

3.2.2 Endocrine Control of Reproduction

In sexually mature fish, reproduction is controlled by the brain–pituitary–gonad (BPG) axis, which regulates sexual maturity, gametogenesis, reproductive behaviour, integrating external stimuli, both environmental (e.g., photoperiod and water temperature), and social (e.g. courtship), and internal ones (e.g. nutritional status) (Muñoz-Cueto et al. 2020). Despite the wide spectrum of reproductive strategies, the endocrine system which controls reproduction is similar among teleost fish (Zohar 2021).

The BPG axis involves the hypothalamic gonadotropin-releasing hormone (GnRH), the pituitary hormones, the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH), and the gonadal sexual steroid hormones (progestin, oestrogen, and androgen hormones) which are altogether key factors in the endocrine control of reproduction. GnRH stimulates the pituitary gland to produce and release FSH and LH into the bloodstream, which in turn induces gonadal steroidogenesis hormones (oestrogens, androgens, and progestogens) for the control of gonadal development and gametogenesis (Muñoz-Cueto et al. 2020; Zohar 2021).

3.2.3 Gametogenesis

Gametogenesis is the process that includes the formation, growth, and maturation of gametes in females and males, named oogenesis and spermatogenesis, respectively (Lubzens et al. 2010; Kagawa et al. 2013).

3.2.3.1 Oogenesis

Oogenesis is a dynamic process that occurs continuously in the ovary and consists of the development of female gametes from oogonia to eggs (reviewed by Le Menn et al. (2007), Babin et al. (2007), Lubzens et al. (2010), Kagawa et al. (2013), Carnevali et al. (2020)). Oogenesis is commonly divided into primary and secondary growth phases which are gonadotropin independent and dependent, respectively.

The first is characterised by oogonia proliferation and transformation into a primary oocyte (PO). Oogonia are diploid cells that enter meiosis but arrest it in the diplotene stage of the first meiotic division. Once enter meiosis and reach the PO stage, they could last at this stage for several years, at least the entire juvenile period, although in some species (e.g. iteroparous species), they are still present in mature fish. The secondary growth gonadotropin-dependent overlaps with three different phases. During the first one PO develop into the cortical alveoli stage (CA), characterised by the accumulation of alveolar vesicles in the cortical zone of the cytoplasm; during this phase, an accumulation in the form of oil droplets of neutral fatty acids synthesised by the liver may occur however not all species develop this phase. The second phase is represented by vitellogenesis, the main feature of which is yolk/ lipid accumulation. Components derived from vitellogenins (Vgs) represent a huge portion of the yolk mass. The Vgs are phospholipoglycoproteins commonly found during vitellogenesis in the blood of oviparous vertebrate females. Teleost Vgs circulate as a large phosphoglycolipoprotein dimer, which is taken up by growing oocytes via receptor-mediated endocytosis. They are synthesised mainly in the liver, under the regulation of estradiol. During this phase, indeed, the oocyte increases in size as it sequesters vitellogenin from blood and increasingly fills the cytoplasm with yolk granules or globules; vitellogenesis is the more protracted oogenesis stage, ranging from days to several months. The final phase consists of the final oocyte maturation characterised by the resumption of meiosis, formation of large oil droplets or lipid coalescence (only in some species), yolk coalescence, and hydration. The maturation phase is fast (less than 1 day) and ends with eggs spawning. Once the oocyte reaches maturation, the energetic investing becomes irreversible. Indeed, vitellogenic oocytes can be reabsorbed before reaching maturation via a degenerative process named follicular atresia, although it has been observed in some oocytes before vitellogenesis. High rate of atresia is usually associated with inadequate fish conditions such as nutritional deficiency. Starting from proliferation, the oocyte is surrounded by a monolayer of somatic granulosa cells which in turn are surrounded by a somatic theca cells layer. These two somatic components represent the follicular layer of oocytes and are responsible for steroidogenesis. After spawning, the empty follicle cell layer degenerates into a post-ovulatory follicle (POF) (reviewed by Le Menn et al. (2007), Babin et al. (2007), Lubzens et al. (2010), Kagawa et al. (2013), Carnevali et al. (2020)).

3.2.3.2 Spermatogenesis

Spermatogenesis is a dynamic process that leads to the formation of mature sperm from spermatogonia and occurs in the testis (reviewed by Schulz et al. 2010; Schulz and Miura 2002; Miura and Miura (2003); Uribe et al. (2014)). In males, spermatogenesis can be categorised into three phases: (1) the spermatogonial phase (SG), during which spermatogonia undergoes several mitotic divisions, followed by Volkoff and London (2018) (2) the meiotic phase, including primary and secondary spermatocytes and finally Saborido-Rey and Kjesbu (2005) the spermiogenic phase consisting in the production of mature spermatozoa. Spermatogenesis takes place in the testis, in which a germinal epithelium, corresponding mainly to somatic Sertoli cells, surrounds germ cells giving rise to spermatogenic cysts formation. Sertoli cells, continuously interacting with germ cells, are essential for the support of spermatogenic cell growth, development and survival. Inside cysts, germ cells develop synchronously among them and with respect to Sertoli cells, until mature spermatozoa are released into the lumen of the testicular lobule. Somatic Leydig cells, responsible for androgen synthesis, are present among cysts in interstitial compartments containing also blood and lymphatics vessels) (reviewed by (Schulz and Miura 2002; Miura and Miura 2003; Schulz et al. 2010; Uribe et al. 2014)).

Mature gametes are the successful completion of gametogenesis, and their quality consists in the ability of spermatozoa to fertilise an egg, or an egg to be fertilised, resulting in the formation of a viable embryo (Bobe and Labbé 2010).

3.2.4 Spawning Dynamics

The spawning dynamics are crucial in reproductive success, as they must give suitable conditions for offspring survival (Saborido-Rey 2019). The size of the fish and hence energy availability is a key factor affecting spawning frequency, as well as the total number of batches produced during the spawning season. Although several species reproduce once per year, some species exhibit shorter cycles, more than one reproductive cycle per year or other species even spawn nearly continuously, i.e. tropical and subtropical species (e.g. *Oreochromis niloticus*). However, reproductive cycles can be interrupted prior to the spawning entering into a regressing phase until the onset of the next breeding season. This event, termed *skipped spawning*, can be caused by inadequate diet and nutritional condition, and it is characterised by a high rate of atresia, clearly indicating that female spawning success is strictly related to energy availability (Carnevali et al. 2017; Saborido-Rey 2019; Yoshida 2020; Alix et al. 2020).

3.2.5 Puberty

Puberty is considered the developmental period covering the transition from a juvenile (immature) to an adult (mature) fish (Taranger et al. 2010; Okuzawa 2002). The main feature of puberty is the associated initiation of the two main gonadal functions: the synthesis of reproductive hormones (oestrogens, androgens, and progestogens) and the production of mature gametes (Taranger et al. 2010). Sex hormones are required during gametogenesis in both males (androgens for spermatogenesis) and females (oestrogens for vitellogenesis). According to Okuzawa (2002), the successful production of the first batch of fertile gametes determines puberty’s end.

The timing of puberty onset in fish is mostly determined by several internal (such as genetic) and external factors (including food availability and somatic growth), all of which could be manipulated under farming conditions. Sexual maturation includes physiological, behavioural, and energetical changes and represents a critical shift in the life of an individual (Taranger et al. 2010). Before sexual maturation, assimilated energy is fully expended for growth and survival. When immature fish reaches a determined threshold of the energetic state, sexual maturation can occur. Therefore, puberty involves several endocrine and metabolic changes which lead to the mobilisation and reallocation of energy and depends on the achievement of a critical size/age (size/age of first reproduction) corresponding to a determined physiological state which could support reproductive processes (Taranger et al. 2010).

3.3 Tilapia Reproductive Behaviour and Physiology

3.3.1 Tilapia Reproductive Strategies

Tilapia species are gonochoristic and oviparous with external fertilisation, but the trademark of their reproduction is parental care, which is not typical of most teleost fish (Rana 1988; Bhujel 2000; Coward and Bromage 2000; Dieudonne et al. 2017).

Tilapia are classified into two main generic groups based on their mode of parental care:

1. ‘Substrate spawners’ (guarders), which contain the fish belonging to the genus Tilapia (e.g. *T. zillii*, *T. rendalli*). In this case, both males and females contribute to the defence of the nest containing fertilised eggs until hatched larvae become independent of their parents (Coward and Bromage 2000).
2. ‘Mouthbrooders’, which include the genus *Sarotherodon* and *Oreochromis* in which fertilised eggs are incubated in the parents’ buccal cavities. In particular, ‘mouthbrooders’ could be: biparental (both males and females brood and defend the fertilised eggs and hatched fry) such as *S. galilaeus*, or mono-parental which in turn could be paternal mouthbrooders (only males brood the eggs) such as *S. melanotheron* Coward and Bromage (2000); Rana (1988); Turner and Robinson (2000), or maternal mouthbrooders (only females brood the eggs) such as the most cultured tilapia *O. niloticus*, *O. aureus,* and *O. mossambicus.*

Oreochromis males build and defend the spawning territory; courtship between a visiting female and a resident male takes only a few hours and ends with egg release and fertilisation. The Nile tilapia female releases a string of about 20–50 eggs which will be immediately fertilised by the male. After fertilisation, the female takes the fertilised eggs into her mouth and moves to a safe place to incubate. The male no longer participates in incubation and breeding while lures further females to the nest. During egg incubation, the female, holding the eggs in her mouth and continuing to turn them continuously, is unable to eat. After about 5–10 days, egg hatching occurs, but the newly hatched fry continues to develop in the mouth. After yolk-sac absorption, the fry leaves the mouth of the mother and returns to it in the presence of danger. (Rana 1988; Coward and Bromage 2000).

Tilapia are multiple spawners, releasing eggs several times per year, if environmental and/or farming conditions are favourable. At the end of the spawning, the tilapia ovary regenerates very rapidly, and previtellogenic oocytes start vitellogenesis within a week (Coward and Bromage 2000; Elizabeth Cruz Suárez et al. 2006). Parental care characterising tilapia reproduction has led to an increase in egg size and a corresponding reduction of fecundity, which in turn, are directly related to fish size, length, or age. Larger females usually produce a higher number of eggs per clutch with respect to smaller ones. Conversely, "mouthbrooding tilapia" produce a lower number of bigger eggs with respect to "substrate spawners" (Rana 1988; Coward and Bromage 2000). It was also demonstrated that bigger eggs contained more yolk and led to larger fry, more resistance to unfavourable environmental conditions and adverse starvation (Rana 1988).

3.3.2 Tilapia Seed Production Systems

In tilapia farming, three seed production systems are commonly used. The first and most widely used is seed production in earth ponds. In these ponds, a limited number of tilapia grow and reproduces naturally. The seed remains with the parent fish until the time of the seasonal or annual harvesting of the entire pond. During harvesting, new broodfish are selected and restocked into new ponds for the next production cycle. Alternatively, the seed may be harvested separately and restocked in fattening or on-growing ponds.

The second system of seed production is based on managed spawning ponds. In this system, seed production is improved by enhancing the quality and quantity of the broodfish diet. Feed quality improvement could consist of organic and/or inorganic fertilisers or supplementary feeding with leaves, rice bran, oil seed cake, or commercial diets containing about 20% protein. Fertilisers and feed availability and costs determine the combined rate of the two methods.

Finally, the third system for seed production is represented by ponds of special design. In this system, there is an upper egg-laying area from which the eggs drain by gravity into an underlying nursery area through a series of graduated screens. In this way, the broodfish remain confined to the spawning area while the fry enters the nursery. The spawning pond is soon available for the next spawning cycle.

In some tilapia farming situations, land for ponds may not be available due to environmental restrictions or competition with other farming sectors. In these cases, seed production occurs in net enclosures suspended in nutritionally rich shallow water areas of lakes and lagoons, traditionally known as hapas. In recent years, the usage of ad-hoc manual or semi-automated seed production tanks has been developed.

3.4 Feed Additives Administration to Control Tilapia Reproduction

3.4.1 Reproduction Improvement by Using Feed Additives

The fast breeding of tilapias in various tropical and sub-tropical environments makes them ideal farming species; however, their extensive cultivation is still challenged by their low reproductive efficiency together with advanced sexual maturation (as early as 3 months) (Coward and Bromage 2000). As stated above, the complex reproductive strategy, together with the asynchronous spawning and low fecundity, require rigorous management of large numbers of broodfish fish. Feeding management of broodfish tilapia can significantly improve reproductive performance enhancing eggs quality and, in turn, hatchery efficiency and profitability (Bhujel 2000; Izquierdo et al. 2001; Migaud et al. 2013; Bombardelli et al. 2017). Female nutrition is extremely important because dietary constituents are employed for vitellogenesis Izquierdo et al. (2001) and affect gonadal maturation and the quality of yolk (Bobe and Labbé 2010). This determines the quality and survival of the offspring Bombardelli et al. (2017); El-Sayed and Kawanna (2008a, b) because the embryos and larvae, in the initial developmental stages, solely depend on the yolk for sustenance (Fernández-Palacios et al. 2011). Limiting the feed during this period reduces the percentage of eggs that finally mature and ovulate, resulting in a reduction in fry production. Furthermore, nutrition controls the health and survival of females and establishes their capability to allow metabolic stress imposed by the intense reproduction process and rearing characteristics of tilapia (Lupatsch et al. 2010).

The quality and quantity of broodfish diet depend on the type of seed production system. Fertilisation of ponds led to reducing costs and providing a well-balanced diet. Instead, clear water systems require a completely well-balanced diet. As for fry management, diets containing 35–45% protein allow for excellent growth rates. It is also known that the quality of the protein is important. Fishmeal is the best source of high-quality protein, but it is expensive and often unavailable. Therefore, optimising proper feeding management for tilapia broodfish becomes necessary for maximising seed production sustainability and culture productivity (Bhujel 2000).

Up today there are several live and non-living feed additives available to improve tilapia reproduction which include probiotics, phytobiotic, vitamins, and other organic compounds.

3.4.1.1 Probiotics

Probiotics are live microorganisms which confer a health benefit on the host when administered in adequate amounts. Nowadays, since the growing demand for environment-friendly aquaculture probiotics is considered an integral part of the aquaculture systems to increase production also in tilapia (Nayak 2010; Hai 2015; Standen et al. 2015).

During the last decade, several studies evaluated the efficacy of probiotics on the reproductive performance of different teleost (Carnevali et al. 2017; Aydın and Çek-Yalnız 2019). Regarding tilapia farming, several papers showed the positive effects of probiotics on *O. niloticus* fry and fingerlings (Gioacchini et al. 2018; Lara-Flores et al. 2003; Standen et al. 2013). Recently, Mehrim and collaborators (Mehrim et al. 2015) evaluated the effects of graded levels (0, 5, 10, and 15 g/kg diet) of a commercial probiotic, Hydroyeast Aquaculture®, on different reproductive parameters of both male and female Nile tilapia *O. niloticus* adults. In particular, the authors reached the best results using a 15 g/kg diet and a 10 g/kg diet of Hydroyeast Aquaculture® probiotic. At these concentrations, in males, the probiotic treatment enhanced reproductive efficiency in terms of an increase of gonadosomatic index (GSI) and sperm quality parameters (such as total count, motility, abnormalities, and dead); in females, probiotic-enhanced GSI, egg weight numbers and diameters, and fecundity (Mehrim et al. 2015).

3.4.1.2 Phytobiotics

Phytobiotics have recently attracted significant attention in aquaculture as they showed very potent immunostimulant, antioxidant and antimicrobial functions (Jana et al. 2018). Among them, Wahbi and Sangak Wahbi and Sangak (2017) demonstrated the beneficial effect of dietary inclusion of graded levels of *Spirulina platensis* (0.5, 7.5, 10, and 20 g kg^{-1} diet) in Nile Tilapia *Oreochromis niloticus* reproductive performances. In particular, they found that Spirulina supplementation increased total fish weight, GSI, spawning activity and spawned egg number, hatching rate, fry length, and weight (Wahbi and Sangak 2017).

3.4.1.3 Vitamin C

Many authors highlighted the influence of vitamin C in fish reproduction(reviewed by (Dabrowski and Ciereszko 2001). In those studies, it was evidenced that a diet free from or deficient in ascorbic acid reduces in females the availability of vitamin C in the ovary, impairing reproduction, decreasing fecundity and egg quality, and increasing larval deformities and mortality (Dabrowski and Ciereszko 2001).

Martins and collaborators showed (Martins et al. 2016) that supplementation of 150 and 300 mg kg^{-1} vitamin C to the diet given to Nile tilapia positively increased GSI. Similar results have been obtained by Sarmento and collaborators (Sarmento et al. 2018) after vitamin C dietary supplementation (0, 261, 599, and 942 mg/kg of Nile tilapia (*Oreochromis niloticus*) broodfish. In this study, the best results in terms of GSI, egg weight, and larval measurements have been obtained by using 599 and 942 mg vitamin C/kg diet supplementations. A higher total and relative fecundity were found for females receiving a 599 mg vitamin C/kg diet. An increase in hatching rate and egg and larvae production per female was found for females receiving all doses of vitamin C. Interestingly, the authors also performed two stress tests on fertilised eggs from treated females, and they demonstrated that when eggs were incubated at a different rate of salinity, the worst survival rate was achieved with the 0 mg vitamin C/kg diet at the end of 120 h of incubation. While the tests of exposure to air for 40 and 50 min, showed that females receiving the 942 mg vitamin C/kg diet produced newly hatched larvae showing the highest resistance to the stress, while females receiving 0 and 261 mg vitamin C/kg diets produced newly hatched larvae showing the lowest stress tolerance (Sarmento et al. 2018).

3.4.1.4 Other Chemical Compounds

Taurine (2-aminoethanesulfonic acid) is an end product of the metabolism of sulphur-containing amino acids, and its feed supplementation significantly affects growth rates and physiological functions of marine and freshwater fishes (reviewed by El-Sayed El-Sayed (2014)). Previous studies revealed that taurine plays a positive role in Nile tilapia (*Oreochromis niloticus*) performance (Al-Feky et al. 2016a, b). Al-feki and collaborators (Al-Feky et al. (2016a, b)) demonstrated that dietary taurine (8 g kg^{-1}) improved the reproductive performance of Nile tilapia (*Oreochromis niloticus*) broodfish. In particular, taurine supplementation reduced the size at first maturation and the time to first spawning. At the same time, dietary taurine significantly increased spawning performances, including spawning frequencies, the total number of spawning per tank, the number of spawning per female, and absolute fecundity. Finally, eggs produced from broodfish fed on taurine exhibited significantly higher hatchability, reduced time for hatching and yolk-sac absorption.

Ahamed and co-workers (Ahamed et al. 2020) demonstrated the effects of a diet supplemented with Azomite, a natural mineral of volcanic ash, on gonadal steroid hormone levels and milt quality in *Oreochromis mossambicus*. The Azomite administration enhanced the quantity of milt, the percentage of sperm count, the duration of sperm motility, as well as gonadal sex-specific steroid hormones, such as testosterone (T), 11-ketotestosterone (11-KT), and 17β-estradiol (E2) (Ahamed et al. 2020).

De Lima and collaborators (de Lima et al. 2020) assessed the effects of diet supplementation with purified nucleotides (NT) on the health, growth, and reproductive performance of Nile tilapia females. In particular, they found that NT supplementation induced an increase in the percentage of secondary growth oocytes and egg number production, together with a decrease in lipid peroxidation in the ovaries (de Lima et al. 2020).

Finally, Abdelhamid and collaborator (Abdelhamid et al. 2010) investigated the effects of three commercial feed additives (Therigon®, Nuvisol Hatch P®, and Gibberellic acid) on females and one (L-carnitine) on males tilapia broodfish performances. Results indicated that all treatments positively affected reproduction inducing, in females, an increase in ovaries weight and egg number per fish, while in males, an increase of quality sperm parameters such as motility, forward, sluggish and dead percentages.

3.4.2 Mitigation of Tilapia Reproduction by Using Feed Additives

Even in farming conditions, tilapias reach sexual maturity and easily spawn, and the simplicity by which they breed is one of the reasons why tilapia farming has spread throughout the world. However, their plasticity and readiness for sexual maturation acquiring can trigger difficulties associated with overpopulation and reduced growth (El-Sayed 2019). The age and size at which tilapias mature and breed vary with environmental conditions. Farmed Tilapia females mature at an earlier age and smaller size compared to those raised in natural conditions. Generally, natural or wild populations breed at an age of between 2 and 3 years. Farmed stock in ponds and similar shallow water bodies reach sexual maturation at an age of only 2–-3 months, and sexually mature fish breed at 5–6 months. Among different factors, it was evidenced that feed quantity and quality could affect size at first maturity. Many authors found that the use of feed additives could control tilapia reproduction by impairing fertility through gonads (testes and ovaries) damaging. Among them, *Aspilia mossambicensis* and *Azadirachta indica* have recently been used to mitigate the prolific breeding of *O. niloticus* (Kapinga et al. 2019). *A. mossambicensis* and *A. indica* can mitigate the prolific breeding of *O. niloticus* thanks to the presence of phytocompounds which can affect steroidogenesis reproductive organs structures. Recently Kapinga and collaborators demonstrated that *A. mossambicensis* and *A. indica* leaf powder administration impair reproduction by decreasing hatchlings production in *O. niloticus (*Kapinga et al. 2019). The same authors also studied the effect of *A. mossambicensis* or *A. indica* leaf powders supplemented with four doses (1.0, 2.0, 4.0, and 8.0 g/kg diet) on a juvenile at 3% of their body weight daily for 90 days. All doses of *A. indica* and *A. mossambicensis* used significantly lowered absolute fecundity and GSI values, in a dose-dependent manner, starting from doses of 2.0 g/kg in males and 4.0 g/kg in females. In males fed at a lower dose (2.0 and 4.0 g/kg for both *A. mossambicensis* and *A. indica*) were observed histological alterations such as the degeneration of seminiferous tubules presenting a dilatated lumen and containing very few spermatozoa, and the presence of inflammatory cells scattered in the testicular parenchyma. The dose of 8.0 g/kg for both *A. mossambicensis* and *A. indica,* induced the loss of testicular architecture, a dramatic decrease in spermatozoa production, and the increased presence of squamous cells and inflammatory cells within the tubules. In females fed at higher doses (4.0 and 8.0 g/kg for both *A. mossambicensis* and *A. indica*), was evidenced the presence of numerous vacuoles in the ooplasm and alteration of granulosa cells nuclei and thecal layer thickness (Kapinga et al. 2019). A study by Jegede and Fagbenro (2008a, b) reported that incorporation of *Azadirachta indica* in *Tilapia zillii* basal diet at 2.0 g kg^{-1} induced swollen spermatids nuclei, increased interstitial cells and focal necrosis in testes and hydropic degeneration, ruptured follicles, granulomatous inflammation in the interstitium and necrosis ovaries. Similar findings were reported in *O. niloticus* fed Carica papaya Okomoda (2017); Jegede and Fagbenro (2008a, b), Hibiscus rosa-sinensis Jegede (2010), and Aloe vera

Kushwaha (2013) as well as in *O. mossambicus* fed dietary *Carica papaya* and *Moringa oleifera*, respectively (Ampofo-yeboah 2013).

3.4.3 Hormonal Sex Reversal by Feed Additives

Although the mixed-sex culture of tilapia represented a widespread practice in many countries for decades, nowadays, broad attention has been given to mono-sex culture. The production of mono-sex (all-male) tilapia represented a great improvement in productivity due to their fast growth rates, tolerance to different culture conditions, stress, and diseases, greater energy conservation, decreased aggressiveness, and better uniformity of size at harvest (Elizabeth Cruz Suárez et al. 2006). Today, mono-sex tilapia populations are obtained by applying several methods including manual separation of sex, sex reversal hormone, genetic manipulation, and hybridisation (Elizabeth Cruz Suárez et al. 2006). Among them, hormonal treatment is the most successful and widely used method responsible for the production of all male culture (Megbowon and Mojekwu 2014). Tilapia larvae are sexually undifferentiated up until 2 weeks after hatching, and at this time, there are no differences between sex hormone levels (Besseau et al. 2013). Therefore, the administration of exogenous steroid hormones such as androgen (male) or oestrogen (female) during gonadal development or before sexual differentiation would influence the larvae to become either male or female based on the hormone administered (Megbowon and Mojekwu 2014). Unfortunately, synthetic hormones lower fish immunity and survival and require human and environmental health concerns (Megbowon and Mojekwu 2014).

Consequently, alternative methods to obtain mono-sex tilapia culture that is safe and environmentally friendly have been evaluated. Among them, the use of medicinal plants in aquaculture to control reproduction represents a valid technology (Francis et al. 2002; Gabriel et al. 2017).

Recently, it was evidenced that several medicinal herbs containing phytochemicals (structurally similar to steroid hormones such as phytoestrogens or phytoandrogens) can induce tilapia masculinisation, feminisation, or impair fertility (Gabriel 2019). Saponin extracts from *Quillaja saponins* (QS) Francis et al. (2002, fenugreek) (*Trigonella foenum-graecum*) and soapbark tree (*Quillaja saponaria*) Stadtlander et al. (2013), and *Tribulus terrestris* B.O. Omitoyin et al. (2013), induced masculinisation in a dose-dependent manner in Nile tilapia when were incorporated in their diet, Similarly, masculinisation induction was also described in Nile tilapia fed dietary Aloe vera, *Mucuna pruriens* Mukherjee et al. (2015), *Butea superb* (Kiriyakit 2014). The same results have been achieved in other tilapia species, such *O. mossambicus* fed dietary moringa and paw crude extracts (Ampofo-yeboah 2013). Finally, El-Sayed and co-workers revealed that soybean meal significantly induced femininisation in Nile tilapia culture and further warned tilapia farmers to avoid using soybean as a source of protein during sex reversal (El-Sayed et al. 2012).

3.5 Conclusion

Constraints associated with the wide adoption of tilapia intensive aquaculture farming systems such as the increased request for proper seed quality and a monosex culture, among others, cannot be overemphasised. Moreover, the consequent use of drugs (i.e. sex reversal hormones) to improve production represents a prominent risk not only for animals but also for the environment and humans. The overview of the results achieved in recent years on the application of feed additives (often of natural origin and not of synthetic origin) for the control of Tilapia reproduction is presented in this chapter, showing that the use of feed additives could represent a valid alternative to drugs. However, the rate of implementation of this innovation is still too slow, despite the numerous advantages associated with it, and there is still the need to deepen knowledge to further validate the daily use of feed additives in tilapia farming.

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Chapter 4
Intestinal Microbiota of Tilapia: Characterization and Modulation

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Abstract Tilapia (*Oreochromis* sp.) are cultured in many countries of the world. Its production is increasing from time to time to fulfill the demand for fish for the fast-growing population of the world. The significant importance of intestinal microbiota in several fish conditions including growth, digestion, energy homeostasis, immunity, and gut-brain axis were reported in a plethora of studies. Characterization of the intestinal microbiota is important to deal with the detailed function of these microbes. The majority of the bacteria species found in the intestine of fish are not culturable and to date, due to the advancement of molecular techniques including denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and next-generation sequencing (NGS) has been employed to investigate and characterize intestinal microbiota. Researchers have been working to increase the production and productivity of tilapia in aquaculture by altering the different microorganisms found in the intestinal of the fish. Studies showed types of diets and their ingredient compositions have a significant role in improving and modulation of the intestinal microbiota of tilapia. Furthermore, the role of several "-biotics" including prebiotics, probiotics, paraprobiotics, and postbiotics in the improvement of the intestinal microbiota was described in many studies. The significance of dealing with intestinal microbiota is to provide a scientific basis for

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the establishment of useful approaches for the effective manipulation of these microorganisms to improve fish health and increase production. The intimate knowledge of the usefulness of intestinal microbiota on the overall fitness of tilapia is necessary. Therefore, the objective of this review was to entirely understand the importance and function of these microorganisms invading the intestine of tilapia on the growth, development, metabolism, immunity, and overall fitness of tilapia. Moreover, we highlight the existing information on the strategies for changing the structure of the microbiota community of tilapia via diets or/and "-biotics" and recommendations for future research with the final objective of the applications of these approaches in the tilapia aquaculture industry.

Keywords Diet · Intestinal microbiota modulation · Probiotics · Prebiotics · Tilapia

4.1 Introduction

Next to carp, tilapia, which consists of several species in the family of *Cichlidae*, is the most common cultured fish in many countries of the world (Watanabe 2002). Tilapia are hardy, prolific, and fast-growing tropical fishes. Tilapia is the most popular culture fish in the world and is the second most important finfish species group cultured worldwide in terms of production. Its production has been increasing fourfold in the past decade due to its appropriateness for aquaculture in different environments, preferable for consumers, and stable market prices (Wang and Lu 2016). Studies demonstrated that many species of tilapia have been produced in more than 140 countries, and this data showed that this fish species is one of the most useful aquatic animals in the fish farming sector throughout the world (Fitzsimmons 2016; Junning et al. 2018). Even though tilapia are produced in several developed and developing countries of the world, most producers of the fish are from developing countries such as China, Indonesia, the Philippines, Thailand, Ecuador, and Egypt are the major producers (Yue et al. 2016). FAO's (2020) fish production data showed that the production of tilapia in 2018 accounted for more than 10% of overall cultured fish species in the world. The increasing trends in the production of tilapia were because of the improvement of the production performances of several breeds and strains of the fish via the development of new breeding strategies (Ponzoni et al. 2011; Nguyen 2015; Fitzsimmons 2016).

Several studies demonstrated that tilapia farming is common practice in these countries with low income and has a great contribution to the improvement of the nutritional security status and increase the economic status of the people. Because of this, researchers have been recommended to promote the production of tilapia. Furthermore, they have been working to improve the production of tilapia via several strategies, and one of the strategies was through modulation of the intestinal microbiota using different types of modulators. Identification and characterization of these microorganisms found in the intestinal of tilapia were done through culture-dependent methods or using modern technologies including high throughput.

To date, researchers performed culture-dependent methods of exploration of the intestinal microbiota of tilapia, and they identified fewer microbiota species, but later due to molecular improvement in high-throughput sequencing technology and advancement of bioinformatics have allowed more accurate data on identification and in-depth analysis of intestinal bacterial communities of fish (Opiyo et al. 2019). Especially, the discovery of new and sophisticated molecular technologies and techniques to identify and characterize these via sequencing genes of these microorganisms has widened our understanding of intestinal microbial community structure and their specific function in fish (Zarkasi et al. 2014; Falcinelli et al. 2015). Using high-throughput sequencing identified that the dominant phyla found in the intestinal microbiota of tilapia include *Proteobacteria, Cyanobacteria, Fusobacteria, Actinobacteria,* and *Bacteroidetes* (Standen et al. 2015). The advancement of molecular techniques for the isolation and identification of microorganisms was not only important for investigation but also for appreciating the significant role of the microbiota that occupy the tilapia intestine. Accordingly, a plethora of studies revealed that the intestinal microorganisms of fish have a significant role in the physiology, growth, and immunity of the fish (Burns et al. 2016; Yan et al. 2016; Nie et al. 2017; Smith et al. 2017). Consequently, these and other studies showed these microorganisms found in the intestine of tilapia play a vital role in overall fish fitness. Currently, the advancement of bacteria identification and characterization methods including next-generation sequencing plays a significant role in the detailed characterization of these microorganisms isolated from the intestine of tilapia. Though, the information on the composition, community structure, and role of the intestinal microbiota of tilapia is not fully understood. Therefore, here below, we provide detailed information on the microorganisms found in the intestinal of tilapia to permit their importance on the overall fitness of tilapia to increase the production and productivity of the fish.

4.1.1 Characterization of the Intestinal Microbiota of Tilapia

To date, our knowledge of the characterization of the microorganisms originating from the intestinal of fish results from the collection of information in several studies; the majority of them were focused on cultured fish species, and these tilapia species have gained much consideration and attention. Characterization of the intestinal microbiota of fish has a significant role in understanding the function of this microbiota of the fish. Studies indicated that the intestine of fish was a harbor with approximately 10^8 bacterial cells belonging to more than 500 beneficial and harmful bacteria species (Rawls et al. 2004; Romero and Navarrete 2006). Colonization of the intestinal of fish starts at the early larval stage and it continued with the fish growth and reaches its complex assemblage of microbes (Nayak 2010). Yet, the compositions of intestinal microbiota were changed rapidly at the fish developmental stages (Del'duca et al. 2013). The intestinal microbiota has multiple functions in the fish host physiology, metabolism, and immunology. Previous studies provide a number of evidence on the significant role of intestinal microbiota using germ-free fish and gnotobiotic models. Oyarbide et al. (2015) demonstrated that gnotobiotic zebrafish (*Danio rerio)* (Zebrafish developed in germ-free conditions and inoculated with few known microorganisms) *is* more affected by pathogenic bacteria compared with the conventional zebrafish. The specific taxa of bacteria found in the intestine as well as the diversity of these microorganisms have been applied as an indicator (biomarker) of the metabolism and health status of the fish (Clarke et al. 2014). Studies revealed that most of the time low diversity of these microorganisms isolated from the intestine of fish was associated with abnormal health of the fish (He et al. 2017; Nie et al. 2017). Several external factors' results reduced the diversity of intestinal microbiota these studies indicated that the application of antibiotics resulted in a reduction in the diversity of intestinal microbiota and affected fish health via facilitating colonization by pathogenic microbes (He et al. 2017).

Most of the time, more diversified intestinal microbiota was an indicator of good fish health because they provide greater protective effects against pathogenic bacteria (De Schryver and Vadstein 2014; Zhu et al. 2016). Therefore, maximizing the diversity of intestinal microbiota is useful since tight mutual interaction between the fish and intestinal microbiota is vital to reduce the effect of harmful microbiota on the host, and increase the production of fish in aquaculture (McFall-Ngai et al. 2013). Analysis of OUT is a good indicator of the diversity formed within the sample (alpha diversity) and the other diversity observed among different samples (beta diversity) while interpreting the composition of the intestinal microorganisms. Recently de Bruijn et al. (2017) demonstrated the relationship between intestinal microbiota diversity, function, and fish health status. Another study also revealed the interaction between intestinal microorganisms and fish innate immunity (Nie et al. 2017).

Studies revealed that the intestinal microorganisms of fish were not easily identified using culture-based techniques, and less the 1% of the total intestinal microbiota of fish was culturable, and this does not show an accurate image (Ray et al. 2012; Banerjee and Ray 2017). Culture-based techniques of identification and isolation of intestinal microbiota were used in many specific media, including nutrient agar, tryptone soya agar, peptone gelatin agar, cellulose agar, and starch agar (Ray et al. 2012). Nowadays, many researchers tried to identify and characterized the intestinal microbiota of tilapia using advanced technologies such as DGGE, TGGE, and NGS (Banerjee and Ray 2017). The function and factors that affect the intestinal microbiota of tilapia were summarized (Fig. 4.1).

4.1.1.1 Common Intestinal Microbiota in Tilapia

Understanding the usefulness of microorganisms found intestines of fish in improving the development and well-being of tilapia was explained by many researchers. A plethora of studies identify and characterize the microorganisms isolated from the intestine of tilapia via culture-dependent and independent methods. The culture-

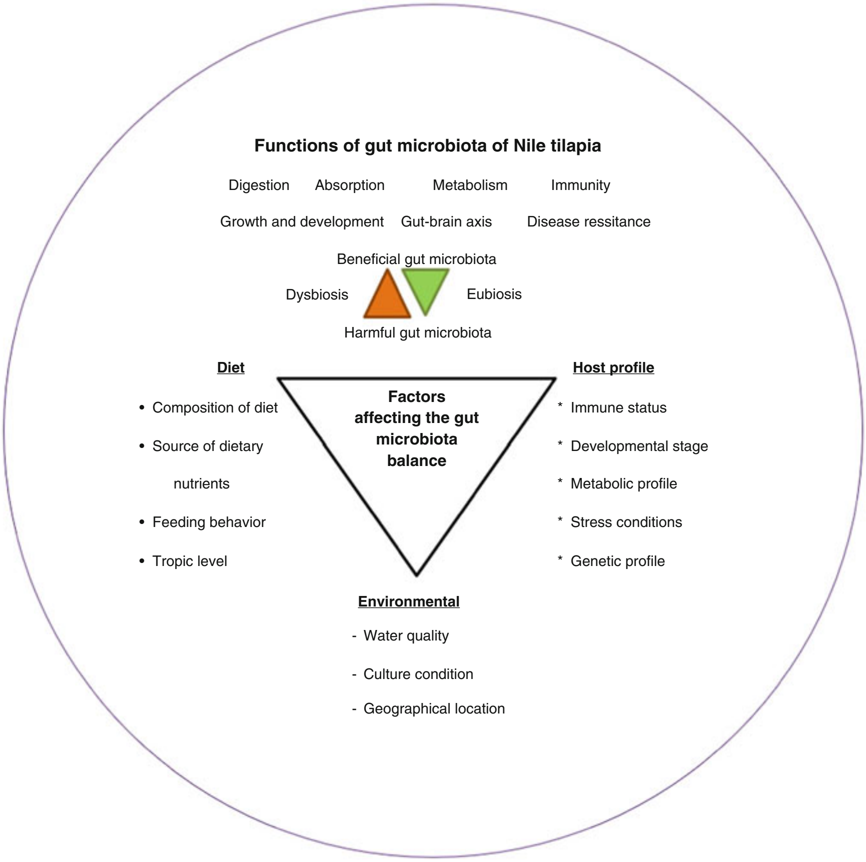


Fig. 4.1 Environmental, diet-associated, or host—associated factors affect the structure and function of the intestinal microbiota of tilapia. These factors can result in either a healthy state (normobiosis) or altered microbiota (dysbiosis) of Tilapia

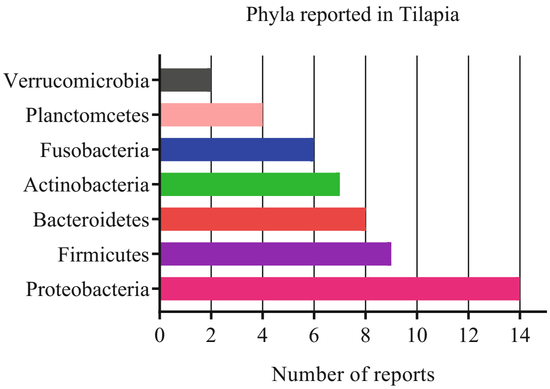
dependent technique of identification of bacteria from the tilapia intestine was accomplished by Cahill (1990). Before three decades, the identification and characterization of the intestinal microbiota of fish were restricted to the most common and easily culturable bacteria such as *Pseudomonas* sp., *Vibrio* sp., *Aeromonas* sp., and other unidentified species (Cahill 1990). Molinari et al. (2003) identified several bacteria including *Aeromonas hydrophila, A. veronii, Citrobacter freundii, Escherichia coli,* and *Plesiomonas shigelloides* from the intestine of tilapia. The advancement of molecular technologies in the identification of microbiota has increased our understanding of fish microbiota (Nayak 2010). The intestinal microbiota of tilapia was largely affected by several external and internal factors and has dynamic composition and community structure (Wang et al. 2016).

Studies demonstrated that the intestinal of tilapia harbor a complex microbiota, and the main phyla are *Proteobacteria, Fusobacteria, Firmicutes,* and *Bacteroidetes* (Fan et al. 2017; de Souza et al. 2020; Souza et al. 2020). Foysal et al. (2019) identified that the most common intestinal bacteria of tilapia fed on the control diet were *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Fusobacteria*, *Verrucomicrobia*, and *Acidobacteria*. *Fusobacteria*, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Planctomycetes* were identified from the intestinal of farmed Nile tilapia (Melo-Boliver et al. 2019). Furthermore, Bereded et al. (2020) identified the core intestinal microbiota of Nile tilapia were *Proteobacteria*, *Firmicutes*, *Cyanobacteria*, *Fusobacteria*, and *Actinobacteria*. In addition to this, Baldo et al. (2015) assessed the composition of intestinal microbiota from different species of tilapia using 16S rRNA pyrosequencing DNA sequencing and identified seven bacteria phyla such as *Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Planctomycetes,* and *Verrucomicrobia.* Besides, several major typical taxa of bacteria such as *Cetobacterium somerae, Clostridium perfringens, Plesiomonas* spp., *Clostridium XI* sp., *Aeromonas* sp., *Neisseriacea, Clostridiaceae, Acromobacter* sp., *Bacillus* sp., *Citrobacter koseri, Edwardsiella* spp., *Enterobacter cloacae, Pasteurella pneumotropica, Photobacterium damselae, Shewanella putrefaciens, Staphyloccocus* sp., *Vibrio* spp., and unidentified species were identified for the intestine of tilapia (Baldo et al. 2015; Pakingking et al. 2015).

All these and other studies demonstrated that the intestinal of tilapia harbor a complex microbiota, and the main phyla are *Proteobacteria, Fusobacteria, Firmicutes,* and *Bacteroidetes*. Figure 4.2 indicated that summarization of the most common intestinal bacteria phyla of tilapia was reported in several studies.

There are a number of factors that can influence the composition and diversity of the intestinal microbiota of tilapia. Administration of antibiotics such as oxytetracycline for the long-term has been negatively affecting the intestinal microbiota, especially *Bacteroidetes* and *Firmicutes* phyla of tilapia, and this, in turn, affects the growth and immunity (Limbu et al. 2018). Numerous studies reported that the characterization and modulation of microorganisms found in the intestinal of tilapia

Fig. 4.2 Bacteria phyla observed in the intestinal of tilapia



have a great role and are used as a model for other commercially important fish species.

4.1.2 Function

Studies demonstrated that the intestinal microbiota of fish has several functions including digestion, absorption, development of the mucosal system, and disease (Rawls et al. 2004). In the intestinal of fish diverse microbiota including archaea, protoctista, fungi, yeast, viruses, and bacteria were identified. The intestinal microbiota of fish was dominated by *Proteobacteria* and *Fusobacteria* bacteria phyla. The different sections of the intestine of fish consist of various species of microbiota, and these bacteria were different in density, composition, and function. The bacteria communities were grouped into allochthonous (free-living, transient microbiota associated with the digesta) and autochthonous (colonize the mucosal surface of the digestive tract), and these autochthonous bacteria are considered the core bacteria species of the fish intestine (Banerjee and Ray 2017).

The intestinal microbiota of fish has several functions and regulates many genes. Rawls et al. (2004), confirmed that fish intestinal microbiota regulates the expression of 212 genes, and these genes were related to nutrient metabolism, innate immunity, and stimulation of epithelial proliferation and growth. Studies demonstrated that the specificity of the host response depended on the bacterial species that colonize the digestive tract (Rawls et al. 2004). The flagella of *Pseudomonas* were able to interact with the host cells, and the motility of *Pseudomonas* spp. was also important to stimulate inflammatory signals in zebrafish (Rawls et al. 2004). Furthermore, they demonstrated that the interaction between *Pseudomonas* and the host epithelium was improved by the flagella-dependent swimming motility of the bacteria.

Moreover, Bates et al. (2006) demonstrated the significant role of intestinal microbiota on fish digestive tract differentiation and development, and function. They also revealed that in the absence of intestinal microbiota, the intestine was developed without brush-border intestinal alkaline phosphate activity, immature patterns of glycan expression, and a reduction of goblet cells and enteroendocrine cells. Finally, these effects lead to the abnormal function of the intestine, which proves how much intestinal microbiota is vital for the development and normal function of the intestine of fish. Remarkably, yet, the normal function and structure of the intestine were reversed by the reintroduction of the common intestinal microorganisms of the fish. The intestinal microbiota of fish including *Proteobacteria, Bacteroidetes, Firmicutes,* and *Fusobacteria* phyla also involved in fish nutritional metabolism.

Some species of bacteria which colonize the intestine of fish can produce short-chain fatty acids and vitamins), which may have a beneficial effect on fish growth and health. A large quantity of vitamin B12 (cobalamin) was produced by *Cetobacterium somerae (C. somerae),* which colonized the intestine of several fish species including tilapia (Sugita et al. 1991; Tsuchiya et al. 2008). Some fish species such as carp and tilapia, due to the presence of *C. somerae* in their intestine, have no requirement for dietary vitamin B12.

Furthermore, studies demonstrated that fish intestinal microbiota other than *C. somerae* bacterium contribute a significant role in host metabolism via synthesizing several enzymes and co-enzymes nutrition by providing enzymatic activities corresponding to the fish host (Ray et al. 2012). As well, some bacteria species, *Microbacterium*, *Micrococcus*, unidentified anaerobes, and yeast have also affected the digestion of the host positively, since these and other microbiota were isolated, and identified from the intestine of fish and can produce a wide range of digestive enzymes including *amylase, protease, lipase, chitinase, cellulose,* and *phytase* (Ray et al. 2012). Although estimation of the contribution of the specific microorganism that colonizes the intestine of the fish is difficult, it is rational to think on the overall microenvironment would be strongly influenced by the predominant populations of microorganisms. To date, our knowledge of the function of a specific microorganism that colonizes the intestine of fish and its effect on the host was increasing due to the modernization and advancement of molecular approaches and new sequencing technologies.

4.1.2.1 The Contribution of the Intestinal Microbiota of Tilapia on Growth, Development Metabolism, and Immunity

The significant role of intestinal microbiota to host digestion, metabolism, and immunity was principally explained in humans (Fouhy et al. 2012) and then in economically valuable animal species including cattle (Li et al. 2019), pig (Jha and Berrocoso, 2015) and chicken (Brisbin et al. 2008). In the previous two decades with the advancement of molecular technologies, the function of intestinal microbiota fish in digestion and metabolism was studied. The intestinal microbiota of fish can break down nondigestible fibers and provides nutrients to the host, and metabolites of the microbes such as enzymes, vitamins, and short-chain fatty acids were absorbed and utilized by the host for growth and development.

In addition to growth and metabolism, the intestinal microbiota of fish can also control the gut-brain axis, innate immunity, and overall fitness of the fish. Intestinal microbiota contributes a significant role in the host health using mechanisms including the development and maintenance of the epithelial cell of the intestine, reducing the growth of pathogenic microbes, supporting the process of angiogenesis, and stimulating the expression of genes related to intestinal development and innate immune system. Besides, these intestinal beneficial bacteria compete for available nutrients with pathogenic bacteria and modify the ecological niche of the bacteria community to prevent the colonization and proliferation of arriving harmful microbes in the fish intestine (Kamada et al. 2013). Fukuda et al. (2012) demonstrated that the beneficial bacteria *Bifidobacterium* can inhibit the growth and invasion of pathogenic *Escherichia coli* using acidification of the fish's intestinal environs. Some other probiotic bacteria species can also synthesize bacteriocins (ribosomal synthesized antimicrobial peptides) and proteinaceous toxins that hinder the growth of members of the same or similar bacterial species. Hence, these and other studies provided evidence that the effect of pathogenic bacteria depended on the stability of the intestinal microbiota of the fish (Galindo-Villegas et al. 2012; He et al. 2017). However, the strong interaction of intestinal microbiota and host health can be affected via several factors, and disease was the main reason (Li et al. 2019; Nie et al. 2017) and mostly resulted in dysbiosis (imbalance of microbiota) of the microbes found in the intestine of the fish (He et al. 2017).

Currently, due to the advancement of molecular technologies, more investigation will require to understand the environmental interactions between members of the microorganisms found in the intestine and host intestinal microbiota interaction in terms of the function and importance of the intestinal microbiota on tilapia health. Development and improvements of techniques applied in genetics and molecular data analyses can provide detailed information on the ecological interaction of microbiota found in the intestinal of tilapia. Roh et al. (2010) used DNA microarrays to determine the regulation of the expression of microbial genes in different parts of the fish intestine. Furthermore, studies revealed that DGGE, TGGE, RNA-seq, and NGS have been used to quickly detection of much autochthonous intestinal microbiota of fish. Moreover, the development of digital transcriptomics techniques was applied to govern the expression level of several microbial genes (Ekblom and Galindo 2011).

4.1.3 Techniques of Modulation of the Intestinal Microbiota of Tilapia

It is clear that aquaculture contributes much to the world's food supply via aquatic products has been increasing these dates, and the main aim of aquaculture is to provide a high-quality product for human consumption. However, several factors negatively affect the production of tilapia in aquaculture, and the primary factor is diseases. Currently, several pathogenic bacteria such as *Streptococcus* sp. and *Francisella noatunensis* subsp. *orientalis*, was affecting the intestine microbiota and health of tilapia (Amal and Zamri-Saad 2011; Soto et al. 2013). These pathogenic bacteria symbolize a wide range of virulence and have different modes of infection. At present, for many bacteria diseases of tilapia, there is no effective treatment, and this results in huge economic loss in tilapia production. Researchers recommend using prevention methods and substitutions to antibiotics including probiotics, prebiotics, paraprobiotics, postbiotics, and feed additives. Understanding the functional significance of intestinal microbiota in many aspects of host physiology and modulation of this intestinal microorganism of fish is a reasonable approach to reducing the number of emerging infectious diseases in aquaculture. To attain this goal, a fundamental understanding of the interaction among fish health, beneficial intestinal microbiota, and pathogens microbes is a prerequisite. These clarifications focus on existing knowledge concerning the links between fish diseases, dysbiosis, and immune responses.

To accomplish the positive alteration of the intestinal microbiota of tilapia, researches used different types of modulators including prebiotics, probiotics, paraprobiotics, postbiotics, and optional feed ingredients in fish diets (Aguilar-Toalá et al. 2018; Wu et al. 2020). Due to the increasing number of fish pathogens, the application of "-biotics" an alternative means to antibiotics, has shown benefits in aquaculture. Improvement of the immunity of farmed fish species via the establishment of normal intestinal microbiota is important, as it affects various biological processes such development and assembly of gut-linked lymphoid tissue and the capability to compete for infectious disease caused by several microorganisms (Merrifield et al. 2011; Ringø et al. 2016).

Several studies indicated administration of probiotics plays a significant role in improving the intestinal microbiota balance and modulate the nonspecific immune system (Gibson & Roberfroid, 1995; Nayak 2010). Banerjee and Ray (2017) also demonstrated the importance of probiotics in the improvement of the immune response and resistance against infection. Furthermore, the probiotics administrated to the fish remain viable in the intestinal of the fish throughout the growth period, and this indicates that probiotics are important to the host via balancing the microbiota communities in the fish intestine (Li et al. 2019; Sayes et al. 2018). Other than probiotics, there are several "-biotics" which have a great contribution to the modulation of the gut microbiota and the improvement of the health of tilapia. Consequently, it is compulsory to start the incorporation of prebiotics, probiotics, or alternative feed ingredients to effectively modify the composition and diversity of the intestinal microbiota of tilapia.

4.1.3.1 Modulation by Watery Environments

The surrounding water contains several species of microbiota, and most of the time; the intestine of tilapia is colonized with these microbiota species at an early stage of growth. Studies revealed that biotic factors, abiotic factors including salinity, the trophic level of the host, and host phylogeny affect the composition and diversity of the intestinal microbiota of different fish species (Sullam et al. 2012). Connected with this, Del'duca et al. (2013) explained the type of different bacteria species isolated from the intestine of tilapia and demonstrated the similarity of the intestinal bacteria and the bacteria collected from the water and its sediments. These are valuable concerns in the experimental design during the exploration of tilapia intestine microorganisms. The type of aquaculture system and the water system are some of the factors that affect the intestinal microbiota of tilapia. Besides, rearing conditions also have a significant role in the intestine microbiota. Studies recommended that rearing conditions should be more similar to the stocking density and system design of the aquaculture since the environment has a great influence on the intestinal microbiota of the host.

Even though the composition of the intestinal microbiota of fish changes quickly at the time of transition from fry to fingerlings stages, many factors contribute to the change. The fish ingested the microbiota found in the water, contributes a lot to the modulation of the intestinal microbiota of the host (Giatsis et al. 2015; Del'duca et al. 2013). The different types of aquaculture systems affect the diversity and establishment of the intestinal microbiota of tilapia, which is explained how a type of aquaculture system (recirculating versus active suspension) influences the majority of the differences of microorganisms to colonize the intestine of the fish larvae (Giatsis et al. 2015). All these studies give evidence that the quality of the water used in the aquaculture system is the most important for the colonization of the gut microbiota of tilapia and is the primary indicator of host-microbial composition.

4.1.3.2 Modulation by Diet

Diet has a great potential to alter the fish intestinal microbiota (Ingerslev et al. 2014). A plethora of studies reported a strong link between the intestinal microbiota of fish with diet, yielding an understanding of the mutual interaction between some microbes and the host fish. These intestinal microbiota communities are also vital for several biological functions of the host including nutrition and metabolism by promoting nutrient uptake and utilization, increasing the production of enzymes, amino acids, short-chain fatty acids, and vitamins, and enhancing the digestion system of the fish (Merrifeld et al. 2010). Digestion enzymes such as carbohydrases, cellulase, phosphatases, esterases, lipases, and proteases were produced by the intestinal microbiota of fish which are responsible for the digestion of different types of diets (Ray et al. 2012; Wu et al. 2015). SCFAs are produced by gut microbiota from indigestible food components such as fiber, oligosaccharides, and polysaccharides via different metabolism channels (Layden et al. 2012; Tan et al. 2014). The SCFAs have a wide range of positive effects on the host, such as providing energy sources for colonic epithelium cells (Pascale et al. 2018), maintaining metabolic homeostasis (Canfora et al. 2015), regulating T regulatory cells (Smith et al. 2013; Furusawa et al. 2013), and anti-inflammatory effects (D'Souza et al. 2017; Ferrer-picón et al. 2019; Sun et al. 2018). Generally, they are indispensable for the growth and well-being of the host when present in sufficient amounts (Leblanc et al. 2017). Intimate relationships also exist between gut bacteria and teleost, and these gut bacteria have a role in supplying the host with volatile fatty acids (Ramirez and Dixon 2003) that provide energy for intestinal epithelial cells (Clements 1997). Furthermore, the gut microbiota of aquatic vertebrates can also produce vitamins and amino acids (Balcázar et al. 2006; Nayak 2010). Sugita et al. (1991) demonstrated that the amount of vitamin B_{12} was positively correlated with the abundance of *Bacteroides* and *Clostridium* in *Oreochromis niloticus*. Nile tilapia fed with a diet deficient in B12, was able to produce 11.2 ng vitamin B12/gram of body weight per day (Lovell and Limsuwan 1982). Tsuchiya et al. (2008) also demonstrated that strains of *Bacteroides* identified from the intestine of tilapia synthesized vitamin B12 at a rate of approximately 8 ng/mL culture in 48 hr.

Here, we discuss the modulation of the gut microbiota of tilapia using diet. Some studies revealed that the diet altered the intestinal microbiota of tilapia (Table 4.1).

4.1.3.3 Modulation by Prebiotics, Probiotics, Paraprobiotics, and Postbiotics

Currently, due to the increase in the number of reports on the negative effect of antibiotics, scientists have been working to develop alternative methods to antibiotics and examining the importance of the application of prophylactic measures such as probiotics, paraprobiotics, postbiotics, and prebiotics (Table 4.2). The word probiotics were defined by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) "as living microorganisms, which, once administered in appropriate amounts, confer a health profit on the host." With the advancement of molecular technologies and increasing our knowledge of the health benefits and their interaction with the host cells, the concept of paraprobiotics was proposed to indicate the use of inactivated microbial cells or cell fractions that confer a health benefit to the host (Taverniti and Guglielmetti 2011; Posadas et al. 2012). Moreover, postbiotics which are the soluble products or metabolites secreted by probiotics that have physiological benefits to the host as defined by (Aguilar-Toalá et al. 2018). The effect of the different types of "-biotics" on the modulation of the intestine microbiota has been reported (Nayak 2010; Wu et al. 2020). In this section, our focus is to review the ability of these compounds to modulate the function of the gut microbiota of tilapia and their role in host physiology and function through shifts in the gut microbiome. The role of different types of prebiotics and probiotics on growth, metabolism, composition, and diversity of gut microbiota communities, and the innate immune system of tilapia have been comprehensively studied and reviewed detailed these days (Goutam and Arun Kumar 2017). A plethora of studies confirmed that some probiotics such as *Bacillus* sp. and *Lactobacillus* sp. have a positive effect on the modulation of the gut microbiota of tilapia and their consequences on the host innate immune system and are recommended to provide an alternative strategy to governing many infectious diseases in aquaculture. The impact of live and dead probiotic cells on the nonspecific immune system of *O. niloticus* was investigated, and probiotics treatment increased the nonspecific immune parameters like lysozyme activity, migration of neutrophils, and plasma bacteriocidal activity, leading to improvement of resistance to *Edwardsiella tarda* infection (Taoka et al. 2006).

Manipulation of useful gut bacteria via improving the intestine of tilapia is one of the strategies applied in aquaculture. Studies reported these modulated intestinal microbes could exert positive effects on fish growth by altering the gut morphology, improving digestion and metabolism, and regulating the expression of microbial genes related to growth and appetite control (Rawls et al. 2004; Bates et al. 2006; Round and Mazmanian 2009). The role of intestinal microbes on the gut-brain axis was studied. Probiotic *Lactobacillus* bacteria within the intestinal microbiota of fish were associated with improved learning/memory capacity (Borrelli et al. 2016).

Table 4.1 Studies assessing the influence of diet on the microbiota and health of tilapia

Type of diet	Effect on gut microbiota	Effect on the host	References
Resveratrol	Increased the ratio of beneficial microbial taxa (*Acetobacteraceae* and Methylobacteriaceae), while the proportion of harmful microbial taxa decreased, e.g., Streptococcaceae deceased	Improve the growth and immunity of fish	Zheng et al. (2018)
Potassium diformate	Improved the relative richness of some intestinal allochthonous bacteria	Improved growth performance and feed conversion ratio	Zhou et al. (2009)
Garlic	Increased bacterial diversity, increase the abundance of *Proteobacteria* and *Tenericutes,* the phyla associated with healthy intestinal flora	Increase resistance against *streptococcus iniae*	Foysal et al. (2019)
Nucleotides	Moderate the intestinal microbiota	Improve the growth, feed utilization, intestinal growth, antioxidant status, and nonspecific immune response	Xu et al. (2015)
Betaine and the antibiotic florfenicol	Betaine can promote intestinal autochthonous bacteria	Improve immunity	He et al. (2012)
DL-methionyl-DL-methionine	Improve the diversity of gut microbiota	Increase growth performance and antioxidant ability	Guo et al. (2019)
Turmeric (Curcuma longa)	Total bacterial count and total lactobacillus count of intestinal content	Improve growth performance	Yusuf et al. (2017)
Low protein diet	Significantly decreased intestinal microbial diversity, *Bacteroidetes* were more abundant, and *Proteobacteria* and *Firmicutes* were less abundant	Restrict growth and weaken disease resistance	Zhu et al. (2020)
Sodium chloride	Increase in microbial diversity	Increased protein digestibility, dietary ions, and nutrient transporters	Hallali et al. (2018)

Administration of a combination of probiotic bacteria, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Aspergillus* to tilapia showed a strong association among these microbes in the host intestine (Iwashita et al. 2015). Besides, this study demonstrated that the intestinal microbiota-host association permitted the upregulation of immune response and reduced the mortality rate of tilapia against *A. hyrdophila* and *S. iniae* challenge. Another study performed using a commercial probiotic Aquastart® Growout (a combination of *B. subtilis*, *E. faecium*, *Lactobacillus reuteri,* and *Pediococcus acidilactici*) were applied to tilapia and resulted improved growth

Table 4.2 Studies evaluating the influence of probiotic microbes on the function and composition of intestinal microbiota and the health of tilapia

Type of prebiotics or probiotics	Effect on gut microbiota	Effect on the host	
Rummeliibacillus stabekisii	Increased the abundance of *bacillus* and *lactobacillus* spp. and reduced abundances of pathogenic bacteria (*streptococcus* and *staphylococcus* spp.) in intestines	Improved weight gain, feed conversion ratio, and feed efficiency, increased intestinal digestive enzymes; improved immunity	Tan et al. (2019)
Lactobacillus rhamnosus JCM1136 and *Lactococcus lactis* subsp. *lactis* JCM5805	Decease the abundance of *Plesiomonas* and increased *rhizobium* and *Achromobacter*	Improved immunity of the host	Xia et al. (2018a, b)
Saccharomyces cerevisiae or *Bacillus subtilis*	Significant higher number of yeast and b*acillus* spp. cells counts in the intestinal of fish fed on a diet supplemented with probiotics	Improved immunity of the host	Opiyo et al. (2019)
Pediococcus acidilactici	Increase the abundance of lactic acid bacteria	Enhanced some aspects of the nonspecific immune response	Ferguson et al. (2010)
Lactobacillus reuteri, B. subtilis, enterococcus faecium, and *Pediococcus acidilactici*	Improve the growth of beneficial bacteria	Enhanced intestinal morphology by elevating the absorptive surface area	Standen et al. (2015)
Lactobacillus plantarum CCFM8610	Modulate gut microbiota	Promote growth performance and prevent the death of fish exposed to cd	Zhai et al. (2017)
Lactobacillus plantarum	Improve the growth of beneficial gut bacteria	Improve immunity	Foysal et al. (2019)
Lactobacillus plantarum	Lactic acid bacteria were increased	Improved growth and feed efficiency	Jatoba et al. (2011)
B. subtilis	Modulate the gut microbiota	Improve growth performance	Giatsis et al. (2015)
Lactococcus lactis subsp. *lactis* JCM5805	Modulate gut microbiota	Upregulated the expression of *IFNα* via the *TLR7/TLR9-Myd88* pathway and enhanced disease resistance of larvae	Xia et al. (2018a, b)
Bacillus cereus	Affected the autochthonous gut bacteria community of tilapia and	Enhance the immune status	Wang et al. (2016)

(continued)

Table 4.2 (continued)

Type of prebiotics or probiotics	Effect on gut microbiota	Effect on the host	
	stimulated various potentially beneficial bacteria		
Lacillus subtilis C-3102	The increased amount of gut bacteria altered the autochthonous gut bacterial communities	Improve fish immunity	He et al. (2013)
Schizochytrium species	Enhanced the richness (represented by the Chao index) of bacteria and increase the abundance of the bacterial phylum *Firmicutes*	Improve structure and integrity of the intestinal villi Modulatory effects on blood cells	de Souza et al. (2020)
Lactococcus lactis JCM5805	Altered the composition of host gut microbiota	Improve metabolic pathways including carbohydrate metabolism, nucleic acid metabolism, energy metabolism, and translation	Xia et al. (2020)
Saccharomyces cerevisiae or *B. subtilis*	Improved the growth of beneficial intestinal bacteria	Improves immunity and enhances fish flesh quality	Opiyo et al. (2019)
Lactobacillus brevis JCM 1170 and *Lactobacillus acidophilus* JCM 1132	Altered gut microbiota	Improve the immunity of the fish	Liu et al. (2013)
Clostridium butyricum	Improved the increased diversity of the intestinal microbiota and the relative abundance of beneficial bacteria (*bacillus*). Reduced the relative abundance of opportunistic pathogenic bacteria (*Aeromonas*)	Improved the specific growth rate and feed intake and increased resistance against *S. agalactiae*	Li et al. (2019)
Clostridium butyricum	Improve the growth of beneficial bacteria	Promoting growth, feed utilization, gut health	Poolsawat et al. (2019)
Mannan-oligosaccharide	Modulate the intestinal microbiota by increasing the number of beneficial bacteria species	Improve immunity	Levy-Pereira et al. (2018)
Oligochitosan	Improve the growth of beneficial gut bacteria	Improve the growth, and immunity and enhance resistance against *A. hydrophila* infection	Shi et al. (2020)

(continued)

Table 4.2 (continued)

Type of prebiotics or probiotics	Effect on gut microbiota	Effect on the host	
Lactogen 13	Modulate gut microbiota communities by increasing the proportion of *lactobacillus*. Decreasing the proportion of potential pathogens	Improved the expression of genes responsible for growth and appetite control and activating the endocrine system	Giorgia et al. (2018)
β-Glucan	Increased the Chao richness and changed the composition of gut microbiota	Modulating plasma glucose concentrations, decreasing mortality, improving immunity	de Souza et al. (2020)
Chito-oligosaccharides	Changed autochthonous gut bacteria,	Improve intestinal health, and improve resistance to infection by *A. hydrophila*	Qin et al. (2014)
Inulin	Improve the composition of α-diversity, and β-diversity of gut microbiota	Improve growth and alleviate oxidative stress o	Zhou et al. (2020)

and enhanced intestinal immunological status (Standen et al. 2016). Administration of a mixed probiotic *Bacillus licheniformis* and a prebiotic, yeast extract can improve the growth and feed utilization of Nile tilapia (*O. niloticus*) (Hassaan et al. 2014). Likewise, the administration of a mixture of exogenous enzymes phytase, protease, and xylanase with a probiotic containing *B. subtilis*, *B. licheniformis*, and *Bacillus pumilus* results in enhanced growth and fiber breakdown (Melo-Boliver et al. 2019). Correspondingly, this combination of probiotics and prebiotics increased the digestion of indigestible nonstarch polysaccharides and trypsin inhibitors, which may produce necrotic enteritis in Nile tilapia (Adeoye et al. 2016). Some microbes such as *Enterococcus*, *Myroides,* and *Exiguobacterium* isolated from the intestine of Nile tilapia were improved the survival rate of the fish after challenged with *Edwardsiella tarda* via enhancing the innate immunity (Villamil Diaz and Esguerra Rodriguez 2017).

Probiotics *L. rhamnosus* GG can improve the host's intestinal microflora balance and enhance the immune system and disease resistance of tilapia after being challenged by *Edwardsiella tarda* and *Streptococcus agalactiae* (Pirarat et al. 2006; Lahti et al. 2013; Pirarat et al. 2015). In addition to several species of bacteria, some yeast species including *Saccharomyces cerevisiae, Kloeckera apiculata, Candida* sp., *Metcschnikowia* sp., and *Rhodotorula* sp., have been used as a probiotic for tilapia (Ayyat et al. 2014). Besides these and other species of yeast have immunostimulants and play a significant role in the growth of fish (Gatesoupe 2007).

4.1.4 Conclusions and Future Perspective

Tilapia is one of the most cultivable fish species in the world. In the previous three decades, studies on the intestinal microbiota of tilapia increased with the aim of optimum manipulation of these microorganisms. The intestine of tilapia was harbored by different species of microbiota, and the dominant bacteria phyla were *Proteobacteria, Firmicutes, Fusobacteria,* and *Bacteroidetes.* The intestinal microbiota has been affecting the host in more than one way. Considering the importance of these bacteria phyla and other microbiota species in the overall fitness of tilapia in aquaculture, scientists have been characterizing and developing many techniques to manipulate the intestinal microbiota to increase the production and productivity of the fish. Conversely, the detailed mechanisms of these intestinal microbiota modulators are required to be fully explored. Presently, several infectious diseases of tilapia have been treated using antibiotics. However, a plethora of studies revealed that antibiotics affect the dynamics of fish intestinal microbiota and mostly cause dysbiosis. Furthermore, increasing the application of antibiotics has fortified antibiotic resistance microbiota, and these results in a condition difficult to fight against pathogens (Kalia et al. 2014). To date, the advancement of "forward microbiomics" was played a great role in manipulating the intestinal microbiota to improve the growth and health of tilapia. Modulation of the intestinal microbiota of tilapia using different diets and "-biotics" have been applied. Most of the time, probiotic bacteria including *Lactobacilli* species, have been used in a number of fish species including tilapia (He et al. 2017). But to date, the function of each intestinal microbiota taxa and modulation of these beneficial microorganisms is not fully understood. The research highlighted above is an important starting point for optimally manipulating the beneficial microbiota of tilapia.

Like other fish species, the intestinal microbiota of tilapia was influenced by many factors; however, the effect of each of these factors on the growth, metabolism, immunity, and physiology of fish remains poorly understood. For drawing meaningful conclusions, this review summarized the importance of intestinal microbiota and the modulation methods of microbiome structure and function. Furthermore, to create a comparative picture of the intestinal microbiota of tilapia needs a careful review of the studies employed using the different advanced molecular technologies such as DNA microarray, next-generation, and third-generation is required to increase our understanding of the role of intestinal microbiota to increase the production and productivity of tilapia. To date, scientists have been working to manipulate the intestinal microbiota to use as biomarkers for the health status of the fish and to assess the detail to uncover functional variation with diet or host factors. Conversely, the detailed mechanisms of these intestinal microbiota modulators are required to be fully explored. This review highlighted collecting information on the characterization and function of intestinal microbiota on tilapia growth, development, and immunity and its modulation methods. Gathering information from different studies would help to identify and characterize the intestinal microbiota of tilapia to use as biomarkers for the status of the fish in aquaculture. As a result, more study is required on the characterization and identification of specific taxa of intestinal microbiota, and detailed work is necessary to investigate the fundamental association between tilapia fitness and the function of each intestinal microbiota, which may allow us to enhance our knowledge on the importance of intestinal microbiota on improving tilapia aquaculture.

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Chapter 5
Probiotics and their Application in Tilapia Culture

Hien Van Doan

Abstract Probiotics are live beneficial bacteria introduced into the gastrointestinal tract through food or water, promoting good health by enhancing the internal microbial balance. Probiotic microbes produce bacteriocins, siderophores, lysozymes, proteases, and hydrogen peroxides, inhibiting the growth of harmful pathogens. Such beneficial bacteria also produce many enzymes such as amylase enzyme by *Aeromonas* spp., *Bacillus subtilis,* Bacteridaceae*, Clostridium* spp., *Lactobacillus plantarum,* and *Staphylococcus* sp., and protease and cellulase enzymes by *B. subtilis*, *L. plantarum*, and *Staphylococcus* sp. In aquaculture, probiotics confer several benefits and play important roles in improving growth performances, disease resistance, immunity, health status, intestinal epithelial barrier integrity, gut microbiome, and water quality. In addition, the practical application of probiotics in aquaculture diets could minimize antibiotic side effects. Promoting these feed additives for fish would help to improve their product performance and feed utilization and, therefore, boost fish production and safeguard human health. This review provides updated information regarding definitions, sources of bacterial probiotics, probiotic use in fish diets against pathogenic bacteria, mechanisms of action, beneficial aspects, and potential applications of probiotics in fish. It is anticipated that these will be of significant value for nutritionists, agricultural engineers, researchers, pharmacists, scientists, pharmaceutical industries, and veterinarians.

5.1 Introduction

Aquaculture is one of the most important occupations, particularly in underdeveloped nations, as it not only helps to increase food security but also helps fish farmers to generate and enhance revenue (Dorji et al. 2022; El-Saadony et al. 2021). Moreover, the development of aquaculture has been necessitated by the exponential

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growth of the human population, the high demand for low-cost sources of proteins, and the decrease in fish capture from natural inland water bodies (Gebremedhin et al. 2021). As aquaculture is potentially the most promising activity for meeting the increasing global food demands, governments as well as development agencies have started to pay more attention to fish farming and aquaculture. The growth rate of aquaculture has been the fastest as compared to all other food production sectors, and thus it can play a vital role in combating malnutrition and increasing food security by supplying food to the ever-increasing population (FAO 2018, 2020).

The use of good husbandry techniques in aquaculture can decrease the stress faced by fish due to different reasons. However, this stress can never be eliminated and can eventually lead to an increased susceptibility of fish that are being produced to diseases and infections (Abdel-Latif et al. 2020; Eissa et al. 2018; Stevens et al. 2017). Vaccinations, biocontrol agents, chemicals as well as antibiotics are most commonly used to prevent or treat diseases in aquaculture (Assefa and Abunna 2018; Shao et al. 2021). However, in some countries, these treatments have either been restricted or prohibited, but many other countries are still widely using them (Shao et al. 2021; Guardone et al. 2022). As chemicals and antibiotics tend to bioaccumulate in the tissues of the fish, their use can pose a risk to the environment as well as consumers (Okocha et al. 2018). The potential development of antibiotic-resistant bacteria is another key concern related to the use of antibiotics in aquaculture (Pepi and Focardi 2021; Henriksson et al. 2018). Exposure to antibiotics at subtherapeutic doses for a long period can lead to the development of antimicrobial resistance in bacteria by allowing the plasmids or genes having antibiotic resistance to evolve (Zalewska et al. 2021; Amin et al. 2020). Moreover, these plasmids and genes can be horizontally passed on to other bacteria (Kent et al. 2020; Lerminiaux and Cameron 2019).

The transfer of antibiotic resistance genes and bacterial species from aquatic animals and environment to terrestrial animals and environment and consequently to humans and their environment, and vice versa, is very well known and has negative effects on the health of animals and humans as well as aquatic ecosystems (Santos and Ramos 2018; Koch et al. 2021). As aquaculture is at the crossroads of antimicrobial resistance, recent research has highlighted the importance of corrective and mitigative measures to protect the health of fish as well as humans (Reverter et al. 2020; Vaiyapuri et al. 2021). A large number of research studies have proved that there are many safe, economical, and ecologically appropriate supplements such as probiotics, prebiotics, and medicinal plants (Ghosh et al. 2021; Hernández-Contreras and Hernández 2020) as well as essential oils (Dawood et al. 2021) that can be added to feed and have significant benefits for the health of farmed fish (Butt et al. 2021; Rohani et al. 2021).

In this chapter, probiotics, their advantages for health, their sources, modes of action, safety concerns, categorization, applications, and side effects in the nutrition, production as well as the health of Nile tilapia are discussed.

5.2 Modes of Action

The most commonly known mechanisms of action of probiotics include (i) competitive exclusion by the production of inhibitory compounds, (ii) competition for energy, nutrients, or chemicals, (iii) competition for adhesion site, (iv) contribution to digestion, (v) contribution to macro- and micronutrients, (vi) augmentation of the immune response, and (vii) reduction of virulence by manipulation of quorum sensing (QS) (Balcázar et al. 2006; De Bidhan et al. 2014; Zorriehzahra et al. 2016; Noor et al. 2020; Dawood et al. 2020).

Probiotics being engaged in the competitive exclusion of pathogenic pathogens was one of the earliest theories to be put out (El-Saadony et al. 2021). It was thought that when beneficial microorganisms entered the host's digestive tract, they would either create inhibitory compounds or engage in competition with pathogens for adhesion sites, nutrients, chemicals, or energy sources, thus interfering with the growth or other activities of the pathogens (Verschuere et al. 2000; Decamp et al. 2008; Kuebutornye et al. 2020a, b, c). Incidentally, advantageous isolates from the intestines of both freshwater and marine species have shown antagonistic action against several fish and shellfish diseases. For instance, extracellular products of five potential probiotics isolated from common clownfish's (*Amphiprion percula)* stomach and intestine displayed possible inhibitory effects against *Carnobacterium piscicola, A. salmonicid, V. harveyi, A. hydrophila, V. anguillarum, V. damsela* and *V. alginolyticus* (Vine et al. 2004). *Saprolegnia* sp. was strongly inhibited by *Aeromonas* media obtained from rearing water used for eel culture (Lategan et al. 2004). Diffusible inhibitors against *E. tarda, V. parahaemolyticus, V. harveyi*, and *A. hydrophila* were discovered to be produced by *B. amyloliquefaciens* (Das et al. 2013).

Contrarily, substances that are known to have bactericidal or bacteriostatic effects such as hydrogen peroxide, lysozymes, bacteriocins, proteases, carbon dioxide, and siderophores, etc. Furthermore, the pH of the gut might change as a result of the generation of organic acids and volatile fatty acids (such as lactic, propionic, butyric, and acetic acids) (Tinh et al. 2008; Vine et al. 2006). By doing so, probiotics may prevent the growth of opportunistic pathogenic bacteria in vivo.

By competing for sites of attachment, some probiotic microbes can reduce the growth of pathogens on the surface of the gut (Dawood 2021). According to research by Chabrillón et al. (2005) lactic acid bacteria such as *L. lactis, L. plantarum*, and *L. fermentum*, respectively, reduced the adhesion of the fish pathogens *A. hydrophila, A. salmonicida, V. anguillarum*, and *Y. ruckeri* to the intestinal mucus of rainbow trout in vitro. The production of antimicrobial agents like antibiotics or siderophores may account for this anti-adhesion activity (Chabrillón et al. 2005).

Indeed, *Pseudomonas fluorescens*, a probiotic that produces siderophores, prevented the growth of *V. anguillarum* by competing for freely available iron (Gram et al. 1999; 2001). One of the most significant defenses against pathogen invasion by probiotics is their capacity to colonize the GIT by adhering to and proliferating in the mucus and epithelial cells (Amoah et al. 2019; Azad et al. 2019).

5.3 Probiotics in Tilapia

Probiotics work in a variety of ways to assist increases in aquaculture production, by encouraging improved growth (Silva et al. 2012), increasing feed consumption and nutrition (Zhou et al. 2010), lowering disease rates (Irianto and Austin 2002), and developing immunological responses (Nayak et al. 2007). Probiotics not only enhance the well-being of domesticated animals but also contribute to consumer safety (Rohani et al. 2021). The most commonly used probiotic bacterial strains include *Arthrobacter* sp., *Enterococcus* sp., *Bifidobacterium* sp., *Bacillus* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Streptomyces* sp., *Phaeobacter* sp., *Streptococcus* sp., *Microbacterium* sp., *Lactococcus* sp., *Micrococcus* sp., etc., yeast probiotics include cell wall of yeast, *Debrayomyces hansenii, Saccharomyces cerevisiae,* etc., micro-algal probiotics include *Spirulina platensis*, etc., and *Tetrasehnis suecica,* Bacteriophages probiotics include *Bacteriophages* sp. Table 5.1 lists the potential impacts of adding probiotics to aquafeeds on fish development, immunity, feed consumption, and disease resistance.

5.3.1 **Bacillus** ***Spp.***

Gram-positive, anaerobic *Bacillus* species are known to exhibit a variety of physiological traits (Jahangiri and Esteban 2018; England 2014). *Bacillus* species is one of the most commonly used probiotic organisms in aquaculture. They have also been discovered to be natural members of the intestinal microbiota of some fish species (Kuebutornye et al. 2020a, b, c). The addition of *Bacillus* species to the fish diet has increased growth performance, disease resistance, as well as resistance to infections from pathogenic bacteria in tilapia. According to Liu et al. (2017), Nile tilapia fed 10^8 cfu/g *B. subtilis* HAINUP40 which was isolated from aquatic ecosystems could improve disease resistance, growth performance, and immunological response. *B. subtilis* HAINUP40's capacity to release exoenzymes like protease and amylase may be responsible for the evaluation of these parameters. Additionally, *B. subtilis* HAINUP40 inclusion dramatically increased the activity of serum SOD and TAC, which may be related to its stronger microbial killing capacity of macrophages (Giri et al. 2013) and higher levels of antioxidant enzymes as well as antioxidants secreted in response to *Bacillus antigens* (Zhang et al. 2013). The addition of *B. subtilis* LT3–1 to the diet also dramatically improved weight gain and serum biochemical parameters. In addition, the hematocrit, total red and white blood cell counts, serum catalase, and lysozyme activity, were observed to be increased. While, the levels of serum complement 3, serum malondialdehyde, as well as immunoglobulin M, were

Table 5.1 Effects of probiotics on Nile tilapia

Bacillus subtilis HAINUP40	Aquatic environment	10^8 CFU/g	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ Intestinal probiotic recovery, digestive enzyme activities ↑ Innate immunity and disease resistance ↑	Liu et al. (2017)
Bacillus pumilus AQAHBS01	Farmed tilapia	10^7, 10^8 and 10^9 CFU/kg 60 days	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Resistance against *S. agalactiae* during the critical period of early	Srisapoome and Areechon (2017)
Bacillus cereus	Intestinal contents of tilapia	Experiment 1: 0, 1.0×10^4 and 1.0×10^5 CFU mL^{-1} Experiment 2: 0, 1.0×10^7 and 1.0×10^8 CFU g^{-1} (42 days)	Nile tilapia (*Oreochromis niloticus*)	Lysozyme and peroxidase activities ↑ Alkaline phosphatase and total superoxide dismutase activities ↑	Wang et al. (2017)
Bacillus licheniformis Dahb1		0; 10^5 and 10^7 CFU g^{-1} 4 weeks	Mozambique tilapia (*Oreochromis mossambicus*)	Final weight, WG, SGR FE ↑ FCR ↓ Total protein, alkaline phosphatase, myeloperoxidase, lysozyme, reactive oxygen species, reactive nitrogen species ↑	Gobi et al. (2018)
Bacillus paralicheniformis SO-1	Marine sediment samples	0, 5, 10 and 20 g/kg 50 days	Nile tilapia (*Oreochromis niloticus*)	Growth rates, digestive enzymes activities ↑ Immunological parameters ↑	Makled et al. (2019)
Bacillus licheniformis strain HGA8B	Commercial probiotic	10^6 and 10^8 CFU g^{-1} 60 days	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Hepatosomatic index, modulated digestive and antioxidant ↑ Enzyme activity ↑	Midhun et al. (2019)

(continued)

Table 5.1 (continued)

Bacillus velezensis LF01	Nile tilapia (*Oreochromis niloticus*)	$1,0 \times 10^9$ CFU/g 9 weeks	Nile tilapia (*Oreochromis niloticus*)	Lysozyme (LZY) and superoxide dismutase (SOD) activities ↑ Resistance against *Streptococcus agalactiae* ↑	Zhang et al. (2019b)
Bacillus subtilis LT3-1	Isolated from the gut of tilapia	0, 3.8×10^{10}, 7.6×10^{10}, 1.14×10^{11} and 1.52×10^{11} CFU kg^{-1} 6 weeks	Nile tilapia (*Oreochromis niloticus*)	FW, WG, and SGR ↑ Serum biochemical indices, haematocrit, the serum catalase and lysozyme activities, parameters for intestinal morphology ↑	Zhu et al. (2019)
Bacillus subtilis strains SB3086, SB3295, SB3615, or AP193	Commercial probiotic	4×10^7 CFU/g 21 days	Nile tilapia (*Oreochromis niloticus*)	Lysozyme activity ↑ Resistance against *Streptococcus iniae* ↑	Addo et al. (2017)
Bacillus strains	Commercial probiotic	(*Bacillus subtilis* 3.25×10^9 CFU/g, *Bacillus licheniformis* 3.50×10^9 CFU/g and *Bacillus pumilus* 3.25×10^9 CFU/g; Sanolife PRO-F, with a total number 1.0×10^{10} CFU/g)	Nile tilapia (*Oreochromis niloticus*)	FW, WG, and SGR ↑ FCR ↓ Intestinal lengths, anterior and terminal intestinal villi heights and anterior goblet cells ↑	Elsabagh et al. (2018)
Bacillus spp. (B. subtilis, B. megaterium and *B. licheniformis)*	Commercial probiotic	Add in water environment: 1×10^6 CFU g^{-1} 90 days	Nile tilapia (*Oreochromis niloticus*	FW, WG, and SGR ↑ Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ↓	Sutthi et al. (2018)
Bacillus velezensis TPS3N; *Bacillus subtilis* TPS4; *Bacillus amyloliquefaciens* TPS17	Gastrointestinal tract of Nile tilapia, *O. niloticus*	0, 1×10^8 CFU/ml (*B. velezensis* TPS3N), 1×10^8 CFU/ml (*B. subtilis* TPS4), 1×10^8 CFU/ml (*B. amyloliquefaciens* TPS17) and combination at a ratio of 1:1:1 with same concentration.	Nile tilapia (*Oreochromis niloticus*)	Mucosal immunity, intestinal health ↑ Proteobacteria ↑ Resistance of Nile tilapia against *A. hydrophila* ↑	Kuebutornye et al. (2020a, b, c)

Bacillus sp. KUAQ1 and *Bacillus sp.* KUAQ2,	Nile tilapia *Oreochromis niloticus*	0, 1×10^{8}, 3×10^{8} and 5×10^{8} CFU/g 8 weeks	Nile tilapia (*Oreochromis niloticus*)	Lysozyme, phagocytic, and respiratory burst activities ↑	Sookchaiyaporn et al. (2020)
Lactobacillus lactis D1813 (PowerLac™)	Commercial probiotic	For laboratory experiment: 0, 0.25, 0.5, 1.0 and 2.0 g kg^{-1} for 8 weeks For field experiments: 0.25, 0.5 and 1.0 g kg^{-1} for 22 weeks	Nile tilapia (*Oreochromis niloticus*)	FW, WG, and SGR ↑ FCR ↓ Resistance against *A. hydrophila* ↑	Suprayudi et al. (2017)
Lactobacillus delbrueckii	Commercial probiotic	0, 1×10^{5}, 1×10^{6}, 1×10^{7} and 1×10^{8} CFU g^{-1} 8 weeks	Nile tilapia (*Oreochromis niloticus*)	FW, WG, and SGR ↑ FCR ↓ Resistance against *A. hydrophila* ↑	Zhang et al. (2017)
Lactobacillus plantarum CCFM8661	Commercial probiotic	10^{8} CFU g^{-1} 4 weeks	Nile tilapia (*Oreochromis niloticus*)	Growth performance ↑ Prevented the death of Pb-exposed fish. Pb accumulation in kidney, liver, gonad, brain, gills and muscle ↓	Zhai et al. (2017a)
Lactobacillus plantarum CCFM8610		10^{8} CFU g^{-1} 4 weeks	Nile tilapia (*Oreochromis niloticus*	Growth performance ↑ Prevented the death of the Cd-exposed fish	Zhai et al. (2017a)
Lactococcus lactis subsp. *lactis* JCM5805	Commercial probiotic	0; 10^{4} and 10^{8} CFU ml^{-1} 15 days	Nile tilapia (*Oreochromis niloticus*)	Disease resistance Resistance against *Streptococcus agalactiae* ↑	Xia et al. (2019)
Bacillus subtilis and malic acid		1.1 1.1. × 10^{5} CFU/g 84 days	Nile tilapia (*Oreochromis niloticus*)	FBW, WG, SGR, PER, PPV and ER ↑ Haematocrit, haemoglobin, red blood cells, white blood cells, total protein, albumin and globulin ↑	Hassaan et al. (2018)

(continued)

Table 5.1 (continued)

Saccharomyces cerevisiae and *Bacillus subtilis*	Commercial probiotic	*S. cerevisiae* at 2; 4 and 6 g kg^{-1} *B. subtilis* at 5; 10 and 15 g kg^{-1} 7 months	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR ↑ FCR ↓ Body composition ↑	Opiyo et al. (2019)
Rummeliibacillus stabekisii	Commercial probiotic	10^6 and 10^7 CFU/g 8 weeks	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Innate immune response ↑ Resistance against *Streptococcus* and *Staphylococcus spp.*	Tan et al. (2019)
Paenibacillus ehimensis NPUST1	Water of local tilapia culture pools	0; 10^6 and 10^7 CFU/g	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Resistance against *Streptococcus iniae* and *A. hydrophila* ↑	Chen et al. (2019)
Traditional Chinese medicine (TCM), *Bacillus subtilis* and *Bacillus lincheniformis*	Commercial probiotic	0, (TCM at 3 and BS at 7 g/Kg), (TCM at 5 and BS at 5 g/kg), and (TCM at 7 and BS at 3 g/kg) 4 weeks	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Lysozyme, superoxide dismutase, catalase, protease and antiprotease activities ↑ Resistance against *Streptococcus agalactiae* ↑	Abarike et al. (2019a)
Lactobacillus plantarum N11 *Bacillus velezensis* H3.1	Nile tilapia (*Oreochromis niloticus*)	0, 10^7 $CFUg^{-1}$ (*B. velezensis* H3.1), 10^8 $CFUg^{-1}$ (*L. plantarum* N11), and 10^7 CFU g^{-1} (*B. velezensis* H3.1) + 10^8 CFU g^{-1} (*L.plantarum N11*).	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ Skin mucus lysozyme and peroxidase activities ↑ Serum lysozyme activity ↑	Van Doan et al. (2018)
Lactobacillus rhamnosus (LR) JCM1136 và *Lactococcus lactis* subsp. *Lactis* (LL) JCM5805	Commercial probiotic	10^8 CFU/g 6 weeks	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Gut microvilli length and microvilli density ↑ Resistance against *Streptococcus agalactiae* ↑	Xia et al. (2018)

Lactococcus garvieae	From raw cow mill	10^7 cells/g 10 days	Nile tilapia (*Oreochromis niloticus*)	Resistance against *Staphylococcus aureus* ↑	Abdelfatah et al. (2018)
Bacillus subtilis and *Lactococcus lactis*	Intestine of juvenile Japanese eel	*Bacillus subtilis* at 10^7 and at 10^8 CFU/g; *Lactococcus lactis* at 10^7 and at 10^8 CFU/g	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Lysozyme activity ↑ Superoxide dismutase and myeloperoxidase activity ↑ Gene expression ↑	Won et al. (2020)
Lactobacillus plantarum and *Bacillus velezensis*	Isolated from tilapia gut	10^7 and 10^8 CFU/g 30 days	Nile tilapia (*Oreochromis niloticus*)	FW, WG, and SGR ↑ FCR ↓ Resistance against *S. agalactiae* ↑	Van Doan et al. (2018)
Clostridium butyricum	Commercial probiotic	0, 0.5, 1, 2, 4 and 8 g/kg commercial probiotic (containing *C. butyricum* 1.5×10^8 CFU/g) 8 weeks	Tilapia (Oreochromis niloticus × O. aureus)	WG ↑ FCR ↓ Protein retention, lipid retention and apparent digestibility coefficient ↑ Intestinal *E. coli* ↓ Resistance against *A. hydrophila* ↑	Poolsawat et al. (2019)
Micrococcus MCCB 104 and *Bacillus* MCCB 101		10^3 CFU animal^{-1} day^{-1} 28 days.	Mozambique tilapia (*Oreochromis mossambicus*)	Intestinal digestive enzymes, intestinal alpha-amylase and total alkaline proteases ↑	Sankar et al. (2017)
Bacillus sp., Pediococcus sp., Enterococcus sp., Lactobacillus sp.	Commercial probiotic	0; 1.0×10^6; 2.3×10^6 CFU/g 8 weeks	Nile tilapia (*Oreochromis niloticus*	FW, WG, and SGR ↑ Villi height and goblet cell counts ↑	Ramos et al. (2017)
B. subtilis and *B. licheniformis* with traditional Chinese medicine	Commercial probiotic	0, 3, 5, 7 and 10 gkg^{-1} 4 weeks	Nile tilapia (*Oreochromis niloticus*)	Final weight, WG, SGR FE ↑ FCR ↓ Lysozyme, protease, antiprotease, superoxide dismutase activities, and immunoglobulin M level ↑	Abarike et al. (2019b)

all observed to be decreased. Fish-fed diets enriched with *B. subtilis* appeared to have healthier intestines than fish fed the control diet, according to parameters for intestinal morphology. In tilapia-fed *B. subtilis*, the survival rate following *Streptococcus agalactiae* exposure increased (Zhu et al. 2019). In tilapia production, *Bacillus licheniformis* has been used as a functional feed supplement. According to Gobi et al. (2018), feeding tilapia (*O. mossambicus*) with *B. licheniformis* Dahb1 significantly improved growth rate, immunological parameters, feed conversion ratio, as well as antioxidant parameters in serum and mucus. *B. licheniformis* HGA8B can considerably improve growth parameters including feed conversion ratio, weight gain, specific growth rate, and hepato-somatic index when added to the diet, according to Midhun et al. (2019). The probiotic preparation adjusted the activity of the digestive and antioxidant enzymes. In the liver tissue, there was a significant modulation of growth-related genes such as the growth hormone receptor genes (GHR-1 and GHR-2) and the insulin-like growth factor genes (IGF-1 and IGF-2). IGF-1 gene expression was markedly elevated in the muscular tissue. Significant immune-related gene upregulation, as evidenced by an increase in TLR-2 and anti-inflammatory cytokines such as IL-10, was seen. *B. licheniformis* secreted digestive enzymes, which were found to be the cause of fish growth and health promotion. Furthermore, the presence of *B. licheniformis* can influence the expression of regional growth factors (IGF-1 and IGF-2) and the receptors (GHR-1 and GHR-2) that bind growth factors and hormones (growth hormone and steroid hormones) to promote greater growth. A new potential probiotic for Nile tilapia is *Bacillus pumilus* AQAHBS01. Srisapoome and Areechon (2017) have suggested that in both laboratory and field culture conditions, the addition of *B. pumilus* at 1×10^8 and 10^9 CFU/kg to diet could boost immune response as well as resistance against streptococcosis. An important topic that needs to be discussed is the mechanism that *Bacillus pumilus* uses to improve fish health and disease resistance. The possible mechanisms by which *B. pumilus* may shield fish from harmful bacterial infection include boosting superoxide anion generation, phagocytic activity, and the number of essential leukocytes. Theoretically, *B. pumilus* may possess pro-inflammatory tumor necrosis factor, bacteriocin-like activity, powerful anti-spore IgG titers, and complement activity against other Bacillus species (Duc et al. 2004; Sun et al. 2010), all of which may significantly increase the survival rate of experimental fish fed with *B. pumilus* (Aly et al. 2008a, b). Unknown are other *B. pumilus* mechanisms of activity, such as the colony and adhesive capacity inside and outside the fish body. *B. pumilus* has not been used much lately in Nile tilapia. Compared to various earlier findings using this probiotic species, there have been only minor improvements in growth properties. A study by Aly et al. (2008a, b) is the only one to demonstrate the potential value of *B. pumilus* as a probiotic for enhancing Nile tilapia growth. In a different study using *Bacillus cereus* isolated from tilapia's stomach, Wang et al. (2017) found that *B. cereus*, when added to water or feed, might improve tilapia's immunological status and have an impact on their gut microbiota. When used as a feed supplement as opposed to a water additive, *Bacillus cereus* was more successful at boosting tilapia's immune system. Similarly, Makled et al. (2019) observed that Nile tilapia were given *B. paralicheniformis* SO-1 isolated from marine habitats greatly increased growth rates, digestive enzyme activities, and immunological parameters with increasing supplementary SO-1 up to 10 g/kg. However, when the bacterial load was increased further to 20 g/kg, fish performance and immune response were significantly reduced. When Nile tilapia were fed SO-1-treated diets, the expression of the IL-1, IL-4, and IL-12 genes was considerably increased. Based on the results of this investigation, *B. paralicheniformis* SO-1 can be recommended as an effective probiotic growth promoter and immune system stimulator for farmed Nile tilapia. According to Zhang et al. (2019a, b), growth performances were observed to be significantly improved in tilapia-fed diets supplemented with LF01 (1.0×10^9 CFU/g) as compared to the control group. Lysozyme (LZY) as well as superoxide dismutase (SOD) activity was also considerably elevated in tilapia-fed diets supplemented with LF01. The gills, gut, and head kidney of tilapia from the continuous feeding group had higher expression levels of three immune-related genes (C3, lyzc, and MHC-II) as compared to the control group. After being infected with *S. agalactiae*, the survival rates of tilapia-fed diets supplemented with LF01 were noticeably higher, and an examination of the gut pathogens showed that the number of Edwardsiella as well as *Plesiomonas* had decreased considerably as compared to the control group. The effect of *Bacillus altitudinis* B61-34b (BAA) isolated from the intestine of Nile tilapia, on immunological response, growth, as well as disease resistance against *Streptococcus agalactiae* has recently been studied (Van Doan et al. 2021a, b, c). The results proved that BAA treatment increased skin mucus as well as peroxidase activity in comparison to the control, with maximal values found in BAA4 and BAA5. In comparison to the control, BAA1, BAA2, and BAA3 dietary groups, higher serum immunities, including serum lysozyme, serum peroxidase, complement, phagocytosis, and respiratory burst activities, were seen in the BAA4 and BAA5 dietary groups. The BAA4 and BAA5 groups also showed considerably greater relative percentages of survival, growth performance, and food conversion ratio (FCR). The authors hypothesized that nutritional supplementation with *B. altitudinis* at 108 CFU ml-1 can enhance Nile tilapia's disease resistance, growth performance, skin mucus as well as serum immunity. Numerous mechanisms have been suggested to achieve these improvements. Increased growth performance is a direct outcome of increased intake of feed and utilization of nutrients that have been digested (Dawood 2021). By improving feed digestion through the production of digestive enzymes, probiotics boost feed intake as well as utilization and make the nutrients that have been digested available for metabolic processes for the body (Wang et al. 2020). Beneficial bacterial cells increase the intestinal villi's surface area for absorption of digested nutrients, protect the villus from harmful microorganisms, and boost transcription of IGFI and inhibition of myostatin (Zaineldin et al. 2021). The starch-degrading enzymes known as α-Amylases, which are released by the bacteria *Bacillus altitudinis* play a critical role in digestion by catalyzing the hydrolysis of internal −1,4-o-glycosidic linkages in polysaccharides (Kumar et al. 2014). Furthermore, *B. altitudinis* can produce pectinase and xylanase enzymes simultaneously (Thite et al. 2020). Although the exact mechanism by which probiotics increase mucosal immunity is unknown, the improvement in mucosal immunity of fish that result is likely because of the impact of derivatives of beneficial bacteria on local intestine immunity (Guardiola et al. 2017). According to Caipang and Lazado (2015), probiotic cell derivatives are likely to penetrate via the local epithelial immune cells and into the stream of systemic lymphoid tissues, where they work to increase innate immunity. Probiotics' metabolic impact on the feed composition, which results in functional nutrients being available, is likely responsible for the increased systemic immunity that is responsible for the increased serum immunity (Terpou et al. 2019). Indirectly competing with pathogenic invaders, probiotics prevent their ability to decrease the immunity of intestine (Dawood and Koshio 2020). In this way, compounds which are derivatives of probiotics migrate via intestinal enterocytes, enter the blood circulation, and ultimately end up in the immunological organs (Kelly and Salinas 2017; Zhang et al. 2019a, b).

5.3.2 Lactobacillus *Spp.*

According to Zhai et al. (2017a, b), adding *Lactobacillus plantarum* CCFM8661 to feed improved the growth performance and kept fish exposed to Pb from dying. When Pb and CCFM8661 were combined in the treatment, Pb buildup in the kidney, liver, muscles, brain, gonads, and gills was found to be significantly reduced. Additionally, administering this probiotic reduced oxidative stress induced by Pb, recovered the activities of blood δ-aminolevulinic acid dehydratase as well as digestive enzymes, reversed changes to innate immune status, and reduced the occurrence of nuclear abnormalities in fish peripheral blood erythrocytes. These findings suggested that, at least in tilapia, CCFM8661 might be a potential dietary supplement against toxicity caused by Pb. The *L. plantarum* CCFM8661 probiotic used in this study has been shown to efficiently bind Pb in vitro, and its theoretical monolayer biosorption capacity (*Qmax*) has been calculated to be 86.96 mg g^{-1} dry biomass, outperforming some commercial lactobacilli like *L. casei* Shirota (70.40 mg g^{-1}) as well as *L. rhamnosus* GG (46.80 mg g^{-1}) (Yin et al. 2016). Because of this, the intestinal CCFM8661 may bind such released Pb before it gets reabsorbed from the intestines, increasing the excretion of Pb through fish feces and decreasing its intestinal absorption (Ringø et al. 2020). The significant reduction in the level of Pb in tissues of the Pb-plus-CCFM8661 group could be explained by these findings. A higher number of this probiotic colonizing in the gut of fish after a longer feeding duration, providing further beneficial effects against Pb toxicity, may also be the cause of the significant protection on improving growth performance, reducing tissue oxidative stress, and regulating innate immune responses in fish after supplementation for 2 months. Similar to this, Zhai et al. (2017a, b) found that adding *L. plantarum* CCFM8610 to the diet reversed the alterations in the composition of gut microbiota in the fish exposed to Cd and also decreased the abundance of *Flavobacterium* as well as *Pseudomonas*. The probiotic treatment greatly improved growth performance and stopped the death of the fish exposed to Cd as compared to the Cd-only group. Supplementing with *L. plantarum* CCFM8610 also reduced Cd accumulation, reduced oxidative stress in tissues, and reversed changes in hemato-biochemical parameters in fish blood. Suprayudi et al. (2017) used *Lactobacillus lactis* D1813 at three different levels, that is, 0.25, 0.5, 1.0, and 2.0 g kg^{-1} in feed as compared to a control group having 0 g kg^{-1}, in both laboratory and field conditions. They found that treatments with dietary probiotic supplements of 0.25 and 0.5 g kg^{-1} significantly enhanced the final body weight of fish, increased growth, and lowered food conversion ratio (FCR) compared to controls (0 g kg^{-1}) in laboratory conditions. In field conditions, higher growth, retention of proteins and lipids, lowered FCR, and reduction in death rate by *Aeromonas hydrophila* challenge was observed by dietary inclusion of 0.5 g kg^{-1}. The beneficial effects of *L. lactis* D1813 on Nile tilapia's growth, as well as feed conversion ratio, may be explained by the probiotics' well-established benefits for the targeted species, including improvements in feed digestibility (Dawood et al. 2015) and uptake of nutrients and their utilization through (1) the involvement of digestive enzymes (Yanbo and Zirong 2006; Suzer et al. 2008), (2) changes in gut microbiota (Yang et al. 2014), (3) contribution on the development of morphology of the digestive tract (an increase in microvilli) that works to increase the surface area of the intestine allowing for the uptake of nutrients (Frouël et al. 2008), and (4) increase in the activity of enzymes that are related to the utilization of nutrients in intestines, such as alkaline phosphatase and leucine aminopeptidase which are the enzymes involved in the absorption of nutrients in the intestinal brush border (Sáenz de Rodrigáñez et al. 2009; Panigrahi et al. 2010). Probiotics have been shown to promote antibacterial activity (Aly et al. 2008a, b), enhance phagocytic and respiratory burst activity (Pirarat et al. 2006), and stimulate complement activity (Wang et al. 2008) as part of their ability to stimulate innate as well as adaptive immune system responses. Different fish can produce more lymphocytes, erythrocytes, macrophages, and granulocytes as a result of some probiotics, and the levels of B-lymphocyte proliferation as well as immunoglobulin levels can also be actively stimulated (Nayak 2010). To improve immunological responses, probiotics interact with immune cells like natural killer (NK) cells, mononuclear phagocytic cells (monocytes, macrophages), and polymorphonuclear leucocytes (neutrophils) (Nayak 2010; Selim and Reda 2015). Similarly, supplementing the diet of *Cyprinus carpio* with a commercial probiotic (*L. delbrueckii*) considerably improved the growth performance, disease resistance against *A. hydrophila*, and immunity of the fish (Zhang et al. 2017).

According to recent research by Van Doan et al. (2021a, b, c) Nile tilapia given *Lactobacillus paracasei* l61-27b dramatically increased rate of growth, serum immunities, feed conversion ratio, disease resistance, and skin mucus. The immunostimulant factor associated with probiotics can be due to the influence of derivatives of beneficial bacteria which can reach mucosal tissues like the skin and systemic immune organs like the spleen and trigger their response (Lazado and Caipang 2014). Additionally, probiotics improve the digestion of functional nutrients like vitamins, which strengthen the local gut immunity and in turn strengthen the systemic immune system (Pérez-Sánchez et al. 2014). Another theory that probiotics have an immunostimulant effect on the body is that they reduce the impact of pathogenic microorganisms in fish intestines, which causes a pronounced effect of good probiotics on intestinal immune cells (Akhter et al. 2015).

5.3.3 Lactococcus *Sp.*

According to Xia et al. (2019), the probiotic strain JCM5805 from *Lactococcus lactis* subsp. *lactis* increased the expression of IFNα through the TLR7/TLR9-Myd88 pathway and improved larval disease resistance. Since JCM5805 was only transiently identified, it was excluded from the stable microbiota of the larva. Tilapia larvae's early exposure to microbes has an impact on the gut microbiome later in life. Further research is necessary to determine whether the upregulation of associated genes is connected to the presence of JCM5805 strain in the intestine. Abdelfatah and Mahboub (2018) reported that fish fed with a feed having dietary inclusion of *L. garvieae* isolated from raw cow milk exhibited no signs of sickness and showed a greater survival rate as compared to others. According to Xia et al. (2018), the dietary addition of *Lactobacillus rhamnosus* (LR) JCM1136 and *Lactococcus lactis* subsp. *lactis* (LL) JCM5805, as a feed additive at 108 CFU/g feed, may enhance the immune status and disease resistance, affect tilapia's gut microbiota, and improve its intestinal morphology. These additives may therefore be used as probiotics for juvenile Nile tilapia. When it came to improving growth, immunological function, and increasing disease resistance, JCM5805 was found to be more beneficial than JCM1136 or the two combined. The different effects of probiotics on the growth performance of tilapia can be attributed to variations in the antibiosis activities of particular strains of probiotics as well as the interactions between probiotics, diet, the host, conditions of research, as well as handling practices, among other factors. These different factors may have influenced the results, which in turn affected the success or failure of probiotics and their combinations to improve growth in each study. The growth performance and feed utilization of tilapia in this study were not impacted by feeding diets made by the combination of JCM1136 and JCM5805 or JCM1136 singly containing diets, in contrast to the effect of feeding JCM5805 singly.

5.3.4 Rummeliibacillus Stabekisii

Rummeliibacillus stabekisii was suggested as the type species for the genus *Rummeliibacillus*, and its entire genome sequence was revealed (Vaishampayan et al. 2009; Da Mota et al. 2016). The categorization of three *Rummeliibacillus* species, including *R. stabekisii, R. suwonensis,* and *R. pycnus*, has only been discussed in two publications, and no application studies have been published. In the current study, *Rummeliibacillus stabekisii* was isolated from the stomach of Nile tilapia, and its effects on the fish's growth, resistance to disease, immunity, and gut microbiota were examined. According to the findings, Nile tilapia's growth, immunity, disease resistance, and gut microflora were all enhanced by dietary supplementation with *R. stabekisii*. According to this study, *R. stabekisii* can be added to feed to help Nile tilapia grow and maintain good health. This is the first study to propose that the genus *Rummeliibacillus* may be a promising probiotic in animals (Tan et al. 2019). The improvement of the analyzed parameters in this study has been attributed to several different mechanisms. The mechanism of *R. stabekisii* growth improvement in fish may have been due to enhanced nutrient utilization through the secretion of protease- and xylanase enzymes and stimulation of intestinal digestive enzyme synthesis after *R. stabekisii* treatment. The mechanism by which *R. stabekisii* enhanced fish immunity may have been due to an increase in lysozyme, phagocytic, and respiratory burst activity as well as the release of cytokines (IL-1, TNF-α, and transforming growth factor (TGF)). Additionally, *R. stabekisii* can influence the intestinal microbiota of Nile tilapia, which helps with disease resistance, immunomodulation, and growth promotion.

5.3.5 Paenibacillus Ehimensis

Due to their shared morphological and physiological traits with the type species *Bacillus subtilis, Paenibacillus* species were first included under the *Bacillus* genus. *Paenibacillus* was given a new genus in 1993 based on phylogenetic research using 16S rRNA gene sequences. There are numerous habitats important to humans, animals, as well as plants where the genus *Paenibacillus* has been identified. Numerous *Paenbacillus* species produce bacteriocins, which are proteins or antimicrobial peptides that are used as pesticides in biocontrol or medicine (Abdel-Latif et al. 2020). In the current investigation, the impact of *P. ehimensis* NPUST1 supplementation on Nile tilapia (*Oreochromis niloticus*) growth and immunity against pathogenic infection was assessed (Chen et al. 2019).

5.3.6 Clostridium Butyricum

Clostridium butyricum is an endospore-forming, gram-positive, and obligate anaerobic bacterium (Liu et al. 2018; 2019). It can create short-chain fatty acids (SCFAs) like butyric acid to offer nutrients for the growth of microbes and sustain epithelial cells in the gut (Junghare et al. 2012). Additionally, *C. butyricum* can resist some antibiotics, thrive in low-acidity and high-temperature environments, and protect fish from infections (Gao et al. 2013; Zhang et al. 2014). In aquaculture, *C. butyricum* has been shown to improve immune responses in *Miichthys miiuy* (Pan et al. 2008), promote growth performance in silver pomfret (Gao et al. 2016), improve nutrient digestibility in hybrid grouper (He et al. 2017), and increase the activity of antioxidants in the intestine as well as the content of short-chain fatty acid in kuruma shrimp (Duan et al. 2017; 2018). In tilapia, Poolsawat et al. (2020) demonstrated that the C-2, C-3, and C-4 groups saw considerably higher weight gain and lower feed conversion ratios. Except for the C-1 group, probiotic supplementation groups showed a considerable increase in the retention of proteins, lipids, and apparent digestibility coefficient of dry matter (ADC); improvement was also observed in the ADC of proteins in the C-4 group. Probiotic supplementation greatly enhanced anterior intestine villus height and decreased intestinal *Escherichia coli* counts. High-throughput sequencing revealed that the top three phyla had higher levels than the NC group, including *Planctomycetes* in all groups containing probiotics, *Proteobacteria* in the C-1 and C-2 groups, and *Chloroflexi* in the C-3 group. After the challenge with *Aeromonas hydrophila*, dietary probiotics helped to lower the overall mortality. To conclude, *C. butyricum* can be added to feed at a rate of 1–2 g/kg to support tilapia's growth, intestinal health, feed utilization, and gut microbiota.

The beneficial effects of dietary *C. butyricum* on feed consumption and growth performance may be related to the production of short-chain fatty acids (SCFAs) in the gut, such as butyric acid, acetic acid, and propionic acid. The production of these acids, especially butyric acid, is stimulated by the presence of this bacteria in the gut, which is an essential source of energy for the proliferation of epithelial cells (Junghare et al. 2012; Van Doan et al. 2018). To lower the intracellular pH, butyric acid can release protons into the neutral cytoplasm. The gut microbiota may then use the leftover protons to retain cells that have a lower mortality rate during metabolism (Duan et al. 2017). Butyric acid may also improve feed utilization by achieving balance in the micro-ecological environment of the stomach (Liang et al. 2021). The immunological indices, as well as disease resistance, were improved because probiotics can boost antibiotic substances as SCFAs in the stomach to support the antioxidative enzyme activities and reduce reactive oxygen metabolites, which improves feed utilization, growth, and overall health of the host (Liao et al. 2015a, b). Probiotics can also boost mucus production, which inhibits the adherence of pathogens and increases the competitive exclusion of pathogens from the spaces as well as food present in the gut (Monteagudo-Mera et al. 2019). The increased intestinal morphology caused by probiotic dietary supplementation may be related to the germination as well as the growth of probiotic cells in the gut that can produce gastrointestinal peptides and raise intestinal epithelial barriers and villus height (Gāliņa et al. 2020). Increased intestinal absorption surface area can help the host's nutrient digestion and absorption, producing favorable impacts on growth performance and feed utilization (Reda and Selim 2015).

5.3.7 Combination of Probiotics

It has been shown that combining different Bacillus species improves tilapia's growth and health. According to Addo et al. (2017), Nile tilapia fed four different strains of *Bacillus subtilis* (SB3086, SB3295, SB3615, and AP193) and a mixture of

SB3086 and SB3615 did not show any differences in growth performance. However, results from serum bactericidal and lysozyme activities demonstrated a substantial difference between treatments and the control, except for the diet modified by SB3295. The challenge's results also revealed a sizable distinction between treatments and controls. Fish fed with strain SB3615 had the lowest death rate (44.0 7.2%), while fish fed the control diet had the highest. Since the fish were grown under ideal conditions in the experiment, there may not have been any discernible growth improvement in this study. Due to the essential and unique metabolic and trophic roles played by the intestinal microbiota, the results may have been affected by the colony of the gastrointestinal (GI) tract that was present (Denev et al. 2009). According to Gutowska et al. (2004), the bacterial flora in fishes' gastrointestinal tracts generally has a very significant and varied enzyme potential with the ability to produce lipolytic, amylolytic, proteolytic, cellulolytic, and chitinolytic enzymes. These enzymes are crucial for the digestion of lipids, proteins, cellulose, carbohydrates, and chitin to promote growth. Several mechanisms have been put forth to explain the impact of probiotics on disease resistance in fish. Competition for nutrition and energy sources, generation of secondary metabolites with bactericidal effects on other microbial populations, competition for adhesion sites on the epithelium of the intestine or other tissue surfaces, and improvement of the host immune response are a few of these. The ability to cling to intestinal wall surfaces and enteric mucus is necessary for probiotic bacteria to establish themselves in fish intestines (Lesel 1990; Westerdahl et al. 1991) because bacterial attachment to tissue surface is crucial during the early phases of pathogenic infection (La Ragione and Woodward 2003; La Ragione et al. 2004).

Montes (1993) postulated that competition with pathogens for adhesion receptors would be a fundamental probiotic trait. When given in sufficient doses, probiotics improve the host's health, according to FAO/WHO (2001). The probiotic diets used in this study likely provided some health benefits to the fish because the survival rate of Nile tilapia when challenged with *S. iniae* infection was found to be much higher than the control diet (Addo et al. 2017). Elsabagh et al. (2018) discovered that feeding farmed Nile tilapia a diet containing certain Bacillus strains (*Bacillus pumilus* 3.25×10^9 CFU/g; *Bacillus licheniformis* 3.50×10^9 CFU/g; *Bacillus subtilis* 3.25×10^9 CFU/g, and Sanolife PRO-F, INVE Aquaculture, Belgium, with a total number 1.0×10^{10} CFU/g) improved the stress responses, gut health and function, growth, immunity, and water quality of farmed Nile tilapia. *Bacilli* can invade the guts of fish, enhancing the generation of organic acids, activating digestive enzymes, and detoxifying hazardous feed ingredients. This all helps to maintain a healthy gut, which then improves nutritional digestibility and absorption (Adeoye et al. 2016; Silva et al. 2015). Additionally, it has been shown that *Bacillus* can remove harmful bacteria from the stomach, increasing disease resistance and enhancing fish performance (Addo et al. 2017; Hostins et al. 2017; Srisapoome and Areechon 2017) Sutthi et al. (2018) also reported that fish reared in water treated with probiotic *Bacillus* spp. and yeast (*S. cerevisiae*) showed a synergistic effect that significantly improved growth performances such as average daily growth gain (ADG), final body weight (FBW) and weight gain (WG) as compared to the control group having lower alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Malondialdehyde (MDA) levels did not alter significantly between any of the treatments. The findings indicated that adding *S. cerevisiae* or *Bacillus* spp. to water as a probiotic improved Nile tilapia growth performance. This strategy can be used to boost production of tilapia in the Thai aquaculture sector. A recent study by Kuebutornye et al. (2020a, b, c) suggested that adding host-associated probiotics to Nile tilapia's diet, such as *B. velezensis* TPS3N, *B. subtilis* TPS4, and *B. amyloliquefaciens* TPS17, either separately or in combination, could improve the fish's mucosal immunity, gut health and resistance against infection by *A. hydrophila.* According to the authors, the greater mucosal immune parameters seen in the CB group may be the result of the three *Bacillus* species' synergistic effects (Kuebutornye et al. 2020a, b, c). Additionally, *Bacillus* species added to the diet of Nile tilapia may improve the health of the fish by positively influencing its gut microbiota. Similarly, Sookchaiyaporn et al. (2020) found that the dietary inclusion of two potential probiotics, *Bacillus* sp. KUAQ1 and *Bacillus* sp. KUAQ2, isolated from the intestine of Nile tilapia *Oreochromis niloticus*, could be beneficial in terms of disease control as well as the stimulation of the immune response of cultured tilapia. In their study, Ramos et al. (2017) sought to determine the effects of a blend of probiotics (*Lactobacillus* sp., *Pediococcus* sp., *Bacillus* sp., *Enterococcus* sp.) given to juvenile Nile tilapia (*Oreochromis niloticus*) on growth performance, innate immunity, feed utilization, and oxidative stress. The findings showed that after a long time of supplementation, no benefit of probiotic feeding was seen. A beneficial nutritional strategy for sustainable tilapia aquaculture may be the dietary supplementation of mixed species probiotics. In a competitive and expanding industry like aquaculture, outcomes like improved immune responses and intestinal morphology are crucial for boosting growth performance, nutritional absorption, and disease resistance in fish. Similar to this, Nile tilapia have been shown to have improved growth performance, immunological responses, digestive enzymes, and disease resistance when given the dietary strains *Bacillus* MCCB 101 and *Micrococcus* MCCB 104 or *Bacillus velezensis* H3.1 and *Lactobacillus plantarum* N11 (Sankar et al. 2017; Van Doan et al. 2018). An eight-week feeding trial was carried out to assess the effects of a basal control diet (CON), *Lactococcus lactis* at 10^7 CFU/g (LL7) and at 10^8 CFU/g diet (LL8), *Bacillus subtilis* at 10^7 (BS7) and at 10^8 CFU/g diet (BS8), and oxytetracycline (OTC) at 4 g/kg diet on Nile tilapia, by Won et al. (2020). The findings demonstrated that fish given the BS8, LL8, and LL7 diets experienced considerably greater feed efficiency, weight increase, lysozyme activity, specific growth rate, and protein efficiency ratio, than fish fed the CON diet. Fish fed the BS8, LL8, BS7, LL7, and OTC diets had considerably higher superoxide dismutase and myeloperoxidase activities than fish fed the CON diet. Fish fed the BS8, LL8, and LL7 diets had much longer intestinal villi and thicker muscle layers than fish fed the CON and OTC diets. Additionally, fish fed the BS8 and LL8 diets had considerably higher levels of interferon-gamma (IFN-γ), heat shock protein 70 (HSP70), interleukin (IL-1 β), and tumor necrosis factor (TNF-α) gene expression than fish fed the CON diet. Following a 13-day challenge test, fish fed the BS8 and LL8 diets had a cumulative survival rate that was considerably greater than fish fed the CON, BS7, and OTC diets. These findings suggest that *B. subtilis* and *L. lactis* at 10^8 (CFU/g) could substitute antibiotics and have positive effects on Nile tilapia growth, histology, immunology, gene expression, and disease resistance. The increased intestinal histology and enzyme activity of Nile tilapia fed probiotic diets may be the cause of their superior growth performance and feed utilization. By enhancing intestinal villi length, trypsin activity, and muscle layer thickness, probiotics may have promoted efficient food absorption. The improvement of phagocytic activity and reactive oxygen metabolites by macrophages is one of the causes of increased immunity. Recent research by Van Doan et al. (2021a, b, c) demonstrated that a mixture of Bacillus species (*B. licheniformis* DF001, *Bacillus subtilis* TISTR001, and *B. megaterium* TISTR067) increased Nile tilapia growth, immunological response, and disease resistance.

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Chapter 6
Prebiotics and Synbiotics in Nile Tilapia Culture

Hien Van Doan

Abstract With the increase in the human population, the need for protein-rich food is increasing. To cope with the increased demand for fish and fish-related products, aquaculture is shifted towards semi-intensive and intensive culture practices. These cultivation practices exacerbate disease outbreaks. Farmers use extensive antibiotics to manage disease outbreaks and lessen the economic impact due to fish mortality. Inappropriate and extensive use of antibiotics results in the development of resistant strains and the presence of antibiotic residues in fish muscles pose a serious threat to food safety for human consumption. Water and dietary supplements that can be effectively used as alternatives to antibiotics are an active area of research. Prebiotics and synbiotics are promising candidates to replace antibiotics and have been used for the treatment and control of pathogens in tilapia aquaculture. In this chapter, the use of prebiotics and synbiotics as growth promoters and as alternatives to antibiotics in tilapia aquaculture has been addressed, along with their possible mechanism of action. Moreover, the positive effects of pre and synbiotics on tilapia health are also highlighted with emphases on growth, immune modulation, and alteration in gut microflora.

Keywords Prebiotic · Gut · Microbiota · Oligosaccharide · Fermentation

6.1 Introduction

Aquaculture is one of the fastest food-producing sectors that can provide protein-rich food to the growing human population (Fiorella et al. 2021; Edwards et al. 2019; Butt et al. 2021). In 2020, fisheries and aquaculture production reached a record of 214 million tons, worth about USD 424 billion (FAO 2022). Tilapia, a member of the Cichlid family, is the second most commonly farmed fish worldwide (Prabu et al. 2019), with a production of 5.584.4 million tons (FAO 2022). Production of tilapia is

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projected to increase to 7.3 MT by 2030 (FAO 2022; Kobayashi et al. 2015). Currently, tilapia is grown and traded commercially in more than 140 nations worldwide (Munguti et al. 2022). Tilapia is a great choice for warm-water aquaculture because of its many advantages, such as high growth rate, well-adapted, tolerance to stress and diseases, high fertility, rapid rate of generation, acceptance of artificial feeds right away following yolk-sac absorption and capacity to feed at a low trophic level (Ng and Romano 2013). To enhance tilapia production and financial returns, there is currently a paradigm shift away from an extensive aquaculture approach toward super intensive system due to its socioeconomic significance (Henriksson et al. 2021). However, super-intensive aquacultural practices have led to over-reliance on commercial feed, environmental contamination from the release of nutrient-rich water into the environment, and disease outbreaks are all consequences of these aquaculture operations (Abdel-Latif et al. 2020a). One of the biggest issues facing aquaculture growers today is a drop in production caused by pathogens. The most prevalent bacterial infections in tilapia that significantly reduce its yield are *Aeromonas hydrophila*, *Streptococcus iniae*, and *Streptococcus agalactiae* (Eissa et al. 2021; Gewaily et al. 2021). As a result, throughout the past few decades, disease outbreaks have been managed by using antibiotics and chemotherapeutics (Rico et al. 2014). However, their unauthorized use has significantly altered the fish's microbiota, which may harm their immunity (Kokou et al. 2020; Payne et al. 2021). Additionally, the unrestricted use of antibiotics in aquaculture has resulted in the development of antibiotic-resistant bacteria and their metabolites, which have the potential to contaminate aquaculture products intended for human consumption (Henriksson et al. 2018; Miranda et al. 2018). Consuming such tainted aquaculture products could alter human gut microbiota, which could impact human health (Cabello and Godfrey 2016; Henriksson et al. 2018). Since the use of antibiotics in aquaculture has recently been prohibited, research on the use of prebiotics and synbiotics against pathogenic bacteria has come to the fore as one of the most effective environmentally friendly and sustainable alternatives to control bacterial outbreaks in aquaculture and to enhance fish growth and immunity (Wee et al. 2022; Yilmaz et al. 2022; Rohani et al. 2022). In light of the importance of tilapia production, this chapter provides an in-depth analysis of the use of prebiotics and synbiotics in tilapia aquaculture while highlighting the growth, immunological parameters, and disease resistance utilized in evaluating fish health following their application.

6.1.1 Prebiotics

Gibson and Roberfroid were the first to introduce the idea of prebiotics, which they described as "an indigestible fiber that can enhance the growth and activity of health-promoting bacteria in the intestine and beneficially affect the host" (Gibson and Roberfroid 1995). Similarly, Bindels et al. (2015a) described prebiotics as "non-digestible food ingredients that are broken down into simpler substances, enhancing the growth and activity of preferred microorganisms found in the gastrointestinal tract and benefiting the health of the host". Guerreiro et al. (2017) presented them as “useful food ingredients that have numerous favorable effects on the host”. Due to the development of antibiotic-resistant strains, the appearance of drug residues in the host, changes in the microbial community of the gut and the culture system, and lowered immunity, the use of antibiotics in aquaculture have recently come under heavy fire (Watts et al. 2017; Ibrahim et al. 2020; Henriksson et al. 2018). The manufacture, preservation, and pelleting processes for the probiotic-based feed could prevent probiotics from surviving or growing. The efficiency of the feed may be decreased if it is processed in an improper or unfavorable environment (Lauzon et al. 2014a). Prebiotics has so recently gained popularity as a probiotic and antibiotic substitute. Numerous studies and remarks have been published highlighting the advantages of prebiotics in aquaculture (Guerreiro et al. 2017; Akhter et al. 2015; Cavalcante et al. 2020; Nawaz et al. 2018). Prebiotics that are widely used in tilapia farming includes β-glucan, inulin, fructooligosaccharides (FOS), short-chain fructooligosaccharides (scFOS), mannan oligosaccharides (MOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), pectin, raffinose, and chitosan. Depending on how much they are polymerized into monosaccharides, oligosaccharides, and polysaccharides, certain carbohydrates are also categorized as prebiotics (Ringø et al. 2010b).

Short-chain fatty acids (SCFAs) and lactate can be produced by the metabolizing of prebiotics by the helpful bacteria *Lactobacillus* and *Bifidobacterium* (Markowiak-Kopeć and Śliżewska 2020; Davani-Davari et al. 2019). While lactate serves as a stimulant for gluconeogenesis, SCFAs can also be absorbed through the digestive system to serve as an energy source (Denev et al. 2009). The colon's pH is decreased by the formation of SCFAs, which slows the spread of infections and promotes the growth of lactic acid-producing bacteria like *Bifidobacterium* (Markowiak-Kopeć and Śliżewska 2020; Parada Venegas et al. 2019; Tiwari et al. 2019; Nagpal et al. 2018). Prebiotics work similarly by competing with glycoconjugates on the epithelial lining to boost SCFAs, cytokines, and mucus production while decreasing the number of pathogenic bacteria (Lauzon et al. 2014a; Hoseinifar et al. 2017a). They have an advantageous impact on the host's growth characteristics, including ultimate weight, weight increase, daily weight gain, specific growth rate, dietary conversion ratio, feed efficiency ratio, and protein efficiency ratio (Mohammadian et al. 2021; Nazarudin et al. 2020; Butt et al. 2021; Hahor et al. 2019; Jami et al. 2019).

Prebiotics added to the diet can help boost the activity of digestive enzymes. Digestive enzyme levels were found to be higher in animals that gained weight and had better feed conversion efficiency (Nedaei et al. 2019; Abasubong et al. 2019; Li et al. 2019; El Basuini et al. 2020). However, no sustained improvements were seen because prebiotics was also identified in certain investigations to act ineffectively (Hoseinifar et al. 2015a; Eshaghzadeh et al. 2015; Dobšíková et al. 2013). By triggering the non-specific immune system and fostering healthy microorganisms, prebiotics can improve immunity even further (Hoseinifar et al. 2015a; Song et al. 2014). The favorable microbiota enhances immunity in the host, protects against dangerous bacteria in the colon, and maintains a healthy balance between the microbiota of the digestive and immune systems (Gomez and Balcazar 2008). To qualify as possible prebiotics, prebiotic candidates must meet the following requirements in both *in vivo* and *in vitro* studies: they must be able to (i) withstand stomach and abdominal acid, (ii) start a variety of enzymatic reactions and processes, (iii) improve intestinal digesting, (iv) encouragement of microbial fermentation, and (v) encourage the growth of beneficial microorganisms that can improve health status (Bindels et al. 2015b; Schrezenmeir and de Vrese 2001). Yet not all prebiotics meet these requirements (Lauzon et al. 2014a).

6.1.2 Synbiotics

The word "synbiotic" is a mixture of two Greek terms "συν" and "βίος", meaning "joint or together" and "life" respectively (Kolida and Gibson 2011). A dietary supplement known as a "synbiotic" beneficially combines probiotics and prebiotics. A substance that modifies the opportunistic bacterial community of the host species' digestive system to enable probiotics to thrive and survive there (Chauhan and Chorawala 2012; Das et al. 2017). Prebiotics are chosen for synbiotic formulation based on their capacity to subtly encourage the growth and survival of advantageous bacteria within their desired range (Davani-Davari et al. 2019). Probiotics are selected based on how they specifically benefit the host (Hill et al. 2014). The selection of probiotics, on the other hand, is based on their overall advantages for the host, whereas the selection of prebiotics is based on their potential to stimulate the growth of probiotic bacteria as their primary function (Kolida and Gibson 2011; Markowiak and Śliżewska 2017). The synergistic effects of synbiotics have been the subject of most recent investigations, which have been emphasized exclusively (Torrecillas et al. 2018; Cavalcante et al. 2020; Sewaka et al. 2019; Hasan et al. 2018; Kumar et al. 2018; Villumsen et al. 2020).

The prebiotics' mode of action in the host species mostly determines how synbiotics are composed (Zepeda-Hernández et al. 2021; Li et al. 2020). Prebiotics that are low in polymerization, however, is better suited for the generation of synbiotics than those that are high in polymerization (Grimoud et al. 2010), since they hydrolyze the developed beneficial strains and produce primary and secondary metabolites that may have favorable effects on the host (Mazzola et al. 2015). Additionally, the administration of synbiotics is more beneficial than probiotic use alone due to increased microbial survival when combined with prebiotics and vice versa (Merrifield et al. 2010). However, prebiotics offers greater tolerance to large variations in oxygen, temperature, and pH (Cunningham et al. 2021). As a beneficial substitute for Nile tilapia, the following research has demonstrated the usefulness of synbiotics. Further evidence of its efficiency in fish farming comes from the greater stimulation of host species' growth and survival. This happens through the production of pancreatic enzymes, which improve enzymatic digestion, modify intestinal microbiota, and lowers mortality rates by altering potential immune responses, and antagonistic species of harmful organisms.

6.2 Modes of Action

Prebiotics' mode of action has been suggested by several in recent years, but the paths by which they function in aquaculture are not fully understood. Prebiotics' application in tilapia farming has received a lot of recent attention from aquaculturists (Zhou et al. 2020; Poolsawat et al. 2021; Koch et al. 2021; Wan-Mohtar et al. 2021; Souza et al. 2020; Mohammadi et al. 2020). Prebiotics has been shown to boost tilapia's resistance to infections (Poolsawat et al. 2021; Koch et al. 2021; Pilarski et al. 2017), immune system activation and regulation (Koch et al. 2021; Mohammadi et al. 2020), intestinal microbiota augmentation, regulation, and modification (Poolsawat et al. 2021; Zhou et al. 2020; Souza et al. 2020), enhancement of the host species' capacity for development and survival (Pilarski et al. 2017; Poolsawat et al. 2021), antioxidant activity (Abdel-Latif et al. 2020b; Dawood et al. 2020c), improvements in enzyme activity (Poolsawat et al. 2021; Zhou et al. 2020), and opposed to toxicity (Abdelhamid et al. 2020).

The use of probiotics and prebiotics in tilapia farming has been explored independently, while the idea of synbiotics has only lately come into existence (Mugwanya et al. 2022). Since being used in tilapia, synbiotics have proven a variety of action mechanisms. According to Laice et al. (2021), synbiotics can increase production and improve the sustainability of Nile tilapia. *Nile tilapia* fed a combination of probiotic *DBA®, MOS, and chitosan*, led to the enhancement of growth and disease resistance against *Aeromonas hydrophila* (Cavalcante et al. 2020). They can also multiply immunological functions including lysozyme activity and boost resistance to bacterial infection (Sewaka et al. 2019). Additionally, it has been discovered that the tilapia gut has greater digestive enzyme activity, which has led to enhanced growth (Hassaan et al. 2020).

6.3 Application of Prebiotics in Tilapia

6.3.1 Prebiotics as Growth Promoters and Disease Protection

The aquaculture industry with the goal is to achieve maximum production with minimum cost. Prebiotics has been used as an effective tool to obtain this goal. In the past decades, many studies have been conducted to access the effects of different prebiotics on Nile tilapia growth and feed utilization.

6.3.1.1 β-glucan

β-glucan has been used as a growth promoter and disease prevention in fish. In Nile tilapia, Sherif and Mahfouz (2019) reported that dietary incorporation of β-glucans at 0.5 g.kg^{-1} feed for 2 weeks significantly increased relative level protection against

Aeromonas hydrophila. Recently, Souza et al. (2020) indicated that feeding Nile tilapia with β-glucan led to a higher survival rate and disease resistance against the *Vibrionaceae* family. More recently, Nile tilapia supplemented with β-glucan at 0.1% significantly enhanced growth and disease resistance against both *Aeromonas sobria* and *Streptococcus agalactiae* strains (Koch et al. 2021). Similarly, significant enhancement of growth, feed conversion ratio, villus length, villus width, goblet cell count, and disease resistance were observed in Nile tilapia fed β-glucan (Chirapongsatonkul et al. 2019; Dawood et al. 2020e). The beneficial effects of β-glucan on the gut flora may provide a credible explanation for growth enhancement (Meena et al. 2013). β-glucans are non-digestible feed components that function as prebiotics, enhancing the health of the host by promoting the growth and/or activity of specific gut bacteria (Miest et al. 2016; Ghaedi et al. 2016).

6.3.1.2 Fructooligosaccharide (FOS)

FOS has been applied in aquaculture as a functional supplement to improve farmed fish growth and health status (Rohani et al. 2022). Supplementation of FOS could improve Nile tilapia's growth performance (Abd El-Gawad et al. 2016b). However, no significant differences in growth rates were observed in fish-fed scFOS (Liu et al. 2017b). In contrast, gut adherence microbiota and disease resistance was significantly increased in fish-fed scFOS (Liu et al. 2017b). The authors hypothesized that dietary scFOS would have a selective promotion on various Lactobacillus strains in tilapia because it enhanced the relative abundance of *L. brevies*, *L. plantarum*, and *L. rhamnosus*. Although the exact mode of action is unknown, FOS's benefits for growth performance may be related to better digestion, immunity, and intestinal homeostasis maintenance through manipulation of the intestinal microbiota (Hu et al. 2019; Markowiak and Śliżewska 2017).

6.3.1.3 Mannanoligosaccharide (MOS)

MOS is one of the popular prebiotics used in aquaculture. Two studies have been conducted on Nile tilapia. Yuji-Sado et al. (2015) revealed that MOS-supplemented diets significantly enhanced intestinal fold height; whereas no impacts on growth performance were observed. In contrast, fish given MOS showed higher growth and survival rates as well as a lower FCR than CPF-intoxicated fish (Dawood et al. 2020c). The influence of MOS on the GIT microbial community, which can cause beneficial bacteria to generate digestive enzymes, determines how effective feed utilization will be (Jami et al. 2019; Mohammadian et al. 2019). Digestive enzymes disassemble the nutrients so that they are readily available within the GIT, improving their rates of absorption (Dawood et al. 2019a). Since the rate of nutrient digestion and absorption increased, it is anticipated that fish will develop faster and be in better overall health (Dawood et al. 2020b).

6.3.1.4 Inulin

In terms of final weight, weight increase, specific growth rate, and feed conversion ratio, dietary inclusion of inulin at 5 g kg^{-1} improved growth responses and feed utilization efficiency (Tiengtam et al. 2015b). Supplementation of 5 g kg^{-1} inulin led to greater intestinal villi height, larger relative goblet cells, increased lactic acid bacteria, and *Bifidobacterium* spp., but decreased *Vibrio* spp. (Boonanuntanasarn et al. 2018). Likewise, the administration of inulin at 0.4% resulted in a better growth rate and feed conversion ratio (Zhou et al. 2020). Fish administered a higher dose of inulin (0.8%) did not exhibit a higher impact on their ability to grow. Similarly, fish fed with diets enriched with 3% inulin exhibited poorer weight gains than fish fed with basal, 1%, and 2% inulin (Reza et al. 2009). Arctic char *Salvelinus alpinus* suffered negative effects from high supplementation levels of inulin (15%) (Olsen et al. 2001). These adverse effects of higher inulin supplementation may be attributed to the intestinal microbiota's inability to ferment excessive amounts of inulin, and extra inulin that builds up in the gut may be harmful to the enterocytes (Olsen et al. 2001; Hunt et al. 2019), forcing Nile tilapia to expend energy on inulin detoxification rather than using it to benefit the host.

6.3.1.5 Xylooligosaccharides (XOS)

Doan et al. (2018) reported that dietary inclusion of corncob-derived XOS significantly improved the growth performance and survival rate of Nile tilapia against *Streptococcus agalactiae.* Similar results were observed in tilapia fed 1-2 g kg^{-1} feed, where weight gain, nutrient retention, feed conversion ratio, protease, amylase, villus height, muscular thickness, numbers of lactic acid bacteria, and *Bacillus* were significantly enhanced. Furthermore, dietary XOS dramatically decreased tilapia's overall mortality following an *Aeromonas hydrophila* injection (Poolsawat et al. 2021). Although the precise mechanism by which XOS dramatically enhances fish development performance is yet unknown, it may be because XOS serves as a prebiotic and immunostimulant. Additionally, studies on dietary XOS have shown positive effects on gut lactic acid bacteria (LAB) and an increase in the activity of the amylase and protease enzymes (Hoseinifar et al. 2014; Xu et al. 2009). The role of LAB in enhancing growth performance, controlling pathogens by the competitive exclusion of adhesion sites, and producing SCFA, hydrogen peroxide, antibiotics, bacteriocins, siderophores, and lysozyme are all well documented (Hoseinifar et al. 2015a; Xia et al. 2018; Li et al. 2018). LAB also affects the fish's physiological and immune responses (Hoseinifar et al. 2015a; Nayak 2010; Merrifield et al. 2014; Ringø et al. 2010a). On the other hand, the metabolites acetic, lactic, propionic, and butyric acid are increased when prebiotics are supplemented (Ríos-Covián et al. 2016; Hoseinifar et al. 2017b). Further research on tilapia and CDXOS is warranted since, like in vertebrates, these SCFAs may benefit the immune cells in the gut-associated lymphoid tissue. (Bach Knudsen et al. 2003; Hoseinifar et al.

2015a). SCFAs are also taken up by the host and used as an energy source, with butyrate serving as the primary fuel for the colonic wall (den Besten et al. 2013). The dietary XOS, on the other hand, was shown in certain experiments to have just a small impact on the growth performance of white shrimp (Wang et al. 2010; Sun et al. 2019), Caspian white fish (*Rutilus frisii kutum*) (Hoseinifar et al. 2014), white sea bream (Guerreiro et al. 2016), and seabream (*Sparidentex hasta*) juvenile (Morshedi et al. 2018). Intestinal microbiota, administration techniques, diet composition, dosage, and species may all have an impact on how effective XOS is at promoting growth (Hoseinifar et al. 2016a).

6.3.1.6 Other Prebiotics

According to Abu-Elala et al. (2015), dietary regimens containing 1% chitosan have significantly boosted resistance to *Aeromonas hydrophila* and enhanced water quality. This could be a result of chitosan's activating impact on phagocytic cells, which causes them to discharge their mediators, including lysosomal enzymes, cationic peptides, complement components, and reactive oxygen species, all of which have antimicrobial effects against fish infections (Sajid et al. 2010; Niu et al. 2011). At a dosage of 0.1%, chitosan exhibited bactericidal effects that were generally more potent against gram-positive bacteria than gram-negative bacteria (No et al. 2002). According to some theories, its capacity to improve the permeability of the outer membrane of Gram-negative bacteria is what causes it to have an antibacterial effect (Chirkov 2002; Bégin and Van Calsteren 1999). By presumably competing with Ca^{++} for electronegative positions on the membrane without providing dimensional stability, the positive charges of chitosan disrupt the negatively charged residues of macromolecules at the surface of bacterial cells, making the membrane permeable (Bégin and Van Calsteren 1999). According to Meng et al. (2017) study employing chitooligosaccharides (COS), supplementing with 0.4 and 0.8 g kg^{-1} COS considerably improved growth performance, with the best value seen in fish fed 0.4 g kg^{-1} diet. COS could positively enhance growth rate because of its ability to improve feed intake (Tang et al. 2005), nutrient digestibility (Chen et al. 2009; Wang et al. 2009), growth hormone, and IGF-I concentrations (Tang et al. 2005).

Doan et al. (2018) conducted a study using pectin obtained from orange peels and found that Nile tilapia had greater growth metrics and were more resistant to disease from *Streptococcus agalactiae*. This improvement may be the outcome of improved digestive function brought on by the prevalence of beneficial bacteria in the gut (Wang An et al. 2017; Zhigang et al. 2013). Ho et al. (2017) demonstrated that pectin can be fermented by gut bacteria and highlighted its potential for application as a novel prebiotic. Additionally, positive effects on the gut microbiota and an increase in the activity of digestive enzymes including amylase, lipase, and protease (Dawood and Koshio 2016; Eshaghzadeh et al. 2015; Hoseinifar et al. 2016b; Kühlwein et al. 2014) or liver enzyme activities (Zhang et al. 2013; Hoseinifar et al. 2015a), along with improved appetite, increased vitamins, dissection of indigestible elements, and optimizing gut morphology (Irianto and Austin 2002; Hoseinifar et al. 2015c) have been recorded after consuming prebiotics. The fish's innate immune system was activated after being treated with pectin, which explains why resistance was significantly increased. Pectin may be a potential component for preventing diseases in both humans and animals, according to some research (Lattimer and Haub 2010; Wen et al. 2022; Wang et al. 2022).

Dietary inclusion of raffinose and pistachio hull-derived polysaccharide (PHDP) also led to better growth rates and disease resistance to *Aeromonas hydrophila* (Abdel-Latif et al. 2020b; Mohammadi et al. 2020). Raffinose greatly increased the growth rates of tilapia, which is likely due to the induced feed efficiency, as seen by the fish's low FCR. Raffinose causes the intestinal microbiota to secrete digesting enzymes, which improves fish's GIT's ability to absorb nutrients (Berrocoso et al. 2017). Because PHDP serves as a prebiotic and promotes the formation of healthy probiotic flora, it has positive benefits on growth rate and disease resistance (Akbari-Alavijeh et al. 2018; Li et al. 2019), or powerful antibacterial activities of polyphenols, which are implicated in bacterial cell breakdown through their lipophilic characteristics (Stratev et al. 2018).

6.3.1.7 Combination of Prebiotics

A combination of prebiotics with each other or with other bioactive compounds could improve the growth performance and disease resistance of Nile tilapia. Dietary inclusion of β-glucan with MOS led to better growth performance and disease resistance to *L. gravieae*, *A. hydrophila*, and *S. iniae* (Abu-Elala et al. 2018; Chen et al. 2019). The manipulation of MOS on intestinal microbiota may be a factor in the underpinning mechanisms of the current growth results. Increased villus integrity, improved digestion and absorption (Fernandez et al. 2002; Dimitroglou et al. 2009), and the production of energy and building protein from β-glucan degraded by glucanases in the digestive gland were all outcomes of MOS (Misra et al. 2006). The Nile tilapia's growth performance significantly improved when fed β-glucan and *Chlorella vulgaris* (Abdelhamid et al. 2020). This outcome could be attributable to CV high-quality protein, which increases fish weight gain and final weight (Kang et al. 2013). Additionally, CV contains growth-promoting ingredients including S-nucleotide adenosyl peptide complex, which could alter an animal's ability to grow and digest the food (Han et al. 2002). The distinct, varied macro- and micronutrients found in CV include proteins, polysaccharides, -carotene, -carotene, minerals, vitamins, pro-vitamins, chlorophyll, and lutein (Panahi et al. 2016). Additionally, our findings demonstrated that β-glucan enhanced growth efficiency following DZN toxicity. This increase in β-glucan's growth efficiency may be caused by the glucanase's likely breakdown of the glucan, which encourages the transfer of additional proteins for growth. Furthermore, β-glucan enhanced the release of digestive enzymes, which may have contributed to the improvement of growth (Guzmán-Villanueva et al. 2014). Similar to this, combining β-glucan with either propylene glycol or *Aspergillus oryzae* dramatically improved Nile tilapia growth performance and disease resistance (Abd El Tawab et al. 2016; Dawood et al. 2020a).

6.3.2 Prebiotics as Immunomodulatory

6.3.2.1 β-glucan

In teleost fish, β-glucans' ability to modulate immunity has been extensively explored (Petit et al. 2019). β-glucan treatment frequently has an immune-stimulating impact and can lead to an increase in resistance to both viral and bacterial illnesses, regardless of the administration route or fish species (Dalmo and Bogwald 2008; Meena et al. 2013; Petit and Wiegertjes 2016). There have been documented distinct mechanisms for β-glucan identification and/or downstream signaling in both mammalian vertebrates (Brown and Williams 2009; Legentil et al. 2015) and invertebrates (Cerenius et al. 2009; Soltanian et al. 2009), specific pathways for β-glucan identification and/or downstream signaling have been identified. Although β-glucans are frequently used in aquaculture, the precise processes underlying the produced effects for teleost fish are poorly understood (Petit et al. 2019).

Sado et al. (2016) indicated that Nile tilapia fed β-glucan for 15 days significantly enhanced serum lysozyme, leukocyte count, and neutrophil number. In another study, Sherif and Mahfouz (2019) reported that dietary inclusion of β-glucan at 0.5 g kg^{-1} feed for 2 weeks significantly improved the fish immune status both innate and adapted immune response. Similarly, dietary incorporation of β-glucan from mushroom and yeast at 10 μg per fish significantly up-regulated relative immune gene expressions (Chirapongsatonkul et al. 2019). Dawood et al. (2020e) also revealed that dietary inclusion of β-glucan significantly increased lysozyme and phagocytic activities, and up-regulated INF-γ, TNF-α, IL-1β, and HSP70 gene expressions. In a recent study, Koch et al. (2021) also reported that a β-glucan incorporated diet resulted in higher lysozyme, respiratory burst, and myeloperoxidase activities of Nile tilapia. However, no effects of β-glucan on serum lysozyme activity of Nile tilapia were found (Souza et al. 2020). β-glucan is well known for its capacity to stimulate the complement system, reactive oxygen metabolite formation, and phagocytic cells' ability to create antimicrobial compounds like lysosomal enzymes (Ji et al. 2017; Goodridge et al. 2009). Additionally, Engstad (1993) noted that salmon possessed BG receptors on its phagocyte membrane and that these receptors could be influenced by BG supplementation. The interaction between helpful microorganism cells and intestinal epithelial cells, which is thought to improve mucosal immunity in the host gut and tighten the epithelial junction, produce antimicrobial peptides and mucosal immunoglobulin, control inflammatory response, and subsequently stimulate the immune response, is responsible for BG's immunomodulating activity (Raa 2015).

6.3.2.2 Fructooligosaccharide (FOS)

Numerous edible plants, including onions and edible burdock, contain fructooligosaccharides (FOS), which are created commercially when fructosyltransferase reacts with sucrose (Mitsuoka et al. 1987). FOS is an example of a prebiotic, which are foods that pass undigested by pancreatic and small intestine enzymes in the human gut and instead reaches the colon, where they have advantageous effects. According to reports, dietary FOS affects the host by promoting the proliferation and activity of beneficial bacteria in the colon (Cummings et al. 2001), which in turn affects many aspects of intestinal function (Grisdale-Helland et al. 2008). These bacteria produce organic acids that are advantageous to the host (Gibson 1998; Roberfroid 2000). Inhibiting the growth of dangerous germs, enhancing the absorption of critical nutrients, synthesizing specific vitamins, and enhancing immunological function are additional examples of potential health-promoting advantages (Li et al. 2007). According to reports, FOS could increase production through improved growth, stimulated immune response, and higher resistance to fish infections, overcoming the drawbacks and side effects of antibiotics and other medications (Akrami et al. 2013; Guerreiro et al. 2014; Zhang et al. 2013; Soleimani et al. 2012). The dietary addition of FOS at 0, 1, 2, and 3% for 6 weeks significantly increased serum IgM and lysozyme activity in Nile tilapia (Abd El-Gawad et al. 2016b). The enhanced lysozyme activity was likely caused by the increased leukocyte production brought on by dietary FOS (Zhang et al. 2013) because neutrophils and macrophages are the primary producers of fish lysozyme (Fischer et al. 2006). FOS's ability to stimulate the growth of helpful bacteria like lactobacilli and bifidobacteria, which produce lipopolysaccharides with immunostimulatory qualities, may be responsible for its ability to stimulate the immune system (Passos and Park 2003). Additionally, the FOS fermentation's final products, acetate, propionate, and lactic acid, are essential in influencing the immune system (Passos and Park 2003). Additionally, FOS was able to interact with the toll-like receptor 2 (TLR2) found on macrophages (Vogt et al. 2013) and upregulate the expression of antimicrobial peptides, which play a crucial role in fish disease resistance due to their importance in innate immune defense (Zhang et al. 2014).

6.3.2.3 Mannanoligosaccharide (MOS)

One well-known prebiotic that has lately been used in aquaculture is mannanoligosaccharide (MOS). MOS is made from the cell walls of the yeast *Saccharomyces cerevisiae* (Gelibolu et al. 2018). By preventing pathogenic bacteria from adhering to the digestive tract, regulating the intestinal microbiota, and maintaining intestinal integrity, MOS aided in the improvement of fish growth performance and health conditions (Ringø et al. 2010b; Fernández-Montero et al. 2019). The dietary inclusion of MOS at 1% considerably increased lysozyme and phagocytic activities in Nile tilapia, according to Dawood et al. (2020c). Prebiotics can maintain a neutral pH in the GI tract, creating an ideal environment for good bacteria to boost phagocytic activity and upregulate the expression of the CAT, GPX, GPX, IL-1, IL-8, and IFN-γ genes (Dawood et al. 2020b). *However,* Yuji-Sado et al. (2015) reported that MOS had no impact on Nile tilapia's immunological parameters. It is well recognized that the effects of MOS largely depend on the biotic factors of farmed fish, such as species, rearing circumstances, length of supplementation, age, and size of fish (Sweetman et al. 2010; Song et al. 2014).

6.3.2.4 Inulin

Inulin is a non-digestible carbohydrate found in a variety of fruits, vegetables, and grains. It is frequently used as an ingredient in functional foods and is commercially made from the chicory root (Madrigal and Sangronis 2007; de Almeida Gualtieri et al. 2013). Inulin has lately been used as an immunostimulant in aquaculture. Tiengtam et al. (2015b) found that inulin-containing diets at 0, 2.5, and 5 g kg^{-1} for 8 weeks increased lysozyme activity in Nile tilapia. Inulin predominantly stimulates the synthesis of IL-10 and NK cell cytotoxicity in Peyer's patches of immunological cells in animal studies. IgA signaling in the ileum and caecum, splenic NK cell cytotoxicity, and splenocyte cytokine levels are further immunological processes that inulin modulates (de Almeida Gualtieri et al. 2013). According to Watzl et al. (2005) finding, inulin predominantly modifies immunological GALT parameters.

6.3.2.5 Xylooligosaccharides (XOS)

Xylooligosaccharides (XOS), which include xylobiose, xylotriose, xylotetrose, and others, are polymers of sugar oligomers made up of xylose units through -(1, 4)-xylosidic connections (Kumar and Satyanarayana 2011). It is possible to generate xylooligosaccharides using chemical (Samanta et al. 2012), enzymatic (Jayapal et al. 2013), or a mix of chemical and enzymatic processes (Samanta et al. 2015). Apart from specifically promoting the development of healthy gut flora (Manisseri and Gudipati 2012), XOS was also shown to stimulate the immune system in hybrid catfish (Hahor et al. 2019), lessen the activity of pro-carcinogenic enzymes in the gastrointestinal tract of Sprague-Dawley rats treated with 1, 2-dimethyl hydrazine (Hsu et al. 2004), and improve intestinal mineral absorption and immune stimulation in albino rats (Mumtaz et al. 2009). Doan et al. (2018) revealed that consuming XOS produced from maize cobs dramatically improved skin mucus and serum immunity in Nile tilapia. Similar outcomes were observed in Nile tilapia fed XOS for 8 weeks at doses of 0 (CON), 0.5 (X-1), 1 (X-2), 2 (X-3), and 4 (X-4) g kg^{-1} (Poolsawat et al. 2021). The short-chain fatty acids that the gut microbiota produces during prebiotic fermentation, which can serve as an antibiotic substrate and a messenger for enhancing immune function, may be connected to the enhanced immunological and antioxidant characteristics (Bermudez-Brito et al. 2012; Song et al. 2014). According to Yu et al. (2015) adding xylooligosaccharides generated from corncob to the diet had an impact on the rising levels of gut microbiota and short-chain fatty acids (CDXOS).

6.3.2.6 Other Prebiotics

The second-most prevalent biopolymer in nature is chitin, while chitosan is a polycationic linear polysaccharide that is produced when chitin is partially deacetylated (Crini 2022). The pharmaceutical, biotech, and agricultural industries have recently used chitin and chitosan extensively (Wang and Zhuang 2022). In recent years, the aquaculture sector has employed chitin and chitosan as feed supplements in the diets of fish and shellfish (Kamilya and Khan 2020). In Nile tilapia, Abu-Elala et al. (2015) indicated that dietary inclusion of chitosan obtained from shrimp shell at 0, 0.5%, 1%, and 2% significantly increased phagocytic activity/index, NBT, lysozyme activity, and ACH50. In another study with low molecular weight chitooligosaccharide (LMV-COS), Meng et al. (2017) reported that inclusion levels of 0, 0.1, 0.2, 0.4, and 0.8 g kg^{-1} resulted in higher phagocytic and lysozyme activities. Similar results were noticed in Nile tilapia-fed orange-derived pectin, raffinose, and pistachio hulls-derived polysaccharide (Abdel-Latif et al. 2020b; Mohammadi et al. 2020).

6.3.2.7 Combination of Prebiotics

A combination of two prebiotics or prebiotics with other active components has been widely applied in aquaculture. Abd El Tawab et al. (2016) dietary inclusion of β-glucan + propylene glycol at 0; 0.5; 1.0 and 1.5 mL resulted in higher lysozyme and nitroblue-tetrazolium activities of Nile tilapia. Similar results were noticed in Nile tilapia fed β-glucan (βG) and mannan-oligosaccharides (MOS), where they reported significantly enhanced phagocytic and lysozyme activities, as well as immune-related genes expressions (Abu-Elala et al. 2018; Chen et al. 2019). Combination of β-glucan with *Aspergillus oryzae* or *Chlorella vulgaris* significantly improved non-specific immunity and up-regulated TNF-α and IL-10 gene expressions (Dawood et al. 2019b; Abdelhamid et al. 2020).

6.4 Synbiotics in Tilapia

6.4.1 Synbiotics as Growth Promoters and Disease Protection

Due to their positive effects on immune response and, consequently, disease resistance, synbiotics have received more attention in aquaculture recently (Carbone and Faggio 2016; Huynh et al. 2018). Liu et al. (2017b) reported that synbiotics between scFOS and *Lactobacilli* resulted in better growth performance, feed conversion ratio, disease resistance, and gut microbiota. These could result from partial dietary protein or amino acid consumption by allochthonous bacteria (Dai et al. 2012; Dai et al. 2010). Similarly, the dietary combination of lactic acid bacteria with Jerusalem artichoke, low molecular weight sodium alginate, pectin, xylooligosaccharide, and β-glucan significantly enhanced growth performance and disease resistance of tilapia (Sewaka et al. 2019; Van Doan et al. 2016a; Van Doan et al. 2019b; Dawood et al. 2020f; Van Doan et al. 2019c). A combination of kefir and LMWSA, MOS and chitosan with DBA®, and MOS with probiotics also increased growth performance and disease resistance (Van Doan et al. 2017b; Cavalcante et al. 2020; Laice et al. 2021). This may be caused by the addition of an unsuitable prebiotic as the substrate in a synbiotic mixture, which prevented a chosen probiotic from fermenting to form short-chain fatty acids (Hoseinifar et al. 2015b). When fish are fed a synbiotic diet, their growth performance is significantly improved, which could be attributed to the probiotic's increased survival and colonization or the prebiotic's improved digestibility (Rodriguez-Estrada et al. 2009; Ai et al. 2011; Ye et al. 2011; Liew et al. 2015). It is generally known that consuming dietary probiotics and prebiotics can cause the fermentation of bioactive microbial metabolites like organic acids, fatty acids, bioactive peptides, and vitamins (Munir et al. 2016; Stanton et al. 2005). These bioactive substances will speed up digestion in the intestine, which will boost growth rates. Additionally, the presence of advantageous bacteria in the stomach may boost microbial balance, which in turn improves nutrient absorption and utilization (Gatesoupe 1999; Lara-Flores et al. 2003).

6.4.2 Synbiotics as Immunomodulatory

Synbiotic supplementation in aquaculture has been proven to boost innate and specific immune responses (Amenyogbe et al. 2020). Van Doan et al. (2017b) indicated that dietary inclusion of kefir and LMWSA significantly boosted skin mucus and serum immunities of Nile tilapia. Although the precise processes through which kefir affects fish's innate immune system have not been shown, they may be related to kefir's potential probiotic effects (Ali et al. 2022). Previous research has shown that advantageous bacteria can increase host resistance, non-specific humoral responses, and the exclusion of prospective pathogens (Nikoskelainen et al. 2003; Rengpipat et al. 2000; Newaj-Fyzul and Austin 2015; Akhter et al. 2015; Lazado et al. 2015; De et al. 2014; Llewellyn et al. 2014; Nayak 2010). Probiotics have also been shown to modulate the host's immune response through interactions with epithelial cells and by raising the secretion of anti-inflammatory cytokines, which may result in decreasing inflammation (Denev et al. 2009). The activity and number of lymphocytes, intraepithelial cells, and acidophilic granulocytes were all affected at the cellular level by the addition of probiotics (Picchietti et al. 2007). Additionally, fish can have their systemic immune responses triggered by probiotics (Lazado et al. 2015).

Improvements to the Nile tilapia's skin mucus immune response, serum immunities, and gene expression have been documented when various prebiotics and lactic acid bacteria are combined (Van Doan et al. 2016a, 2019b, c, Dawood et al. 2020f). Prebiotics and lactic acid bacteria may have an impact on the fish immunological response, as evidenced by the remarkable improvement that was seen in this study. Prebiotics and LAB may work together to enhance the number of lactobacilli and bifidobacteria in mouse feces while decreasing the viability of Enterococcus, Enterobacter, and *Clostridia* species (Markowiak and Śliżewska 2017; Vieco-Saiz et al. 2019). Corn cob-derived xylooligosaccharide (CDXOS), which was fermented with *L. plantarum*, was found to have strong superoxide anion radical-scavenging abilities, according to an in vitro antioxidant assay (Yu et al. 2015).

6.5 Conclusion

Using prebiotics and synbiotics in tilapia aquaculture has advantages for sustainable food supply to fulfill the rising worldwide need for animal protein. For the host organism to perform better and develop immunity, knowledge of the dosage and duration of administration is crucial. Under both normal and environmental stress circumstances, longer administration times appear to have a more favorable impact on fish performance and immune response. Prebiotics and synbiotics also appear to have effects that are dose-dependent and reliant on the fish's culture circumstances. Although numerous studies have been carried out to clarify their mode of action, further research is required to fully comprehend how they affect the activity of digestive enzymes, and the utilization of carbohydrates, fatty acids, and minerals. Similarly, further work needs to be done to understand how these feed additives affect tilapia's metabolic profile, gut microbiome, and gene expression using omics technology. Additionally, insufficient research has been done, as far as we are aware, to determine how these feed supplements affect tilapia when they are exposed to pesticide, heavy metal, and fungal toxicity, as well as virus and fungus infections. We, therefore, urge future research on tilapia to concentrate on these areas to protect the sustainable production of tilapia under various ecological systems.

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Chapter 7
Medicinal Plants in Tilapia Aquaculture

Mehwish Faheem, Seyed Hossein Hoseinifar, and Farid Firouzbakhsh

Abstract Tilapia are a group of fish belonging to the family Cichlidae. Because of their mild flavor and tasty flesh, tilapia has become the second most important aquaculture species after carp. Two highly cultured species of tilapia are *Oreochromis mossambicus* and *Oreochromis niloticus.* Tilapia aquaculture is expanding enormously and up to 6.6 million tons of tilapia is expected by 2030 annually. With the intensification of tilapia culture and the environmental manipulation of culture systems, there is an increase in infectious diseases and economic losses due to disease and stress. A lot of consideration has to be given to health management and the proper use of prophylactics. Various methods are used for disease prevention and control in tilapia aquaculture. Chemotherapeutic agents like antibiotics, biotherapeutic agents like probiotics, prebiotics symbiotics, and medicinal plants are often used. The use of medicinal plants is gaining much attention as they are eco-friendly, sustainable, and have the least environmental impact. Medicinal plants improve growth, appetite, and disease resistance and also boost the immune system of aquatic organisms. Powder of different parts of medicinal plants and extracts are used while routes of administration include oral, immersion, and in form of injections. Oral administration as feed additives is the most commonly used method. This chapter will focus on the use of different medicinal plants in tilapia

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aquaculture, their routes of administration, and their effects on fish physiology. Moreover, various modes of action of these medicinal plants are also discussed.

Keywords Herbs · Immune parameters · Disease resistance · Antioxidant defense · Bioactive compounds

7.1 Introduction

The human population is increasing at a very high rate and it is expected to reach up to 10 billion by 2050 (Hoseinifar et al. 2020a, b). With the increase in population, the demand for animal protein is also increasing. Considered the fastest-growing food-producing sector in the world, aquaculture is recognized as a possible sustainable solution for food security and increased dietary nutrition in developing countries (Adewole 2014). Fish and shellfish are important animal-based proteins and account for 16.7% of total animal-based protein intake globally. Captured fisheries are declining in past decades and many resources are almost exploited, therefore, the intensification and expansion of aquaculture are required to produce enough animal-based protein to meet increasing demand (Delgado et al. 2003).

Tilapia is the common name used for three genera (*Tilapia, Sarotherodon*, and *Oreochromis*) belonging to the family Cichlidae. Tilapias are important species in aquaculture with growing consumer preference (Ahmad et al. 2009). Aquaculture has observed an increase in tilapia culture in the last few years as these species are distributed worldwide (El-Sayed 2006) and are considered as most important fish species of the 21st century. Currently, more than 135 countries are doing tilapia culture (FAO 2014). Global annual production of tilapia was 4.5 million tons in 2013 and by 2030 it's expected to grow to 6.6. Million tons (Kuebutornye and Abarike 2020). Annual tilapia culture makes up about 12.2% of total finfish culture globally (El-Sayed 2019). Highly cultured species of tilapia belong to the genera *Oreochromis*. Mozambique tilapia (*Oreochromis mossambicus*) and Nile tilapia (*Oreochromis niloticus*) are widely cultured. Around 90% of tilapia culture is of *O. niloticus* (FAO 2016). Tilapia are among a few fish species that can be cultured in almost all types of culture systems viz. pond culture, static pond culture, integrated fish culture, recirculation or closed cycle systems in temperate countries, etc. (Fitzsimmons 2000). Recently the intensive cage culture of tilapia is gaining popularity around the globe.

With the rising global demand, the intensive culture of tilapia is gaining much attraction. However, diet quality, infectious diseases, and resistance to various therapeutics are major constraints in the intensification of tilapia culture (Ahmad et al. 2011; Elkafrawy 2020). Moreover, intensification is stressful to fish and leads to the weakening of the immune system hence leading to infections (Roosta and Hoseinifar 2014). To manage the losses resulting from disease and pathogen infections, farmers are extensively using different chemicals mainly antibiotics

(Hoseinifar et al. 2017a, b, c, d; Nawaz et al. 2018). Antibiotics have been used successfully to treat fish diseases but indiscriminate use of antibiotics has resulted in resistance and accumulation of antibiotic residues in the environment and the fish tissues leading to the emergence of multiple antibiotic-resistant (MAR) strains (Seyfried et al. 2010; Rossolini et al. 2014; Santos and Ramos 2016; Hoseinifar et al. 2017a, b, c, d; Nawaz et al. 2018). Widespread use of vaccines and antibiotics is expensive and impractical in fish farms and a single vaccine works against only one type of pathogen (Harikrishnan et al. 2011; Plant and LaPatra 2011). Therefore, fish farmers are advised to improve fish husbandry and the water quality of ponds and use sustainable dietary supplements to strengthen the immune system of fish (Daniels and Hoseinifar 2014). Organic fish farming or green aquaculture relies mainly on eco-friendly approaches to avoid disease and infection. Feed additives *e.g.* Pro- and pre-biotics and medicinal plants are now being used as an alternative to antibiotics in aquaculture. Among these feed additives, medicinal plants provide the most sustainable and eco-friendly alternative to antibiotics (Abarike et al. 2018; Awad and Awaad 2017) and are used to strengthen the fish immune system (Srichaiyo et al. 2020; Mohammadi et al. 2020a, b).

In the current chapter, a detailed review of existing literature about the use of medicinal plants in tilapia aquaculture is provided along with the possible mode of action. Furthermore, future perspectives and areas to be focused on are highlighted.

7.2 Importance of Medicinal Herbs/Plants in Aquaculture

Medicinal plants include parts of plants (flowers, seeds, leaves, and roots), plant-derived products, spices, herbs, and seaweed (Hai 2015). The use of medicinal and edible plants, vegetable herbs, and seeds dates back in human history (Davidson-Hunt 2000). Traditional medicines and drugs were made by using medicinal plants and they enhanced the utilization of dietary energy and increased performance efficiency. Moreover, medicinal plants act as natural tonics and restoratives (Götti et al. 2014). Herbs and spices were used to improve storage stability and impart flavors to a variety of foods (Ahmad et al. 2011). Medicinal plants/herbs are used in the treatment of different ailments (*e.g.* lowering blood cholesterol and prevention of cancer) by activating the immune system (Desai et al. 2008; Greenwell and Rahman 2015). Antimicrobial substances were used to treat bacterial disease in fish (Van Doan et al. 2020; Ashour et al. 2020; Ceballos-Francisco et al. 2020; Abdel-Tawwab and El-Araby 2021).

Medicinal plants are inexpensive, usually readily available, and have no or minimal effects on fish health and they don't contribute negatively to the environment (Na-Phatthalung et al. 2018). Different parts (leaves, roots, seeds, skin, flowers, and fruit) are used because of their rich nutritional value and therapeutic properties (Akinyemi et al. 2018). Medicinal plants contain a variety of biologically active compounds like carotenoids, flavonoids, terpenoids, polyphenols, tocopherols, glycosides, alkaloids, lectins, saponins, cinnamic acid, polypeptides, quinone, tannins, folic acid, biogenic amines, steroids, phytoandrogens, and phytosterols (Mulat et al. 2020). Medicinal plants also contain many vitamins (riboflavin, thiamine, niacin, and ascorbic acid), and trace minerals (calcium, sodium, zinc, iron, magnesium, and potassium) (Hussain et al. 2011; Ibrahim et al. 2019). All these compounds enhance fish growth, nutrient digestibility, gut health, gut microbiota, digestive enzymes, immunity, and anti-oxidant status (Citarasu 2010; Mohiseni et al. 2017; Astuya et al. 2017; Faheem et al. 2020).

There are almost 17,000 known species of plants and around 8,000 species of plants are being used in traditional medicines in many countries like Pakistan, India, Sri Lanka, China, Japan, and Thailand (Singh 2015; Hai 2015). Medicinal plants are used in traditional medicines as antioxidants (Guardiola et al. 2018; Parham et al. 2020), as antimicrobial, antifungal, antiparasitic, and antiviral agents (Tagboto and Townson 2001; Dhama et al. 2018; Kalsoom et al. 2020; Parham et al. 2020). Medicinal plants also have anti-inflammatory and stress-releasing properties (Reyes-Becerril et al. 2019; Shourbela et al. 2020). The use of medicinal plants in traditional medicines as an appetizing agent, hepatoprotective, gastro-protective, nephroprotective, hematoprotective, and immunostimulatory agent (Bai et al. 2009; Ayaz et al. 2017; Okaiyeto et al. 2018; Asnaashari et al. 2018; Esmail and Ali 2019; Abdel-Tawwab and Hamed 2020; Faheem et al. 2020) also very popular. For example, Cinnamon possesses potent antioxidants, and anti-inflammatory and anti-mutagenic compounds (Ahmad et al. 2011). Parsley is of high nutritive value as the extract can counteract the toxic effects of aflatoxin in fish. Parsley contains a high amount of vitamins A, C, riboflavin, and niacin and is also a rich source of minerals like sodium, calcium, magnesium, iron, and zinc (Peter 2001). Both seeds of *Nigella sativa* and seed oil are used as an immune booster and confer many positive health benefits (Mohammed and Arias 2016; Latif et al. 2020).

7.3 Bioactive Compounds of Medicinal Plants

Medicinal plants have various bioactive compounds, and phytochemicals, which are mainly responsible for their positive health effects. Various types of phytochemicals *e.g.* flavonoids, alkaloids, essential oils, phytosterols, etc. are present in many plants and plant parts. Some anti-nutrient factors are also present in plants along with phytochemicals. These anti-nutrient factors, sometimes decrease the effectiveness of medicinal plants. These anti-nutrient factors are now decreased or eliminated by making extracts or by using enzymes.

7.3.1 Flavonoids

Flavonoids are secondary plant metabolites. Structurally, flavonoids are di-phenylpropanes, a three-carbon ring molecule having two benzene rings attached on both sides of the carbon chain. Flavonoids are present in plants in various forms like flavanols, flavanone, flavones, isoflavones, flavan-3-ol, and anthocyanins. When a different functional group or compound like methyl group, hydroxyl group, sugar, or oxygen attaches to the 3-carbon chain, various classes of flavonoids are formed.

Many plants and herbs contain flavonoids e.g., tea is rich in catechin, epigallocatechin, and epicatechin while onions, olives, grapefruit, barriers, and red wine are rich in quercetin, tamarixetin, myricetin, and kaempferol. Citrus fruits like lemons, grapefruit, and oranges contain a variety of flavanones like hesperidin, taxifolin, naringenin, and naringin. Isoflavones, e.g., daidzin and genistin, are present in soybean. The skin of many fruits is also a source of flavonoids, the skin of tomato and red pepper is loaded with rutin, chrysin, luteolin, apigenin, and luteolin glucosides. Cherry, strawberry, and raspberry contain anthocyanidin and cyanidin. Flavonoids are known for their antioxidant property. However, the antioxidative potential of flavonoids is dependent on the presence and configuration of functional groups. Flavonoids having B-ring configuration have potent radical scavenging potential, they produce stable flavonoid radicals by donating hydrogen and electron to reactive oxygen species like hydroxyl, peroxyl, and peroxynitrite. Flavonoids also have other pharmacological properties like antiviral, antiallergic, antibacterial, anti-inflammatory, and vasodilatory.

7.3.2 Alkaloids

Alkaloids are the organic metabolites of plants having basic properties. Structurally, they are heterocyclic compounds. The pharmacologically active class of alkaloids is isoquinoline. Major and important isoquinoline is sanguinarine which belongs to benzo[c]phenanthridine alkaloid and has immunomodulatory, anti-inflammatory, and antibacterial properties. Sanguinarine also promotes growth by enhancing feed intake and lowering the degradation of amino acids.

7.3.3 Essential Oils

Aromatic plants produce essential oils that are hydrophobic concentrates having a strong odor. Essential oils have antimicrobial, anti-inflammatory, and analgesic properties. The essential oil extracted from oregano, *Origanum heracleoticum*, have thymol, carvacrol, and monoterpenoid phenolic compounds as major phytochemicals. An *in vitro* study with phytochemicals like cymene and carvacrol has revealed that they have bactericidal activities against *Salmonella typhi* (Rattanachaikunsopon and Phumkhachorn 2009). Therefore, it is believed that phenolic compounds present either in plant extracts or essential oil have antibacterial properties, that can modulate gut microflora leading to better utilization of nutrients and hence better growth performance in fish.

7.3.4 Triterpenoids

Triterpenoids are hydrocarbons having six isoprene units with substitution or displacement of the methyl group with oxygen. They are present in a variety of medicinal plants with antitumor antibacterial, and antiviral potential, role in glucose metabolism and diabetes. Examples of triterpenoids include lupol, glycyrrhizin, botulin, oleanolic and glycyrrhetinic, ursolic, maslinic, and betulinic acid. Triterpenoids belonging to ursane, oleanane, and lupane (betulin, betulinic acid, and lupeol) had anti-cancerous properties because of their anti-inflammatory potential. Rainbow trout fed with varying levels of maslinic acid showed improved growth.

7.3.5 Phytoandrogens

Many plants possess bioactive compounds that have similar effects as testosterone, such bioactive compounds are called phytoandrogens as they are produced from plants. Seeds of fenugreek have a bioactive compound called diosgenin which is a steroid-based sapogenin, similarly, soy has an isoflavone compound called daidzein and gutta-percha tree has triterpenoids that have androgenic properties.

7.4 Effect of Medicinal Plants on Fish Physiology and Growth

Fish growth may be defined as the increase in fish weight and length with time. It is a somatic trait and is characterized by an increase in fish weight due to enhanced synthesis of protein. The growth of fish depends mainly on stocking density, water quality parameters, and quality and quantity of food. While the endogenous cues also have an important role in fish growth. Both endogenous and exogenous signals are received and integrated by the central nervous system and regulate fish growth, appetite, and metabolism. In teleost, the primary regulators of fish growth are insulin-like growth factors and growth hormones.

Upon receiving the exogenous signals, the brain produces growth hormone releasing hormone and dopamine that act on the pituitary. Somatotrophs that are located in the anterior portion of the pituitary are responsible for the production of

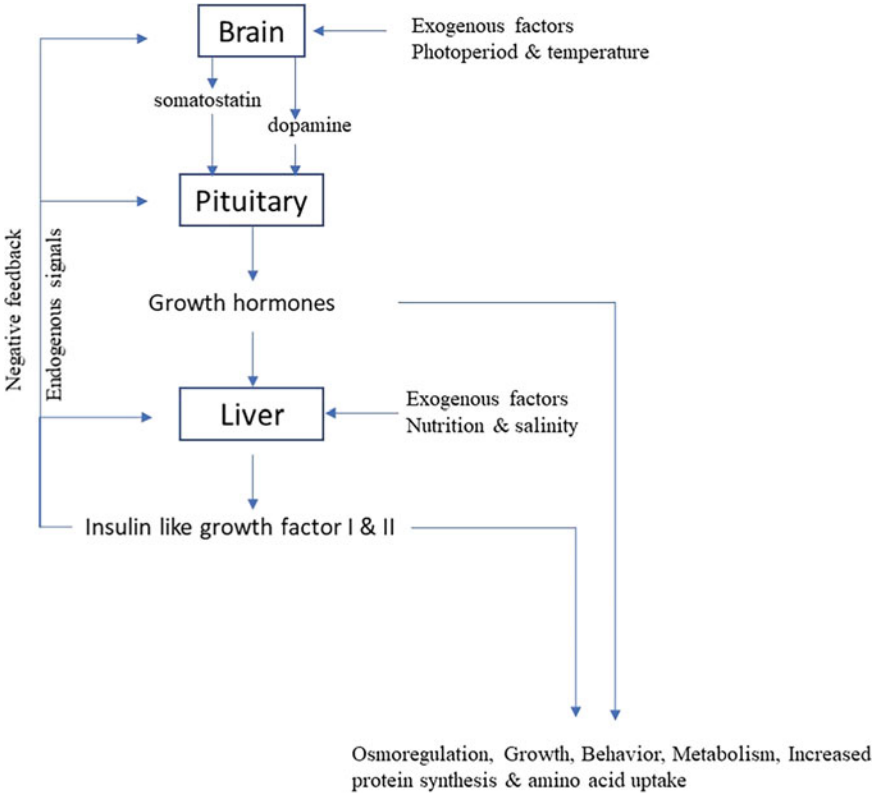

Fig. 7.1 Growth axis in a teleost adapted from

growth hormones (GHs). Once released in the blood circulation, these GHs bind to their receptors, present in various tissues and organs mainly the liver, and activate many metabolic pathways including the production of insulin-like growth factors. Both growth hormones and insulin-like growth factors are responsible for growth, metabolism, osmoregulation, and reproduction. The growth axis in a teleost is elaborated in Fig. 7.1. A balanced feed with an optimum quantity of essential nutrients that enhance fish growth is one of the major challenges in aquaculture. The addition of plants/herbs that can stimulate fish growth is an active area of research and many researchers are working on the addition of plant-based supplements to the diet that enhances the growth of tilapia. Details of such studies are listed in Table 7.1. Medicinal plants can directly act on the pituitary and enhance the synthesis and release GHs or on the liver to stimulate the release of insulin-like growth factors. Supplementing tilapia diets with 0.5% curcumin resulted in enhanced mRNA expression of GHs in the brain and IGFs in the liver, indicating that medicinal plants might directly act on the growth axis. Similarly, when tilapia feed is supplemented with 4 and 6 ppm of D-limonene, it resulted in higher mRNA levels of both GHs and IGF-I. Medicinal plants also stimulate growth by enhancing

Table 7.1 Effect of medicinal plants on growth performance of tilapia

Fish	Plant used	Dietary inclusion	Experimental duration	Observed effects	Optimal dose	Reference
Oreochromis niloticus	*Origanium vulgare* (Oregano) Ethanolic extracts	0.5%, 1%, 1.5%	10 weeks	Growth performance (↑*), Feed consumption (↑*), Total protein (↑*), Serum albumin (↑*), Globulin (↑*)		Ahmad et al. (2009)
Oreochromis niloticus	*Caraway seed meal (Carum carvi)*	0.0, 5, 10, 15, and 20 g/kg	12 weeks	Survival (95–100%), growth performance (↑*), feed utilization (↑*), and whole body composition (+)	10 g/kg	Ahmad and Abdel-Tawwab (2011)
Oreochromis niloticus	Cinnamon	0, 0.5, 1.0, and 1.5%	90 days	Specific growth rate (↑), feed conversion ratio (FCR) (↑), feed efficiency ratio (↑), protein efficiency ratio (↑), apparent protein utilization (APU) (↑), and energy utilization (EU) (↑)	1%	Ahmad et al. (2011)
Oreochromis niloticus	*Ocimum gratissimum* Essential oil	0, 0.5, 1.0, and 1.5%	55 days	Specific growth rate ↓* and feed conversion ratio ↑*	0.5%	Brum et al. (2017)
	Zingiber officinale Essential oil			Specific growth rate ↓* and feed conversion ratio ↑*	1.5%	
Gift tilapia	*Aloe vera*	0.5, 1, 2, and 4 %	8-weeks	Weight gain ↑*, specific growth rate ↑*, feed conversion ratio ↑*	2 & 4%	Gabriel et al. (2015)
Oreochromis mossambicus	*Psidium guajava*	1, 5, and 10 mg/g aqueous and ethanolic extracts	30-days	Final body weight ↑*, specific growth rate ↑* and feed conversion ratio ↓	10 mg/g	Gobi et al. (2016)

Oreochromis niloticus	*Cinnamomum camphora*	2 g/kg	90 days	Weight gain (↑*) and specific growth (↑*), feed conversion ratio (↑*) and condition factor (↑*)	2 g/kg	Kareem et al. (2016)
	Euphorbia hirta			Weight gain (ns) and specific growth (ns), feed conversion ratio (↑*), and condition factor (↑*)		
	Azadirachta indica			Weight gain (ns), specific growth (ns), feed conversion ratio (ns), and condition factor (ns)		
	Carica papaya			Weight gain (↑*), specific growth (↑*), feed conversion ratio (↑*),and condition factor (↑*)		
Oreochromis niloticus	*Astragalus membranaceus*	1500 mg/kg	Astragalus polysaccharides (APS)	Gain (↑*) , specific growth rate (↑*), feed conversion ratio (↑*) and feed intake (↑*)	1500 mg/kg	Zahran et al. (2014)
Hybrid tilapia (O. niloticus × O. aureus) juvenile,	Garlic (*Allium sativum*)	0, 0.5, 1 g/kg	4-weeks	Weight gain ↓* in 0.5% and ns in 1%)	1%	Ndong and Fall (2011)
Oreochromis niloticus	*St John's-wort (Hypericum perforatum)*	Ethanolic extract (0%, 0.2%, and 0.5)	60 days	FBW (ns), WG%,(ns) SGR (ns), and FCR(ns)	0.5%	Mohammadi et al. (2020a, b)
	Lemon balm (Melissa officinalis)			FBW↑* , WG%↑*, SGR↑*, and FCR↑*		
	Oregano (Origanum vulgare)			FBW↑* , WG%↑*, SGR↑*, and FCR↑*		

(continued)

Table 7.1 (continued)

Fish	Plant used	Dietary inclusion	Experimental duration	Observed effects	Optimal dose	Reference
Oreochromis niloticus	*Licorice (Glycyrrhiza glabra*	Root powder (0, 5, 10, and 20 g/kg)	60-days	Weight gain↑*, growth rate↑*, and feed intake↑*	10 g/kg of feed	Abdel-Tawwab and El-Araby (2021)
Oreochromis niloticus	*Assam tea (Camellia sinensis)*	0, 1, 2, 4, and 8 g/kg	4 & 8 weeks	Final body weight ↑*, weight gain ↑*, and specific growth rate ↑*, feed conversion ratio↓	2 g/kg	Van Doan et al. (2019)
Oreochromis niloticus	*Elephantopus scaber*	0, 2.5, 5, 10, and 20 g kg	4 & 8 weeks	Final body weight ↑*, weight gain ↑*, and specific growth rate ↑*	5 g/kg	Van Doan et al. (2019)
Oreochromis niloticus	Thai ginseng (*Boesenbergia rotunda)*	0, 5, 10, 20, 40 g/kg	8 weeks	Final weight ↑*, weight gain ↑*, specific growth rate ↑*, feed intake ↑*, and feed conversion ratio ↑*	10 g/kg	Van Doan et al. (2019)
Oreochromis niloticus	*Berberine*	0, 1, 3, 6, and 9 g/kg	4 & 8 weeks	Final body weight ↑*, weight gain ↑*, and specific growth rate ↑*	1 g/kg	Van Doan et al. (2020)
Oreochromis niloticus	Seaweed (TrueAlgaeMax)	0, 0.5, 1, 1.5, and 2%	70-days	Weight gain↑*, average daily weight gain ↑*, specific growth rate ↑* and achieved the best feed conversion ratio ↑*	2%	Ashour et al. (2020)
Oreochromis niloticus	Watermelon rind powder	0 20, 40, 80, 160 g/kg	8-weeks	Final weight↑*, weight gain ↑*, and specific growth rate ↑*	40 g/kg	Van Doan et al. (2020)
Oreochromis niloticus	White button mushroom	0, 0.5, 1, 2, and 4%	60-days	Final body weight ↑*, weight gain ↑*, specific growth rate↑*, and feed intake↑*, feed conversion ratio ↓	2%	Dawood et al. (2020)

Arrows indicate an increase (↑), a decrease (↓), a significant increase (↑*), and a significant decrease (↓*)

the appetite of fish. The addition of medicinal plants increases the attraction towards food, increased feed acceptance, and palatability.

Intensive aquaculture results in stress on fish which leads to detrimental effects on fish health through improper feed utilization, changes in intestinal functions, and inability to digest consumed feed (Hegazi and Hasanein 2010; Yilmaz 2019). The intestine of fish is the main entrance to the fish body, and various pathogens can alter the local intestinal immunity that results in an increased probability of pathogen infection (Abid et al. 2013; Standen et al. 2016). Many feed additives are used in fish feed, and bioactive components of medicinal plants improve intestinal functions by increasing nutrient digestibility, enzyme secretion, feed utilization, and increasing healthy gut microflora (Van Kooyk and Rabinovich 2008). Natural growth promoters are present in medicinal plants that were found to improve body weight, survival rate, and feed conversion ratio in fish (El-Dakar 2004; Shalaby 2004).

Growth promoting effects of medicinal herbs may be due to the nutrient digestibility and thus leading to enhanced absorbance of nutrients. Many spices stimulate digestion by increasing the secretions of bile and digestive enzymes (Platel et al. 2002). Important compounds present in herbs and spices act as attractants for fish leading to better feeding and improved growth. Growth of *Tilapia zillii* improved after adding olfactory stimulants to feed (Adams et al. 1988).

Organium vulgare extract was found to increase body weight, weight gain%, SGR, and survival rate due to the presence of antioxidants (tocopherols, total polar compounds, fatty acids, and oxidative stability) and antimicrobial compounds (Ahmad et al. 2009; de Souza Pereira et al. 2017). Diets containing feed additives (0.5% cinnamon and 1% marjoram) showed better growth performance when fed to tilapia (Abdel Wahab et al. 2007; Abdel El-Maksoud et al. 1999). 1% supplementation of cinnamon was reported to act as a natural growth promoter in the diets of tilapia. The presence of cinnamon in the diet enhances the sensory stimulation of fish which may result in improved appetite and thus lead to improved growth (Ahmad et al. 2011). Similarly, dietary supplementation of clove and basil at 0.5% improved the feed conversion ratio in tilapia (Brum et al. 2017). An increase in body weight gain was observed after feeding Nile tilapia with *A. vera* (Gabriel et al. 2015). Dietary inclusion of licorice root powder, aqueous, and methanolic extracts of licorice root powder improve growth performance and feed utilization in Nile Tilapia (Abdel-Tawwab and El-Araby 2021).

Many active compounds like phenols, flavonoids and isoflavonoids, saponins, and coumarins present in medicinal plants and herbs improve nutrient digestibility leading to better growth and weight gain in fish (Poolsawat et al. 2020; Bostami et al. 2021).

7.5 Medicinal Plants to Improve Pathogen Resistance in Tilapia

Mortalities due to pathogen infection are huge in tilapia culture and lead to substantial losses. There is a dire need to look for sustainable solutions and prophylactics to manage pathogen resistance and damage caused by pathogens. Many bacterial, fungal, and viral pathogens are responsible for disease outbreaks in tilapia culture. Important pathogens of tilapia are *Branchiomyces*, *Dactylogyrus*, *Saprolegnia*, *Trichodina*, *Dactylogyrus*, *Iridovirus*, *Francisella*, *Nocardia*, *Flavobacterium*, and *Edwarsiella* (Absali and Mohamad 2010; MSD Animal Health 2012). Major bacterial pathogens of tilapia culture belong to the genera *Streptococcus* especially *Streptococcus iniae and Streptococcus agalactoe.* Almost 82% of pathogens isolated from farmed tilapia belonged to *Streptococcus agalactoe* (MSD Animal Health 2012; Li et al. 2014). Other economically important bacterial pathogens of tilapia are *Aeromonas hydrophila, Francisella* spp., and *Edwardsiella* spp.

7.5.1 Streptococcus agalactiae

Streptococcus agalactiae is a gram-positive bacteria of cultured fish both in freshwater and marine water (Kümmerer 2009). Infections with *Streptococcus agalactiae* occurs both in juvenile and adults with a prevalence rate of 40-60% (Pretto-Giordano et al. 2010). *Streptococcus agalactiae* cause infection in tilapia, rainbow trout, channel catfish, and silver sea bream in tropical and temperate climates (Ye et al. 2011). Once infected with bacteria, fish show erratic swimming, injuries in the skin, loss of appetite, swelling in the abdominal region, paleness of the liver, loss of coordination in movement, and softening of the head region (Figueiredo et al. 2006; Pretto-Giordano et al. 2010; Chen et al. 2012). *S. agalactiae* can survive for long periods in pond water and mud; however, water is the primary route of transmission for *S. agalactiae*. Around 90-100% mortalities were recorded in tilapia after infection with *S. agalactiae.* Virulence of this bacteria in tilapia depends upon CFU and strain. The 72-h lethal dose (LD_{50}) of *S. agalactiae* in tilapia is 1.12×10^6 CFU/ml (Bondad-Reantaso et al. 2005; Mian et al. 2009) while the concentration ranging from 1×10^1 to 1×10^5 CFU caused 80-100% mortalities in cultured Nile Tilapia (Hernández et al. 2009).

7.5.2 Aeromonas hydrophila

Genus *Aeromonas* is gram-negative bacteria that belongs to the family Aeromonadaceae which has four species *i.e. Aeromonas hydrophila*, *Aeromonas. salmonicida*, *Aeromonas sobria*, and *Aeromonas piscicola.* These are facultative pathogens and cause dermal red plague or hemorrhagic septicemia syndrome. These pathogens were isolated from tilapia, goldfish, channel catfish, rainbow trout, and salmon (Molnár and Csaba 2005; Rattanachaikunsopon and Phumkhachorn 2009; Austin and Austin 2012). Members of the genus *Aeromonas* are naturally present in many habitats including freshwater, the degree of infection caused by the pathogens depends greatly on environmental conditions, dissolved oxygen, ammonia levels, and species of fish (Beaz-Hidalgo and Figueras 2012). Among four species of the genus *Aeromonas*, *Aeromonas hydrophila* is the most important with a prevalence of up to 80% in freshwater (Beaz-Hidalgo et al. 2012; Soto-Rodríguez et al. 2013) it is present on the skin of fish and in the digestive system (Harikrishnand and Balasundaram 2005). Loss of appetite, weakness, hemorrhages in gills and skin, and necrosis of vital organs are major signs of *Aeromonas* infection (Bastardo et al. 2012). Other symptoms include loss of scales, accumulation of fluid in the body, and loss of vision (Yardimci and Aydin 2011).

7.5.3 Francisella *spp.*

Members of the genus *Francisella* are gram-negative and facultative aerobic bacteria and have two species i.e. *Francisella noatunensis orientalis* occurs in tropical zones and *Francisella noatunensis noatunensis* (Colquhoun and Duodu 2011). Both species are distributed globally with a prevalence of up to 90% in freshwater aquaculture systems (Ottem et al. 2007; Jeffery et al. 2010). Francisella is transmitted horizontally in the freshwater culture system and it has been isolated from Nile tilapia, white and striped bass, salmon, and Atlantic cod (Ostland et al. 2006; Ottem et al. 2007; Kamaishi et al. 2010). Fish infected with *Francisella* spp. shows loss of appetite, erratic swimming pattern, hemorrhage around the pectoral fin, and anemia (Soto et al. 2009). Severe infection results in gill hyperplasia, enlargement of the kidney and spleen, and necrosis of vital organs (Mauel et al. 2007; Ottem et al. 2009).

7.5.4 Edwardsiella *spp.*

Genus *Edwardsiella* is a group of gram-negative pathogens and has four species *i.e. Edwardsiella tarda*, *Edwardsiella Ictaluri*, *Edwardsiella hoshinae*, and *Edwardsiella anguillimortifera.*

Edwardsiella ictaluri is commonly present but *Edwardsiella tarda* is an economically important species that also cause zoonosis (Bastardo et al. 2012). *E. tarda* has been isolated from channel catfish, Nile tilapia, hybrid tilapia, turbot, red seabream, and Japanese eel (Burr et al. 2012). Infection with *E. trada* depends mainly on temperature, the presence of organic matter in the pond, and water quality parameters. General symptoms of infection include bulging of eyes, spiral swimming, swelling of the abdominal region, and pale eyes.

The use of vaccines and antibiotics to control pathogen infection is common practice among tilapia fish farmers around the globe. Uncontrolled use of antibiotics resulted in the emergence of resistance, therefore plants and plants-derived chemicals are now being used in tilapia culture to avoid the spread of disease (Table 7.2).

Tilapia fed with different medicinal plants showed improved growth and resistance to pathogens (Table 7.2). Tilapia fed with Echinacea showed improved survival rates against *P. fluorescens* infections (Aly et al. 2008). Similarly, the addition of Cinnamon (0.5%) to the diets of tilapia resulted in improved gut microbial content and feed absorption. Moreover, guts of tipalia fed with cinnamon containing diet had a smaller number of harmful bacteria like *Campylobacter jejuni*, *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *etc.* (Abdel Wahab et al. 2007). Similarly, the addition of *Cinnamomum camphora* to the diet increased the survival rate in tilapia when exposed to *Streptococcus agalactiae* (Kareem et al. 2016). Several medicinal plants *e.g. Lonicera japonica* (Japanese honeysuckle), *Nyctanthes arbortristis* (night jasmine), *Panax quinquefolium* (American ginseng), *Tinospora cordifolia, Glycyrrhiza glabra* (Licorice) protect tilapia against A. hydrophila challenge (Sudhakaran et al. 2006; Ardo et al. 2008; Kirubakaran et al. 2010; Abdel-Tawwab and El-Araby 2021). Supplementing feed of tilapia with leaf extract and dried leaves of rosemary increases survival rate when challenged with *Streptococcus iniae* (Abutbul et al. 2004). Tilapia showed increased resistance against *Streptococcus agalactiae* when fed with varying levels of *Sophora flavescens* (Wu et al. 2013).

In addition to protection against bacterial pathogens, medicinal plants, and herbs also protect tilapia against parasitic infections. Indian almond (*Terminalia catappa*), garlic (*Allium sativum*), and extracts of green tea protect tilapia against Trichodina sp. infections (Chitmanat et al. 2005; El-Deen 2010).

Many bioactive compounds found in plants have medicinal properties. Active compounds like Thymol and Carvacrol have lipophilic properties and can cause the breakdown of the bacterial cell wall (Lambert et al. 2001; Ultee et al. 1998; Conner 1993; Helander et al. 1998). Other compounds like ρ-cymene present in Oregano cause swelling of the bacterial cell membranes and hence destroy the bacterial cell (Ultee et al. 2002). The addition of herbs increases mucus lysozyme activity which can break down peptidoglycans of the bacterial cell wall (Magnadottir 2010). It is believed that the addition of medicinal plants to the diet of tilapia improved immune response and hence provided better resistance to pathogens.

Table 7.2 Role of different medicinal plants to improve pathogen resistance in tilapia

Fish	Plant used	Inclusion type	Inclusion ratio	pathogen	Route of pathogen administration	Resistance	Optimal dose	Reference
Oreochromis mossambicus	*Tinospora cordifolia*	Ethanolic extract	0.8, 8, 80 mg/kg	*Aeromonas hydrophila*	Intraperitonial 10^9 cells/fish	Decreased mortality	80 mg/kg ether and ethanolic extract	Sudhakaran et al. (2006)
Oreochromis mossambicus	*Solanum trilobatum*	Water and hexane soluble fractions	0, 4, 40, 400 mg/kg	*Aeromonas hydrophila*	Intraperitonial 1×10^9 cells/fish	80% mortality in the control 23% in 400 mg water extract	4 mg/kg hexane soluble fraction & 400 mg/kg water-soluble fraction	Divyagnaneswari et al. (2007)
Oreochromis mossambicus	*Eclipta alba*	Leaf extracts	0, 0.01, 0.1, or 1%	*Aeromonas hydrophila*	Intraperitonial 1×10^8 cells/fish	Decreased mortality	1% aqueous extract-supplemented feed	Christybapita et al. (2007)
Oreochromis mossambicus	*Toona sinensis*	Hot water extract	4 and 8 μg/g injection.	*Aeromonas hydrophila*	Intraperitonial 5×10^7 cells/fish	Decreased mortality	8 μg/g	
Oreochromis niloticus	Cinnamon *C. zeylanicum*	Powder	0., 0.5, 1.0, and 1.5%	*Aeromonas hydrophila*	Intraperitoneal 5×10^5 CFU	Decreased mortality	1%	Ahmad et al. (2011)
Oreochromis niloticus	*Viscum album coloratum*	Aqueous leaf extract	0, 10, 50 g, and 200 mg /kg	*Aeromonas hydrophila*	Intraperitoneal 2×10^5 cells/fish	Decreased mortality	50 mg/kg	Park and Choi (2012)
Oreochromis niloticus	The herbal mixture of *Astragalus, angelica,* hawthorn,	Powder	0.0, 0.5, 1.0, 1.5, and 2.0%	*Aeromonas hydrophila*	Intraperitoneal 10^8 cells/ml	Decreased mortality	2%	Tang et al. (2014)

(continued)

Table 7.2 (continued)

Fish	Plant used	Inclusion type	Inclusion ratio	pathogen	Route of pathogen administration	Resistance	Optimal dose	Reference
	Licorice root and honeysuckle							
Gift tilapia	*Aloe vera*	Powder	0.5, 1, 2, and 4 %	*Streptococcus iniae*	Intraperitonial 7.7×10^6 cells/mL	Decreased mortality	0.5%	Gabriel et al. (2015)
Oreochromis mossambicus	*Citrus limon*	Essential oils of the peel	0, 0.5%, 0.75% and 1%	*Edwardsiella tarda*	Intraperitonial 1.2×10^8 cells/mL	Decreased mortality	0.5%	Baba et al. (2016)
Oreochromis niloticus	*Cinnamomum camphora*	Ethanolic extract	2 g/kg	*Streptococcus agalactiae*	Intramuscular 1×10^6 cells/mL	Decreased mortality	2 g/kg Cinnamomum camphora	Kareem et al. (2016)
	Euphorbia hirta							
	Azadirachta indica							
	Carica papaya							
Oreochromis mossambicus	*Psidium guajava*	Aqueous and ethanolic leaf extracts	1, 5, and 10 mg/g	*Aeromonas hydrophila*	Intraperitonial 10^7 cells/ml	Decreased mortality	10 mg/g ethanolic extracts	Gobi et al. (2016)
Oreochromis mossambicus	*Nyctanthes arbortristis*	Methanolic extracts of seeds	2, 20 or 200 mg/kg (Intraperitonal)	*Aeromonas hydrophila*	Intraperitoneal 10^8 cells/mL	Decreased mortality	20 mg/kg	Kirubakaran et al. (2010)
Oreochromis niloticus	*Zingiber officinale*	Essential oil	0, 0.5, 1, 1.5%	*Streptococcus agalactiae*	Gavage 10^8 CFU g/fish	Decreased mortality	0.5% ginger	Brum et al. (2017)
	Ocimum gratissimum							
Oreochromis mossambicus	*Moringa oleifera*	Leaf powder	0, 3, 6, 9, and 12%,	*Aeromonas hydrophila*	0, 1×10^6, 1.5×10^6, 3×10^6 and 4×10^6 cells. mL			Mbokane and Moyo (2018)

Oreochromis niloticus	Assam tea (*Camellia sinensis*)	Ethanolic extracts	0, 1, 2, 4, and 8 g/kg	*Streptococcus agalactiae*	Intraperitonial 10^7 CFU/ml	Decreased Mortality	2 g/kg	Van Doan et al. (2019)
Oreochromis niloticus	*Elephantopus scaber*	ethanolic extracts	0, 2.5, 5, 10, and 20 g/kg	*Streptococcus agalactiae*	Intraperitonial 10^7 CFU/ml	Decreased Mortality	5 g/kg	Van Doan et al. (2019)
Oreochromis niloticus	Thai ginseng (*Boesenbergia rotunda*)	Powder	0, 5, 10, 20, 40 g/kg	*Streptococcus agalactiae*	10^8 CFU/ml	Decreased Mortality	10 g/kg	Van Doan et al. (2019)
Hybrid tilapia *Oreochromis niloticus* × *O. mossambicus*	*Psidium guajava*	Leaf powder	0, 1.5, and 3%	*Vibrio harveyi*	10^4 cells/mL	Decreased Mortality	1.5%	Ceballos-Francisco et al. (2020)
Oreochromis niloticus	Berberine	Powder	0, 1, 3, 6, and 9 g/kg	*Streptococcus agalactiae*	Intraperitonial 10^7 CFU mL^{-1}	Decreased mortality	1 g/kg	Van Doan et al. (2020)
Oreochromis niloticus	Seaweed (TrueAlgaeMax)	Aqueous extracts	0, 0.5, 1, 1.5, and 2%	*Aeromonas hydrophila*	Intraperitonial 1 × 108 CFU/mL	Decreased mortality	2%	Ashour et al. (2020)
Oreochromis niloticus	Watermelon rind powder	Powder	0 20, 40, 80, 160 g/kg	*Streptococcus agalactiae*	10^7 CFU/mL	Decreased mortality	40 g/kg	Van Doan et al. (2020)
Oreochromis niloticus	Licorice (*Glycyrrhiza glabra)*	Root powder (0, 5, 10, and 20 g/kg)	60-days	*Aeromonas hydrophila*	Intraperitoneal With LD_{50} was 1×10^6 cells/ml	No mortality	10–20 g/kg diet	Abdel-Tawwab and El-Araby (2021)

7.6 Medicinal Plants and Improved Immune System: Possible Mechanism of Action

The immune system is a complex physiological, chemical, and metabolic process that protects the organism against foreign materials such as bacteria, viruses, protozoa, fungi, *etc.*. The immune system is usually divided into two categories, innate (non-specific or natural) immune system, and adaptive (specific or acquired) immune system.

7.6.1 Innate Immune System

The innate immune system is present in almost 98% of all multicellular organisms and from a phylogenetic point of view, and it is the oldest immune system. The innate immune system act as the first line of defense against infection and humoral and cellular components of this system is present even before exposure to foreign material. As it is non-specific, the reaction mounted against a foreign material is always the same.

Components of the innate immune system include

i. Chemical and physical barriers *e.g.* skin and mucus
ii. Cellular and humoral components *e.g.* blood proteins (complement & acute phase, cytokines, natural killer cells, eosinophils, and phagocytic cells (macrophages and neutrophils))

The innate immune system of fish is highly robust (Rombout et al. 2005). Toll-like receptors (TLR) and complement components are the first lines of defense. Toll-like receptors are the principal inducer of innate immune response and complement components of the innate adaptive immune response.

Scales, skin, and mucus along with gills and the gastrointestinal tract provide physical protection against pathogens (Alexander and Ingram 1992). The innate immune response is initiated when there is tissue damage, or any pathogen is sensed by leukocytes. Inflammation is the first response of the innate immune system to evade pathogen replication and initiate the process of cellular repair. Many leukocytes i.e. Neutrophils, macrophages, monocytes, etc. are recruited to the site of inflammation. Each type has a different function but they work in precise coordination to subside the effects of pathogens.

The innate immune system of teleosts works through precise mechanisms to destroy pathogens. These mechanisms include degranulation, diapedesis, phagocytosis, oxidative burst, and opsonic and hemolytic serum activities.

Upon detection of the foreign substance, a large amount of anti-microbial molecules and inflammatory mediators are released from neutrophils and mast cells. This process is known as degranulation. These chemicals induce diapedesis or chemotaxis i.e. movement of leukocytes towards the site of infection so the pathogen and foreign molecule can be eliminated. Upon reaching the site of infection, leukocytes start the process of phagocytosis i.e. engulfing the foreign particles or pathogens. Pattern recognition receptors (PRRs) present on immune cells detect the conserved pathogen-associated molecular patterns (PAMPs) present on microbes and foreign bodies and make a PRR-PAMP complex which is engulfed by lysosomes.

Production and release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from leukocytes cause respiratory burst activity (Swindle et al. 2002). These ROS/RNS takes part in degranulation and diapedesis and also help in the removal of phagolysosomes. Along with these mechanisms, the innate immune response also modifies the foreign bodies/microbes/virus to increase their interaction with phagocytes and cytotoxic cells. This process is called Opsonization. The complement component of innate immune response can neutralize viruses by enveloping them (Whyte 2007).

In fish, cells of innate immune response include neutrophils, macrophages/monocytes, mast cells, non-specific cytotoxic cells, and other nuclear leukocytes.

Neutrophils are round with large granular vesicles. They are mainly present in hematopoietic tissues and the peritoneal exudate. Neutrophils are also present in the blood, gills, gonads, submucosa of the intestine, and skin (Mulero et al. 2007). In teleost, the primary function of neutrophils is phagocytosis (Sepulcre et al. 2002). Neutrophils also release reactive oxygen species, produce cytokines, and express a broad range of toll-like receptors (Sepulcre et al. 2002).

Macrophages/monocytes are horseshoe-shaped or round cells mainly present in the head kidney, spleen, thymus, gills, connective tissue, and peritoneal exudate (Lieschke et al. 2001). Macrophages sometimes form melanomacrophage centers, a dark-colored cluster in hematopoietic tissue (Herraez and Zapata 1986). The main function of macrophages is phagocytosis along with neutrophils, they also release ROS and various cytokines in fish (Uribe et al. 2011).

Mast cells, also known as eosinophilic granular cells (EGCs) in fish are very large, round-granulated cells. They are mainly present in the head kidney, spleen, epidermis, intestinal epithelial, and gastrointestinal-associated hematopoietic tissue (Da'as Uribe et al. 2011; Prykhozhij and Berman 2014). In fish, mast cells produce anti-microbial peptides that induce activation and chemotaxis of neutrophils (Prykhozhij and Berman 2014; Crivellato et al. 2015).

Non-specific cytotoxic cells are small agranulocytes and are present in the kidney, spleen, thymus, blood, and body fluid (Rubio-Godoy 2010; Uribe et al. 2011). They have cytotoxic activity against viruses, and protozoa and also produce a variety of cytokines (Utke et al. 2008).

Humoral components of innate immune response include lysozymes, antimicrobial peptides, protease, complement components, eicosanoids, reactive oxygen and nitrogen species (ROS/RNS), and cytokines.

Cytokines are regulatory molecules of the innate immune system and include tumor necrosis factor α (TNFα), interferons (IFN), interleukin (IL), and chemokines.

Tumor necrosis factor α (TNFα) is produced by leukocytes, it activates macrophages that leads to increased respiratory burst activity and phagocytosis (Whyte

2007; Uribe et al. 2011). It also induces the expression of cyclooxygenase 2 and leukocytes (IL-1β, IL-8) (Zou et al. 2003). Tumor necrosis factor-alpha (TNF-α) is capable of mounting a powerful antimicrobial response. It activates many host-responsive genes, induces apoptosis, and inhibits intracellular replication of the pathogen (Reyes-Cerpa et al. 2012). It also mediates the NF-κB signaling pathway (Rahman and McFadden 2006). Interleukin-8 is a chemokine that is produced by macrophages in response to pathogens and other cytokines like TNF-α.

Interferons (IFN-α and IFN-β) inhibit the viral replication within infected cells and also modify the expression of various immune genes thus providing protection against viral infection in infected and non-infected cells (de Veer et al. 2001; Samuel 2001; Uribe et al. 2011). Another important type of interferon is IFN-γ which is produced by T-cells and non-specific cytotoxic cells. IFN-γ in fish serves the same function as IFN-γ in mammals (Zou and Secombes 2016).

Interleukins include important pro-inflammatory and inflammatory cytokines. Interleukins are classified into 6 sub-categories (IL-1, IL-2, IL-6, IL-10, IL-12, IL-17) (Savan and Sakai 2006). IL-1 includes pro-inflammatory cytokines IL-1β and IL-18. Inteleukin-1β is expressed in macrophages and neutrophils and elicits a humoral response (Savan and Sakai 2006). Interleukin-18 is present in many different cells like T and B cells, and macrophages and induces secretion of interferon-γ (Whyte 2007). IL-2 derives lymphocyte differentiation; production of IFN-γ is also modulated by IL-2 (Díaz-Rosales et al. 2009; Corripio-Miyar et al. 2007). Il-6 and IL-12 are important inflammatory cytokines (Zante et al. 2015) while IL-10 is an anti-inflammatory cytokine that limits the production of pro-inflammatory cytokines (Karan et al. 2016).

Chemokines are a superfamily of cytokines that include CXC, CC, C, and CX3C classes. They are produced by many different cells and their major function is to attract and engage cells at the site of infection and convert innate immune response to adaptive response.

7.6.2 *Adaptive Immune Response*

The adaptive or specific immune response is specific for each pathogen and has a memory so upon successive encounters with the same pathogen; a more pronounced response is mounted (Male and Roitt 2001).

The main components of the adaptive immune response are

i. The cellular component includes lymphocytes (B and T-cells)
ii. The humeral component includes immunoglobulins like IgM, IgD, and IgT/Z.

T-cells are mainly produced in the thymus, spleen, and head kidney (Nakanishi et al. 2015). T-cells have also been detected in blood, gut, and skin (Rombout et al. 2005). In line mammals, T-cells are divided into T-cytotoxic cells and T-helper cells. Cytotoxic T-cells have the same cell-mediated cytotoxic function as their mammalian counterpart (Fisher et al. 2006).

In fish, B-cells are mainly produced by the head kidney and thymus, and spleen (Crowhurst et al. 2002; Salinas et al. 2011) and propagate in the intestine and peritoneal cavity (Rombout et al. 2005; Parra et al. 2015). B-cells produce immunoglobulins which precipitate and agglutinate the antigens for better phagocytosis. Antibodies also activate complement components (Rubio-Godoy 2010).

Antibodies are present in blood, skin, gills, intestine, and bile (Cain et al. 2000; Hatten et al. 2001). Different types of immunoglobulins are present in fish e.g. IgM, IgD, IgT/Z. IgM is the most common and predominant antibiotic found in fish (Rubio-Godoy 2010).

The immune system of organisms is affected by various endogenous and exogenous factors that lead to either stimulation or suppression of the immune system (Trichet 2010; Nawaz et al. 2018). The immune system of fish comprises an innate and adaptive immune system. Goblet cells present in fish skin secrete mucus (Sanahuja et al. 2019). Mucus is the first line of defense and provides a biological, physical, and immunological barrier against pathogens and other exogenous chemicals (Esteban 2012; Cordero et al. 2016). Many enzymes like proteases and antiproteases, lysozyme, peroxidases, phosphatases, and esterases made major components of fish skin mucus. Many immunoglobulins, complement components, and antimicrobial peptides are part of fish skin mucus (Adel et al. 2018; Mohammadi et al. 2020a, b).

In fish, major organs that possess hematopoietic activities are the kidney (head kidney), thymus, and spleen (Reyes-Cerpa et al. 2012). The major cells that mount the innate immune response by secreting cytokines are neutrophils, mast cells, macrophages, and non-specific killer cells (Awad and Awaad 2017). Some important pro-inflammatory cytokines secreted by the cells of the innate immune response are tumor necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8). Interleukin-8 is responsible for attracting lymphocytes and neutrophils and also induces a powerful respiratory burst activity (Laing et al. 2002). Interferon-γ (IFN-γ) is produced by T-lymphocytes and natural killer cells and is responsible for inducing synthesis of nitrous oxide, enhanced respiratory burst activity, and enhanced macrophage-mediated phagocytosis of the pathogen (Arts et al. 2010; Grayfer et al. 2010).

Herbs and medicinal plants can elicit the fish immune system by activating cells of the innate and adaptive immune system, phagocytosis, and complement system which result in protection against pathogens (Watanuki et al. 2006; Hoseinifar et al. 2020a, b). Different medicinal plants have been used in tilapia culture to improve the immune response (Table 7.3). Herbs interact with lymphoid organs associated with the skin (SALT), gills (GIALT), and gut (GALT) to enhance mucus immunity (Vallejos-Vidal et al. 2016; Martin and Krol 2017). The immunostimulatory properties of medicinal plants can also be attributed to various bioactive compounds. Polyphenols such as catechins can modulate the immune system by interacting with immune cells and regulating the secretions of chemokines and proinflammatory cytokines. Moreover, medicinal plants stimulate the immune system through respiratory burst activity. Hydrogen peroxide & superoxide anions produced as a result of respiratory burst activity provide a powerful antimicrobial activity; moreover, these

Table 7.3 Role of medicinal plants as immune-modulators in tilapia aquaculture

Fish	Plant used	Feeding rate	Duration	Endpoints studied	Observed effects	Optimal dose	Reference
Oreochromis mossambicus	*Tinospora cordifolia* (Ethanolic extract & petroleum ether extracts)	0.8, 8, or 80 mg/kg body weigh	2-days post-immunization	Serum immunity	Neutrophil activity (↑*), antibody production (↑*)	All doses of ethanolic extract. 0.8 mg/kg ether extract	Sudhakaran et al. (2006)
Oreochromis mossambicus	*Eclipta alba*	0, 0.01, 0.1, or 1%	1, 2 and 3 weeks	Cellular and humoral immunity	Lysozyme↑*, antiprotease↑* and complement ↑, myeloperoxidase content↑*, production of reactive oxygen and nitrogen species↑*	1%	Christybapita et al. (2007)
Oreochromis mossambicus	Solanum trilobatum	0, 4, 40, or 400 mg kg1 body weight of water or hexane soluble fraction		Nonspecific immune	Lysozyme activity (↑*), reactive oxygen species (↑*), reactive nitrogen species (↑*),	40 mg/kg water-soluble fraction 4 & 400hexanesoluble fraction	Divyagnaneswari et al. (2007)
Oreochromis mossambicus	*Toona sinensis* Hot water extract	4 and 8 μg/g injection.	2-weeks	Serum immunity	Respiratory burst activity ↑*, phagocytic activity ↑*, and lysozyme activity ↑*, total immunoglobulins (ns)	8 μg/g	
Oreochromis niloticus	*Cinnamon* *C. zeylanicum*	0.5%, 1.0%, or 1.5%		Blood plasma profile	Hemoglobin (HB)↑ Red blood cells (RBCs) ↑ Hematocrit (PCV) ↑ Total protein ↑ Total lipids ↑ creatinine ↓, urea ↓, aspartate	1% cinnamon	Ahmad et al. (2011)

					aminotransferase (AST) ↓, alanine aminotransferase (ALT) ↓, and glucose↓		
Hybrid tilapia (*O. niloticus* × *O. aureus*)	Garlic (*Allium sativum*)	0, 0.5, 1 g/kg	4-weeks	Cellular and humoral response	Total leucocyte count (↑*), Respiratory burst activity (↑*). Phagocytic activity (↑*), phagocytic index (↑*), Lysozyme activity (↑*)	0.5% garlic	Ndong and Fall (2011)
Oreochromis niloticus	*Viscum album coloratum*	0, 10, 50, and 200 mg /kg	80-days	Serum immunity	Respiratory burst activity ↑*, lysozyme activity ↑*, alternative complement haemolysis activity ↑* phagocytic Activity ↑*	50 mg/kg	Park and Choi (2012)
Oreochromis niloticus	Astragalus membranaceus	Astragalus polysaccharides (APS)	0.15%	Immunological parameters	Phagocytic activity (↑), the respiratory burst activity (↑), plasma lysozyme (↑), bactericidal activity (↑), superoxide dismutase (↑), glutathione peroxidase (↑), and amylase activity (↑), serum nitric oxide (ns) or Malondialdehyde (ns)		Zahran et al. (2014)
Oreochromis niloticus	An herbal mixture of *Astragalus, angelica*, hawthorn, Licorice	0.0, 0.5, 1.0, 1.5, and 2.0%	4-weeks	Serum immunity and Immune-related gene expression	Serum lysozyme, superoxide dismutase TNFα ↑*, IL-1β ↑*	2%	Tang et al. (2014)

(continued)

Table 7.3 (continued)

Fish	Plant used	Feeding rate	Duration	Endpoints studied	Observed effects	Optimal dose	Reference
	root and honeysuckle						
Gift tilapia	*Aloe vera*	0.5, 1, 2, and 4 %	8-weeks	Hematological parameters	Red blood cells ↑*, hematocrits ↑*, hemoglobin ↑*, white blood cells ↑*, neutrophils ↑*, monocytes ↑*, eosinophils↑* and serum total protein↑*	2 & 4%	Gabriel et al. (2015)
Oreochromis mossambicus	*Citrus limon* Essential oils of the peel	0, 0.5, 0.75, and 1%	60-days	Immunological & biochemical parameters	Total white blood cell ↑*, serum lysozyme activity↑* and myeloperoxidase activity ↑*, total proteins↑* , glucose ↓, cholesterol ↓ and triglycerides ↓	0.75%	Baba et al. (2016)
Oreochromis mossambicus	*Psidium guajava*	1, 5, and 10 mg/g aqueous and ethanolic extracts	30-days	Serum immunity	Serum myeloperoxidase activity ↑*, lysozyme activity ↑*, serum antiprotease activity ↑*, alternate complement haemolytic activity ↑*	10 mg/g ethanolic extracts	Gobi et al. (2016)
				Serum antioxidants	Superoxide dismutase ↑, Catalase ↑*, Glutathione peroxidase ↑*		
Oreochromis mossambicus	*Nyctanthes arbortristis*	2, 20, or 200 mg/kg injection of seed's	Not specified	Serum immune parameters	Lysozyme activity↑*, Myeloperoxidase activity ↑*, antiprotease activity ↑*	2 & 20 mg/kg	Kirubakaran et al. (2010)

		methanolic extracts					
Oreochromis niloticus	*Cinnamomum camphora*	2 g/kg	90-days	Biochemical parameters	Plasma protein (ns), creatine, albumin (ns) and AST (ns), ALT (↑*), plasma protein (ns), creatinine (ns), albumin (ns)	2 g/kg	Kareem et al. (2016)
	Euphorbia hirta				Plasma protein (ns), creatine, albumin (ns) and AST (ns), plasma protein (ns), creatinine (ns), albumin (ns)		
	Azadirachta indica				Plasma protein (ns), creatine, albumin (ns), AST (ns), ALT (↓*), plasma protein (ns), creatinine (ns), albumin (ns)		
	Carica papaya				Plasma protein (ns), creatine, albumin (ns) and AST (ns), plasma protein (ns), creatinine (ns), albumin (ns)		
Oreochromis niloticus	*Zingiber officinale Essential oil*	0, 0.5, 1, 1.5%	55-days	Hematological parameters	Ethtrocytes (ns, ↓, Hematocrit ↓, Hemoglobin ↓, Neutrophils, Monocytes	0.5 % ginger essential oil	Brum et al. (2017)
	Ocimum gratissimum Essential oil			Hematological parameters	Erythrocytes ↓, Hematocrit ↓, Total leukocytes ↑, Thrombocytes ↑,		

(continued)

Table 7.3 (continued)

Fish	Plant used	Feeding rate	Duration	Endpoints studied	Observed effects	Optimal dose	Reference
					Neutrophils ↑, Monocytes ↓		
Oreochromis mossambicus	*Moringa oleifera*	0, 3, 6, 9, and 12%,	45-days	Hematological parameters	WBC↑*, RBC ↑*, HGB ↑* and HCT ↑*		Mbokane and Moyo (2018)
Oreochromis niloticus	St John's-wort (*Hypericum perforatum*)	Ethanolic extract (0%, 0.2 %, and 0.5)	60 days	Digestive enzymes, hepatic antioxidants	Amylase (ns), Lipase (ns), Protease (ns)	0.5%	Mohammadi et al. (2020a, b)
	Lemon balm (*Melissa officinalis*)				Amylase↑*, Lipase ↑*, SOD ↑*, Catalase		
	Oregano (*Origanum vulgare*)				Protease ↑*, SOD ↑*, Catalase↑*		
Oreochromis niloticus	Assam tea (Camellia sinensis)	0, 1, 2, 4, and 8 g kg	4 & 8 weeks	Serum & mucus immunity	Serum lysozyme↑*, peroxidase↑*, alternative complement (ACH50) ↑*, phagocytosis↑*, and respiratory burst activities↑* mucus lysozyme ↑ and peroxidase activities ↑	2 g/kg	Van Doan et al. (2019)
Oreochromis niloticus	*Elephantopus scaber*	0, 2.5, 5, 10, and 20 g kg	4 & 8 weeks	Serum & Mucus immunity	Serum lysozyme↑* , peroxidase ↑*, alternative complement↑*, phagocytosis ↑*, and respiratory burst ↑*,	5 g/kg	Van Doan et al. (2019)

					mucus lysozyme ↑and skin peroxidase ↑		
Oreochromis niloticus	Thai ginseng (*Boesenbergia rotunda*)	0, 5, 10, 20, 40 g/kg	8 weeks	Serum & Mucus immunity	Mucus lysozyme and peroxidase activity ↑*, serum lysozyme ↑*, serum peroxidase ↑*, alternative complement ↑*, phagocytosis index ↑*, and respiratory burst activities↑*	10 g/kg	Van Doan et al. (2019)
Hybrid tilapia *Oreochromis niloticus* × *O. mossambicus*	*Psidium guajava*	0, 1.5, and 3%	21-days	Skin mucus immunity and gene expression	Mucus Protease ↑, antiprotease (ns), peroxidase ↑, and lysozyme activity (ns)	1.5%	Ceballos-Francisco et al. (2020)
Oreochromis niloticus	Berberine	4 & 8 weeks	0, 1, 3, 6, and 9 g/kg	Skin mucus	Skin mucus lysozyme ↑* and peroxidase activities ↑*; as well as improved serum lysozyme ↑, peroxidase ↑, alternative complement (ACH50) ↑, phagocytosis ↑, and respiratory burst activities ↑	1 g/kg	Van Doan et al. (2020)
Oreochromis niloticus	Seaweed (TrueAlgaeMax)	0, 0.5, 1, 1.5, and 2%	70-days	Serum immune response	Serum protein ↑*, lysozyme ↑*and respiratory burst activity ↑*	2%	Ashour et al. (2020)
Oreochromis niloticus	Watermelon rind powder	0 20, 40, 80, 160 g/kg	8-weeks	Serum & Mucus immunity	Mucus lysozyme & peroxidase activity ↑* serum lysozyme ↑*,	40 g/kg	Van Doan et al. (2020)

(continued)

Table 7.3 (continued)

Fish	Plant used	Feeding rate	Duration	Endpoints studied	Observed effects	Optimal dose	Reference
					peroxidase ↑*, phagocytosis ↑*		
Oreochromis niloticus	White button mushroom	0, 0.5, 1, 2, and 4%	60-days	Heamatological & gene expression	Hb ↑*, RBCs ↑*, and WBCs↑* INF-γ, TNF-α, and IL-1β	2%	Dawood et al. (2020)
				Antioxidants	Catalase ↑*, superoxide dismutase ↑*, glutathione peroxidase (ns)		
Oreochromis niloticus	Gotu kola (*Centella asiatica*)	0, 5, 10, and 20 g/kg	61-days	Serum & Mucus immunity	Skin mucus lysozyme ↑* and skin mucus peroxidase activities ↑* serum lysozyme ↑* and serum peroxidase activities ↑*, alternative complement ↑*, phagocytosis ↑*, and respiratory burst activities↑*	10 g/kg	Srichaiyo et al. (2020)
Nile tilapia, Oreochromis niloticus	Licorice (Glycyrrhiza glabra	Root powder (0, 5, 10, and 20 g/kg)	60-days	Digestive enzymes, anti-oxidants & immune parameters	Total protein↑*, albumin↑*, and globulin↑*	10–20 g/kg of feed	Abdel-Tawwab and El-Araby (2021)
					catalase↑*, superoxide dismutase↑*, and glutathione peroxidase↑*		
					Lysozyme activity↑*, respiratory burst↑*, and total immunoglobulin↑*		

Arrows indicate an increase (↑), a decrease (↓), a significant increase (↑*), and a significant decrease (↓*)

ions also influence the release of inflammatory cytokines (Klebanoff 1992; Rieger et al. 2010).

Medicinal plants/ herbs are well known for their immunomodulatory potential. Many herbs have immunostimulatory properties while many have anti-inflammatory properties. The immunoregulatory action of medicinal plants is dependent on the bioactive components. Medicinal plants exhibit anti-inflammatory action by inhibiting or activating many immune pathways. One possible mode of action may be the inhibition of enzymes involved in the arachidonic acid pathway. Arachidonic acid is formed from the lipid of the cellular membranes upon the action of phospholipase A2. This arachidonic acid produces inflammatory mediators. Many anti-inflammatory drugs block phospholipase A2 which leads to the inhibition of arachidonic acid synthesis and ultimately the formation of inflammatory mediators. Bioactive compounds present in the plants act as natural inhibitors of phospholipase A2 e.g., quercetin, a flavonoid, was the first plant-based inhibitor of phospholipase A2. Many other plants such as pimientillo (*Xylopia frutescens*), onion (*Allium cepa*), garlic (*Allium sativum*), and turmeric (*Curcuna longa*) have been reported to have phospholipase inhibitory activity. Medicinal plants also inhibit 15-lipoxygenases (LOX), an enzyme that is involved in the synthesis of leukotrienes from arachidonic acid. Leukotrienes are important mediators of pro-inflammatory and allergic reactions. Inhibition of LOX activity is linked with a decreased production of pro-inflammatory cytokines. Ethanolic and methanolic extracts of many plants can inhibit 15-LOX activity. Ethanolic extracts of *Ajuga genevensis* were found to inhibit 15-LOX up to 30% while methanolic extracts of *Petroselinum crispum* (parsley) and *Apium graveolens* (celery) inhibit LOX activity up to 50%. This inhibition activity may be attributed to the bioactive components like luteolin, luteolin-O-glucoside, caffeic acid, and apigenin present in plants.

Medicinal plants also exert their immunomodulatory action through the inhibition of prostaglandins. Prostaglandins are also known as eicosanoids. These are active lipid compounds produced from arachidonic acid and regulate blood flow, blood clotting, and inflammation. Cyclooxygenases (COX1 and COX-2) are responsible for the synthesis of prostaglandins from arachidonic acid. Non-steroidal anti-inflammatory drugs (NSAIDs) are used to block COX's active sites, lowering the synthesis of prostaglandins and thus decreasing inflammation. Bioactive compounds of many medicinal plants have NSAID-like modes of action that can block the activity of COX. Bioactive compounds, acetyl-β-boswellic acid present in *Boswellia carterii* and betulinic acid present in *Glycyrrhiza uralensis*, are active inhibitors of COX-1 while senkyunolide-O present in *Ligusticum chuanxiong* and cryptotanshinone, present in *Salvia miltiorrhiza* activity inhibit COX-2. Similarly, phenethyl-trans-ferulate present in *Notopterygium incisum* and roburic acid present in *Gentiana macrophylla* can actively inhibit both isoforms of COX.

Plants and their bioactive compounds exert their cellular regulation by activating or inhibiting different protein kinases e.g. protein kinase C and mitogen-activated protein kinase that are involved in various signal transduction pathways. These medicinal plants can directly or indirectly influence the production of pro-inflammatory cytokines. The DNA-binding capacity of different transcription factors (such as activator protein-1 or nuclear factor-kappa B) is also influenced by these compounds resulting in the regulation of downstream targeted genes.

7.6.3 Immunostimulatory Mode of Action

Immunostimulant is a term used for any molecule/compound that can stimulate the immune system of the organism. This immunostimulation can be specific through vaccines and antigens or can be non-specific through drugs or nutrients. Active compounds in plants and herbs can stimulate nonspecific immune responses. In the last decade, a lot of research has been done on finding the plant/herb or their active compounds that can stimulate the immune response in fish so the need for vaccines can be avoided. The use of herbal parts and their extracts are beneficial as they are cheap, can stimulate nonspecific immune responses leave no memory, are sustainable, and have almost no hazardous footprints on the environment.

Phagocytes play an important role in the non-specific immune response and exert an anti-microbial response through NADPH phagocyte oxidase or inducible nitric oxide synthase (iNOS) pathways.

Inducible nitric oxide synthase (iNOS) pathway can be activated by chemokines e.g. interferon gamma (IFN-γ) and tumor necrosis factor-α (TNF-α). Upon activation, this led to the production of reactive nitrogen species by macrophages. In an in vitro study with rat hepatocytes, it was reported that IFN-γ and TNF- α (alone or in combination with LPS) stimulate the inducible nitric oxide synthase (iNOS) pathway. Reactive nitrogen species also activate the NF-kB signaling pathway in the mononuclear cells of peripheral blood which is an important factor for proinflammatory cytokine transcription.

Another possible mechanism through which medicinal plants activate the immune system is through toll-like receptors (TLRs). These are transmembrane proteins and are the first to detect parasites, bacteria, fungi, and viruses. Around 17 TLRs have been identified in teleost. These TLRs may be located in endosomal and lysosomal compartments or maybe cell surface orientated. These TLRs are associated with adaptor proteins, each of which initiates a different signaling pathway. Two important adaptors that initiate immune-related signaling pathways are TRIF-related adaptor molecule (TRAM) and Myeloid differentiation primary-response protein 88(MyD88)> The end product of these signaling pathways may be the production of cytokines or activation of other pathways like MAPK and NF-kB pathway.

Toll-like receptors recognize the pathogen through pathogen-associated molecular patterns and activate the respective adaptor protein (either TRAM or MyD88). These activated adaptors in turn activate transcriptional factors which bind with the DNA and upregulate the synthesis of pro-inflammatory cytokines, interferons, chemokines, and anti-microbial peptides (Li et al. 2014). Medicinal plants and herbs influence the TLR pathway and stimulate the immune response. Supplementing the diet of yellow catfish with *Glycyrrhiza uralensis* stimulated the

TLR pathway and upregulate the expression of TLRs and MyD88 resulting in more production of interleukins. Similarly, supplementing the fish diet with a mixture of Chinese herbs resulted in the activation of TLR5 and MAPK pathways.

Different medicinal plants have been used in tilapia aquaculture as immune stimulants, details of which have been mentioned in Table 7.3.

7.7 Conclusion

The present chapter provides in-depth knowledge about the use of medicinal plants in tilapia aquaculture. In the light of literature reviewed here, it is suggested that the use of medicinal plants provides a sustainable, less costly, and eco-friendly alternative to antibiotics. Moreover, the use of medicinal plants has positive effects on the fish's physiological system by improving gut health, increase in digestive enzymes and antioxidants, and stimulating the immune system hence improving growth and disease resistance. Most importantly, supplementation of herbs/spices improves feed conversion ratio and feed utilization/absorption by fish. However, it must be kept in mind that the addition of a large number of medicinal plants may cause unwanted effects in fish and these effects may be species-specific. Therefore, the optimal range of medicinal plants inclusion and duration of feeding must be determined before the addition of that herb in tilapia feed on a large scale.

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Chapter 8
Alternative Protein Sources for Sustainable Tilapia Farming

Sherine Ragab, Mohmed Hassaan, Kevin Fitzsimmons, and Ehab El-Haroun

Abstract During the past 20 years, there has been a growing concern about how best to replace fish meals with more sustainable, cost-effective, and environment-friendly ingredients. A global crisis of high feed prices, expansion of the aquaculture sector, and the increasing proportion of farmed fish have run into a decrease in the supply of fishmeal products. Therefore, scientists have redoubled efforts to discover alternatives and more renewable ingredients for aquafeeds worldwide. The goal has been to protect ecosystems and increase economic viability based on enhanced efficiency of production facility outputs and nutritional efficiency. A key challenge though is sourcing sustainable, renewable protein ingredients. This chapter reviews the advances, novel ingredients, and new techniques that serve the mission for the future of aquafeed research on alternatives for fishmeal to achieve global aquaculture sustainability, with a focus on partial or full replacement of fishmeal, especially in the feed of tilapia.

Keywords Aquaculture · Sustainability · Plant-based protein · Animal-based Protein

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8.1 Introduction

The world population is predicted to reach 9.8 billion by 2050, which consequently will increase our need for protein by 30%–80% (Hua et al. 2019). However, the aquaculture industry faces big challenges to provide balanced amino acid proteins and other essential nutrients (SOFIA 2020; Barrows et al. 2007). Aquaculture will be considered a major source of animal protein in the near future to satisfy the nutritional needs of a rising human population (Gamboa-Delgado and Marquez-Reyes 2018). Already, animal nutrition plays a main role in aquaculture (Kaushik et al. 2011). Feed costs make up a significant fraction of total fish production costs, representing 40–70% of operating costs (USB 2008). Protein is the most essential nutrient needed for growth to be efficiently utilized and is relatively expensive. Thus, fishmeal (FM) has been considered an essential of aquafeeds, supplying over 60% of the protein total. Therefore, it has been the most costly ingredient in aquafeeds and has been the favorite dietary protein source for many fish feeds due to its essential amino acid profile, vitamin-mineral content, high digestibility, and palatability (Miles and Chapman 2006). The production of fishmeal fluctuates depending on several items including global environmental conditions including El Niño—La Niña oscillation, government fishing subsidies, and availability of fishing crews. The general situation for the last 30 years has been decreasing fishmeal supply as stocks are overfished and fishers resort to capturing juveniles of non-forage fishes., Therefore, the development of alternative sources of protein has become a priority of research in aquaculture. Affordable and sustainable alternative ingredients would compensate for the shortages of fishmeal and fish oil and help to achieve aquaculture sustainability and increase industry profitability (Ayadi et al. 2012; Nathaly et al. 2018; Alsager et al. 2018). The development of alternative ingredients would also address the competition for limited feed resources with other animal livestock, especially in the poultry industry (Hardy 2010). The aquafeed industry currently relies on derived ingredients to formulate cost-effective diets with a target by 2025 to supply 40 million tons of aquafeeds (Aas et al. 2019; Willora et al. 2020; Hua et al. 2019). Hence, FM is increasingly replaced with ingredients such as plant and terrestrial animal proteins, agriculture by-products, and microbial proteins to partially or replace FM without compromising the growth performance of the fish or feed utilization efficiency. In addition, the industry has learned to remove many Anti-Nutrition Factors in certain animal and plant-based ingredients by soaking, cooking, fermentation, chemical and enzyme treatment, and deficiency of particular amino acids by using additives (Archit et al. 2019). In this chapter, we review the beneficial use of alternative protein sources in aquafeeds for a more sustainable and secure future for the aquaculture industry, with the potential to partially or fully substitute for fish feed, especially in the feed of tilapia (Table 8.1).

Table 8.1 Summary of alternative proteins (animal, plant, and microbial) in aquafeeds

Source (specification)	Species fish	Levels (%) inclusion	Supported inclusion level (%)	Species (weight, g)	Type replacement	References
Animal protein sources						
Meat and bone meal	*Oreochromis niloticus*	0, 25, 50, 75, and 100%	50	1.80	Fish meal(FM)	Gomaa and El Moghazy (2014)
Poultry by-products	*O. niloticus*	10, 20, 30, or 40%	Up to 20	—	Soybean meal (SBM)	Dawood et al. (2020)
Poultry by-product	*O. niloticus*	50, 75, and 100	100	1.5	FM	Yones and Metwalli (2015)
Feather meal	Hybrid red tilapia (*Oreochromis niloticus x Oreochromis mossambicus*)	10% and 15	15	37	FM	Yong et al. (2018)
Feather meal	Hybrid red tilapia (*O.niloticus x O.mossambicus*)	10 and 15	15	18	SBM	Farahiyah et al. (2018)
Hydrolyzed chicken feather meal	Red tilapia	3, 6, 9, and 12	Up to 12	24.09	FM	Nursinatrio and Nugroho (2019)
Hydrolyzed feather meal	*O. niloticus*	33, 66, and 100	Up to 33–66	2.30	FM	Suloma et al. (2014)
Chicken feathers	*O. niloticus*	0, 12.5, 25, 37.5, and 50%	12.5	3.21	FM	Diana and Istiyanto (2019)
Black soldier fly larvae meal	Hybrid tilapia, Nile x Mozambique (*O. niloticus x O. Mozambique*)	0, 5, 10, 20, and 30%	30	2.6	SBM	Yildirim-Aksoy et al. (2020)
Black soldier fly maggot meal (BSFM)	*O. niloticus*	0, 25, 50,75, and 100%	Up to 50%	3.07 g	FM	Muin et al. (2017)
Plant protein sources						
Soybean meal	*O. niloticus*	0,32, 42, and 50,9%	Up to 42%	2.09	FM	Obirikorang et al. (2020)

(continued)

Table 8.1 (continued)

Source (specification)	Species fish	Levels (%) inclusion	Supported inclusion level (%)	Species (weight, g)	Type replacement	References
Soybean meal	*O. niloticus*	0%, 25%, 50%, and 75%	50%	15.58	FM	Ahmad et al. (2020)
Soybean meal	*O. niloticus*	0, 22, 44, 66, and 88%	Up to 22%	18.51	FM	Amer et al. (2019)
Soybean meal	*O. niloticus*	0, 5, 10, 15, and 20%	20%	3.22	FM	Sangeera and Reddy (2019)
Soybean meal	*O. niloticus x O. aureus*	0, 10, 20, 30, and 40%)	Up to 30%	32.47	Rubber seed meal	Deng et al. (2015)
Soybean meal	Hybrid tilapia (*O. niloticus x O. aureus*)	0, 33, 50, 67, and 100%	Up to 50	1.54	Shrimp shell meal	Fall et al. (2012)
Soybean meal	*O. niloticus* × *O. aureus*	25, 50, 75, and 100%	50%	4	FM	Lin and Luo (2011)
Soybean meal	Hybrid tilapia *(O.niloticus x O. aureus)*	0, 15, 30, 45, 60, and 100%	30%	56.3	Canola meal	Zhou and Yi-Rong (2010)
Fermented soybean meal (FSBM)	(*O. niloticus,* L.1758)	0, 20, 30, 40, and 50%	Up to 40%	0.74	FM	Yigit (2016)
Cottonseed meal	*O. niloticus*	12, 16, and 23%	12%	11.62	FM	Hassaan et al. (2019)
Cottonseed meal	*O. niloticus*	0, 25, 50, and 75%	50%	4.24	FM	Agbo et al. (2011)
Cottonseed meal	*O. niloticus*	0, 25, 50, 75, and 100%	Up to 25%	1.43	FM	Soltan et al. (2011)
Cottonseed meal	*O. niloticus*	0, 25, 50, 75, and 100%	25%	4.79	SBM	El-Saidy and Saad (2011)
Cottonseed meal	Hybrid tilapia (*O. niloticus x O. aureus*)	15, 30, 45, 60, and 100%	Up to 30%	6.24	SBM	Yue and Zhou (2008)

Sunflower seed meal	*O. niloticus*	0, 25, 50, or 75%	25	10.30	FM	Soltan et al. (2015)
Sunflower seed meal	Tilapia rendalli	10,20,30,40, and 50%	Up to 20	0.93 g	FM	Olvera-Novoa et al. (2002)
Fermented sunflower meal	*O. niloticus*	0, 25, 50, and75%	25	10.28 g	FM	Hassaan et al. (2018)
Canola meal	*O. niloticus*	0, 12.5, 25, 37.5, and 50%	25	0.1	FM	Mohammadi et al. (2019)
Canola meal	*O. niloticus*	0, 15, 30, 45, 60, and 75%	75%	20	FM	Luo et al. (2012)
Canola meal	*O. niloticus*	0,10,20,30,40, and 50%	Up to 10%	1.21	FM	Yigit and Olmez (2009)
Canola seed meal (CSM)	*O. niloticus*	0,25, 50, 75, and 100%	50%	8.42	FM	Soltan (2005)
Fermented canola meal	*O. niloticus*	0, 25, 50,75, and 100%	Up to 50	10	SBM	Pichet and Yakupitiyage (2014)
Lupin seed meal (LSM)	*O. niloticus*	0, 15, 30, 45, and 60%	30%	3	FM	Srour (2007)
Lupin seed meal (LSM)	Hybrid tilapia (*O. niloticus xO. Aureus*)	0, 33%, 67%, and 100%	Up to 33%	0.3	SBM	Chien and Chiu (2003)
Linseed protein	*O. niloticus*	0, 25, 50, 75, and 100%	Up to 75	12.4	FM	El-Saidy and Gaber (2001)
Microbial biomass (single-cell protein)						
Single-cell protein	*O. niloticus*	25, 50, and 75%	Up to 50%	15.39	FM	Al-Hafedh and Alam (2013)
Single-cell protein	*O. niloticus*	0, 10, 20, 30, 40, and 50%	Up to 50%	8	FM	Bob-Manuel and Alfred-Ockiya (2011)

(continued)

Table 8.1 (continued)

Source (specification)	Species fish	Levels (%) inclusion	Supported inclusion level (%)	Species (weight, g)	Type replacement	References
Microalga spirulina	*O. niloticus*	20, 40, 60, 80, and 100%,	40	26.7	FM	Olvera-Novoa et al. (2008)
Spirulina (Arthrospira platensis)	*O. niloticus*	0, 30, 45, 60, and 75%	30	0.89	FM	Stephanie et al. (2016)
Spirulina (Spirulina platensis)	*O. niloticus*	0, 5, 10, 15, and 20%	Up to 20	—	SBM	Abo El-Ward et al. (2016)
Spirulina (Arthrospira platensis)	Hybrid red tilapia (*O.niloticus x O. mossambicus*)	0, 50, 75, and 100%	75	0.23	FM	El-Sheekh et al. (2014)
Green seaweed Ulva	*O. niloticus*	0, 10, 20, and 30%	10	3.5	FM	Suryaningrum and Samsudin (2019)
Seaweed Ulva	*O. niloticus*	0, 10, 20, and 30%	10	3.42	FM	Lusi and Reza (2020)

8.1.1 Animal-Based Protein Sources

Animal by-products are well known as valuable sources of protein in aquafeeds and are recognized for their high content of protein, comparable amino acid profiles, ready availability within the market, and competitive price (Hassaan et al. 2018; Moutinho et al. 2017). The types of animal protein mainly are meat and bone meal (MBM), poultry by-product meal (PBM), feather meal, and insect meal (Bureau et al. 2000; Metts et al. 2011; Wu et al. 2018).

8.1.1.1 Meat and Bone Meal

Meat and bone meal (MBM) is typically 48–52% protein, 33–35% ash, 8–12% fat, and 4–7% water. Meat and bone meal (MBM) has several advantages including (i) high protein content, minerals, good amino acid profile, lack of anti-nutritional factors, and a reasonable price usually less than fish meal (Ferouz, et al. 2012; Suloma et al. 2013). The nutritive value of meat and bone meal depends on the quality of raw materials and the processing techniques (Kureshy et al. 2000). However, the occurrence of bone, high levels of ash and mineral content can limit the amount used in fish diets (Bureau et al. 1999). A recent research stated that meat and bone meal could positively substitute for fish meal for up to 75% of diets for Mozambique tilapia or seabream without compromising performance (Gomaa and El Moghazy 2014; Davies et al. 2020). In addition, Yang et al. (2004) and Hu et al. (2008) found that MBM supplemented with lysine and methionine successfully replaced up to 50% of the fishmeal enhancing growth performance for gibel carp (*Carassius auratus gibelius*).

8.1.1.2 Poultry by-Products

Poultry by-product meal (PBM) is often considered the most important animal protein source for replacing fish meal (Meeker and Hamilton 2006). The AAFCO (USA) defines poultry by-product meal as the ground, rendered, clean parts of the carcass of slaughtered poultry such as necks, heads, feet, undeveloped eggs, gizzards, and intestines (provided their content is removed), exclusive of feathers (Watson 2006). Whole poultry carcass meal can also be obtained from culled laying hens (spent hen meal), notably in areas where there is no market for culled hens (Hertrampf and Piedad-Pascual 2000). Poultry by-product meal (PBM) has been shown to substitute for 80–90% fishmeal in fish feed. With high protein content (55–75%), and an essential amino acids profile similar to FM's AAs profile, except for lower lysine and methionine levels, PBM can lower costs in fish diets (Gupta et al. 2020; Gonzalez-Rodrıguez et al. 2016; Heuzé et al. 2015). However, the composition of PBM depends on processing conditions and drying techniques, and the original raw materials (Johnson and Parsons 1997). Previous trials have been conducted to investigate the effects of replacing FM by PBM for several fish species including Nile tilapia, redbelly tilapia (*Coptodon zillii)*, and hybrid striped bass (Metwalli 2008; El-Husseiny et al. 2006; Yones and Metwalli 2015; Dawood et al. 2020; Yildrim et al. 2009; Rawles et al. 2009). Tacon et al. recommended that 10–30% of PBM could replace FM depending on the type of fish and processing technique. Recent research conducted by Yones and Metwalli (2015) found that up to 100% of FM could be replaced by PBM with positive impacts on the growth of *O. niloticus*. El-Haroun and Burea (2007); El-Haroun et al. (2009) found using blood meal, or a combination of animal protein including PBM up to 30% in rainbow trout diets, could replace fish meal with growth matching the control diet with fish meal as the main source of protein. Furthermore, Dawood et al. (2020) found that PBM could replace up to 20% of FM content, without any negative impacts on the performance and feed utilization efficiency of Nile tilapia. NRC (2011) concluded that two main factors could limit the high inclusion of PBM in aquatic feeds; (i) high lipid content, and (ii) the deficiency of DHA and EPA in lipids of PBM.

8.1.1.3 Feather Meal

Feather meal (FEM) is a by-product of poultry processing, made from poultry feathers by partially grinding them under pressure and heat, and then additional grinding and drying. Although total protein content levels are high, the bioavailability of this nitrogen may be low due to the difficulty of digestion and assimilation from the presence of beta-keratin and fibrous fiber components (Adelina et al. 2019). This has been a constraint of using FEM in aquafeeds. Several protocols and solutions have been developed to mitigate these challenges and enhance- diet quality, flavor, and improvement in terms of digestibility (Nursinatrio Nugroho 2019) including (i) hydrolysis and; (ii) fermentation techniques improving protein digestibility and palatability (Isika et al. 2006; Madigan and Martinko 2005). Laporte et al. (2007) and Davies et al. (2009) demonstrated that using these techniques improved the coefficients of crude protein (CP) from 67 to 87% indicating a significant improvement in the digestibility of feather meal. These enhancements could be attributed to good manufacturing techniques and the optimization of the processing conditions (Poppi et al. 2011). Thus, the inclusion of feather meals in diets can decrease the cost of feed and thus help in the exploitation of these by-products (Bishop et al. 1995). Kumari and Sundarabarathy (2011); Suloma et al. (2014) and Abwao et al. (2017) found that FEM could replace up to 65% of FM in diets for Nile tilapia without negative impacts on performance or feed utilization. Similarly, Farahiyah et al. (2018) and Yong et al. (2018) found that FM could be replaced at 15–30% with feather meal without compromising the growth performance, feed utilization, and protein efficiency in juvenile red hybrid tilapia and Nile tilapia.

Hydrolyzed feather meal (HFM) is another type of feather meal with keratins being hydrolyzed, that is commonly used due to its high crude protein content (74%–91%) (Campos et al. 2017; Divakala et al. 2009; Munguti et al. 2014; Suloma et al.

2014) and is highly digestible ~80% (Davies et al. 2020). Recent research confirmed this idea as Yu et al. (2020) and Suloma et al. (2014) found that up to 25–45% or 66%, respectively, of FM, could be replaced by HFM without affecting growth rate or feed utilization on *O. niloticus*. Nursinatrio Nugroho (2019) and Yones and Metwalli (2015) reported that 12% of hydrolyzed feather meal incorporated into red tilapia and juvenile Nile tilapia diets enhanced growth compared to the control with fish meal as the sole source of protein.

8.1.1.4 Insect Meal

Historically, the European Union, the US, and other countries had strict regulations regarding insects and insect parts allowed in feed ingredients or finished feed. With the advent of farmed insect meals specifically as an ingredient, regulations are being rewritten. In 2021, there are about ten insect species that have received approval for use in animal feeds in major markets and are considered non-pathogenic (Wang and Shelomi 2017). The most heavily researched are the black soldier fly, the common housefly, and the yellow mealworm. Larval meals from these as well as crickets, grasshoppers, and silkworms all have high levels of crude protein (similar to soymeal and fishmeal) and lipids. Insect meals show promise as an ingredient in aquafeed due to their increasing availability and superior protein content of up to 60–70%, depending on the stage and species of insect (Prachom et al. 2021) and balanced amino acid profiles, vitamins, and minerals similar to that of FM (Barroso et al. 2014; Henry et al. 2015; Gasco et al. 2019). Their amino acid composition is comparable to FM and protein digestibility is high, and these can substitute for 25–100% of soybean meal or fishmeal protein in aquafeeds. High oil content in some insect meals can be extracted to produce another value-added product, biodiesel; and the defatted insect meals have protein content even higher than soybean meal or fishmeal. Several insect meals are recognized for providing high productivity and low FCR (Berggren et al. 2019; IPIFF 2018; Bruni et al. 2018). The main challenge of using certain insect meals is their unfavorable fatty acids (FA')s profiles, which can be altered by the feed offered to the insect larvae or by removal of FAs using different processing and drying techniques (Lock et al. 2016; Ferrer et al. 2019).

Defatted insect meal can contain up to 83% crude protein (Makkar et al. 2014a, b). Previous studies confirmed that insect meals can partially or completely replace FM (Silvia et al. 2019; Tran et al. 2015; Henry et al. 2015). Belghit et al. (2019) and Borgogno et al. (2017) found that 100% of fish meal could be replaced with different types of insect meals that could help to boost growth performance, disease resistance, and immune response (Ido et al. 2019). Confirming this hypothesis (Gasco et al. 2016; Iaconisi et al. 2018; Magalhães et al. 2017; Renna et al. 2017; Bruni et al. 2018; Devic et al. 2018; Wang et al. 2017) found that up to 70% substitution of fish meal with the black soldier fly meal obtained growth performance and feed efficiency matching basal diets with the fish meal as the main source of protein. Kurniawan et al. (2018) reported using BSF that up to 14% replacement of fish meal improved growth and FCR ($P \leq 0.05$) in Nile tilapia with replacement of fish meal in the control diet. Successful 75% FM substitution with *Musca domestica* (MD) was reported in Nile tilapia (Ogunji et al. 2007) and in hybrid tilapia up to 30% (Yildirim-Aksoy et al. 2020). The sum of this research highlights the importance of FM replacement with BSF and variation with larval meal level depending on fish species and processing techniques (Henry et al. 2015).

8.1.2 Plant-Based Protein Sources

Modified plant proteins treated with exogenous enzymes or solid-state fermentation (SSF) are finding their way into aquafeeds, as a complete or partial replacement for FM due to their low cost and increasing availability. Modified plant protein sources including soybean oil meal (SBM), sunflower meal, and cottonseed meal are considered promising ingredients to partially or totally replace fish meal in aquafeeds (Puja et al. 2019). The main challenges of using these untreated ingredients are (i) low initial protein content, (ii) high fiber content (iii) the presence of antinutritional factors (ANFs), (iv) unbalanced profile of EAA, and (v) deficit of essential AAs lysine and methionine, and low palatability which negatively impact their use in fish diets (Henry et al. 2015; Naylor et al. 2009; Oliva-Teles et al. 2015). Different strategies are used in ingredient processing to mitigate and avoid the negative impacts of ANF on plant ingredients (Puja et al. 2019). Soaking, heat treatment, fermentation, and enzymatic treatment are available as detoxification methods (Aguihe et al. 2017). Modified plant proteins are providing partial or total replacement of FM in many fish species (Hassaan et al. 2019; Bowyer et al. 2020), based on new advances in feed technology processing and gene technology. Many studies to evaluate alternatives to FM have been conducted with plant proteins, which have achieved success in fish diets (Glencross et al. 2011; Ngo et al. 2016; Agbo et al. 2015; Al-Thobaiti et al. (2017). These studies reported the substitution of FM by a mixture of plant protein sources (soya bean meal, cottonseed meal, groundnut cake, corn gluten meal, and wheat gluten meal) or using new technology including solid state fermentation (SSF) was able to replace FM (50%) in the diets for Nile tilapia (*O. niloticus*) and 75–100% in common carp and seabream respectively without affecting growth performance or feed utilization. The results showed that fish meals can be replaced up to 100% by a mixture of plant proteins without a negative impact on growth, feed utilization, and fish health.

8.1.2.1 Soybean Meal

Soybean meal (SBM) is certainly the most common plant protein source to replace FM protein in fish diets with its high protein content (45–55%) (Ozkan et al. 2015). A challenge of using SBM in fish diets is ANFs (Cabral et al. 2011; Gerile and Pirhonen 2017). To maximize the incorporation of SBM in fish diets it is necessary to eliminate or inactivate its ANFs content (Sales 2009). Different processing techniques have been adapted to remove ANFs to adjust and enhance the nutritive value of SBM including soaking, solvent-extracting, or fermenting processes (Hotz and Gibson 2007). Selective breeding and advanced genetic tools have been employed to increase the level of essential AAs that were otherwise limited in older soybean varieties to mimic FM (Llanes and Toledo 2011; Hassaan et al. 2015).

Feeding trials with several species of fish reported that improved versions of SBM could enhance performance, digestibility, and immune function compared to traditional SMB and FM (Bansemer et al. 2015; Ferrara et al. 2015; Liu et al. 2017; Grisdalle-Helland et al. 2002; Bonaldo et al. 2008; Zhou et al. 2011). Choi et al. (2019) reported that fermented SBM was used to replace 40% of FM without a negative impact on the growth performance and feed utilization of rainbow trout. Ye et al. found that up to 75% of FM could be replaced by a complementary mixture of plant protein (soybean meal, corn gluten meal, and cottonseed meal) without significant differences in growth and feed utilization of hybrid Nile tilapia. Also, Obirikorang et al. (2020) found that up to 42% of fish meals could be replaced with SBM in Nile tilapia and up to 30% in hybrid tilapia. However, as the price for fermented SBM is increasing there is interest in less-traditional plant protein sources to improve the sustainability of aquafeeds, aquaculture, and farmers' profitability (Fawole et al. 2016).

8.1.2.2 Cottonseed Meal (CSM)

The more common end-efficient technique used for the production of cottonseed meal is the extraction of oil from cottonseed meal. Cottonseed meal (CSM) has been used as an alternative ingredient to both fishmeal and soybean meal due to its lower cost, being readily available in some countries, particularly in the USA, China, India, and Egypt. Cottonseed meal has advantages including (i) nutritive value, (ii) good palatability. (iv) low cost and (vi) good protein content which is variable (23–53%) contingent on the processing (Yue and Zhou 2008; Ng and Romano 2013; El-Saidy and Saad 2011). The main challenge of using cottonseed meal in aquafeed could be summarized: (i) low levels of EAAs such as methionine and lysine., (ii) CSM contains high concentrations of the ANFs phytic acid and gossypol, and (iii) CSM contains a toxic pigment that decreases lysine availability (Ayadi et al. 2012). Previous trials reported that CSM was used to replace FM and SBM in the diets of *O. niloticus* without effects on growth performance (El-Saidy et al. 2012; Agbo et al. 2011). Sun et al. (2015) reported that CSM replaced up to 16% of FM in a feed trial for black seabream without negative effects on performance and feed conversion ratio. Additional research proved the benefits of using CSM to replace FM for hybrid tilapia and Nile tilapia (Yue and Zhou 2008; Soltan et al. 2015; El-Saidy and Saad 2011).

8.1.2.3 Sunflower Seed

The nutritive value of sunflower meal is similar to that of soybean meal and contains high amounts of sulfur amino acids. However, it contains relatively high amounts of crude fiber, low lysine content, and contains high ANFs protease and arginase inhibitors which have limited the incorporation of CSM in aquafeed (Gonzalez-Salas et al. 2014; Ochieng et al. 2017). Previous research concluded that the quality and quantity of protein in the sunflower meal depended on the oil extraction technique.

Ochieng et al. (2017) reported that up to 25% of FM could be replaced with CSM in Nile tilapia diets without ($P \geq 0.05$) impacting growth. Soltan et al. (2015) found that up to 25% of FM could be replaced by fermented sunflower meal for Nile tilapia, without negative impacts on performance, feed consumption, or protein efficiency ratio. Abdel et al. (2008) confirmed the possibility of substituting up to 30% of soy protein with sunflower and sesame seed for Nile tilapia without impacts on performance and carcass composition. However, the high inclusion of sunflower could affect protein conversion in the fish. In addition, (Abdel et al. 2010; Mugo-Bundi et al. 2013) found that the protein level in the body composition tended to decrease consistently with the high ash and crude fiber content in the diets. When testing the cumulative presence of sunflower meal up to 100% replacement, increasing the inclusion of CSM led to reduced digestibility and consequently a decrease in the growth rate of fish.

8.1.2.4 Rapeseed or Canola Seed

The chemical composition of rapeseed oil meal or canola protein concentrate (CPC) contains high levels of crude protein equal to FM with high content of lysine and methionine compared to SBM (Gonzalez-Salas et al. 2014). Friedman (1996) stated that the amino acid profile of canola protein is superior compared to many other plant proteins. However, some disadvantages limit the inclusion of rapeseed meals in both fresh and marine water species such as the high amount of fiber and ANFs including glucosinolates, tannins, and phytate (Ayadi et al. 2012). Burr et al. (2013) reported that for *Salmo salar,* there was not a significant difference in growth comparing the salmon fed the 0% CPC and the 10% CPC diets ($p > 0.05$). Canola protein concentrates significantly depressed growth when included in the diet at 20%, but not at 10%, indicating that canola could be used as a minor feed ingredient when available. The nutritive value and quality of canola meal rely on the intensity of heat and drying techniques used within oil extraction. However, excessive heating during the processing leading can lead to the reduction of protein digestibility and amino acid availability, especially lysine (Ayadi et al. 2012). An early trial conducted by Lim et al. (1997) found that rapeseed meal protein was a cost-effective replacement for FM. Soares et al. (2001) stated that rapeseed meal could substitute for 49% of SBM in tilapia diets. Mohammadi et al. (2019) found that canola meal can replace up to 35% of SBM or FM for hybrid tilapia without any impact on the growth of fish. Plaipetch and Yakupitiyage (2014) found that up to 50% of yeast-fermented canola meal in *O. niloticus* could be included in a diet without a negative impact on growth, survival, or feed consumption. Though, a high inclusion level of rapeseed meal up to 75–100% had an adverse effect on nutrient digestion and protein utilization efficiency of Nile tilapia.

8.1.2.5 Lupin Meal and Pea Meal

Lupin meals (LM) are recognized to be a unique replacer of FM or SBM due to their high content of protein (up to 45%), balanced nutritional characteristics, palatability, high digestibility, and stable price in the market (Bartkiene et al. 2015; Rajeev and Bavitha 2015; Lucas et al. 2015). The major concern about the inclusion of lupins in aquafeeds is the presence of some ANFs, which can reduce nutrient availability and digestibility (Petterson 2000; Francis et al. 2001). Burel et al. (2000) found up to 45% extruded lupin meal diets could substitute for fishmeal with positive support of the growth performance of rainbow trout, and turbot (*Psetta maxima*). Abdel-Moneim Yones (2010) found that LM succeeded to replace FM in hybrid red tilapia without affecting growth performance. The appropriateness of lupin meal in aquafeeds could have a positive effect on the fish diet, as it would alleviate the dependence on imported plant-based proteins (Anwar et al. 2020). Furthermore, Vo-Binh et al. (2015) reported that fermented lupin meal succeeded to replace 70% of FM without significant changes in performance and FCR compared to the basal diets in Nile tilapia containing FM as a main source of protein. The findings of these results stated that FM and soybean meal could be replaced by lupin meal without affecting performance, survival, and nutrient utilization efficiency (Pham et al. 2020).

Øverland et al. (2009) reported that pea protein concentrate was shown to be a promising new protein ingredient for salmonids and could replace 20% of high-quality fish meal protein in the feed without any adverse effect on growth performance, carcass composition, or histology of the gut. Schulz et al. (2007) reported that for juvenile Nile tilapia inclusion of 30% protein derived from pea protein isolate in the diet resulted in a growth performance (in terms of WG and SGR) that did not differ significantly from the diet with 100% of protein derived from FM.

8.1.2.6 Linseed Meal

Linseed meal has been recognized for its high protein content (30%), after the extraction of its oil (Oomah and Mazza 1993). However, it has some components limiting its use in fish nutrition (i) high fiber content (37%) and its water retention capacity are considered responsible for the depletion of growth that reduces food intake (Ndou et al. 2017; Goulart et al. 2015), and (ii) trypsin inhibitor and total polyphenolic compounds which may impair the assimilation and availability of the nutrients (Soltan 2005; Francis et al. 2001). The high fiber content could contribute to a high capacity to engross water and increase volume thereby limiting intestinal topography capacity and degrading the nutrients (Eastwood et al. 2009). The application of protein isoelectric precipitation is a solution to mitigate the ANFs, increase the incorporation of linseed in aquafeeds and increase protein concentration and eliminate the antinutrients components in fish diets, (Lovatto et al. 2014, 2015, 2016). Soltan (2005) compared raw LSM with roasted LSM and autoclaved LSM as substitutes for SBM in tilapia diets. The trials demonstrated that there was no significant difference in growth or FCR with up to 25% replacement of SBM with roasted or autoclaved LSM but a 7% decrease in feed cost. At the level of 75% replacement, there were significant decreases in growth and FCR, but feed costs were decreased by 18%. Pianesso et al. (2020) found that up to 40% linseed protein concentrate (LPC) could replace FM in silver catfish without influence on growth and nutrient efficiency. Wei et al. (2018) found that the replacement of fish oil with linseed oil in the fish feed did not affect performance, SGR, feed efficiency, or survival rate in Nile tilapia. Hanafy (2006) tested several diet formulations including LSM and concluded that soybean could be completely replaced by linseed in Nile tilapia diets without adverse effects on the growth rate when diets supplemented with *Yucca schidigera* as an additional nutrient and functional ingredient source.

8.1.3 Microbial Biomass and Macro-Algae (Seaweeds)

Recently feed nutritionists are examining different sources of microbial proteins (bacteria, algae, and yeast) as novel ingredients for fish (Pacheco-Vega et al. 2018). Single-cell proteins (SCP) have the highest protein content (65–85%) and contain balanced essential amino acid profiles. Moreover, SCP products are rapidly increasing in volume, renewable, and constitute a variety of compounds that can be blended to substitute for protein sources such as FM and SBM (Intraco 2020; Sharif et al. 2021; Hua et al. 2019; Jones et al. 2020). The quality and quantity of single-cell proteins vary widely due to feed sources, type of bacteria, algae or yeast, conditions of fermentation, and processing technique (Øverland et al. 2010). Previous research conducted by (Zamani et al. 2020; Gamboa-Delgado et al. 2016; Zhao et al. 2015; Rosales et al. 2017; Tlusty et al. 2017; Kalhoro et al. 2018; Sun et al. 2015; Chen et al. 2020) found that SCP could replace FM of SBM up to 60% in *O. niloticus* diets without effects on growth rate feed consumption. Likewise, Aas et al. (2006) found that yeast biomass could be included in 30% of the diet instead of fishmeal with no effect on fish growth rate and nutrient absorption. The benefits of microalga biomass as a feed additive or substitute for FM or SBM in tilapia fish feed have been documented with positive impacts on growth and feed efficiency (Gamboa-Delgado and Marquez-Reyes 2018; Sarker et al. 2018). The inclusion of *Spirulina* up to 10% has been reported as a feasible alternative to enhance the nutritional value in the feed of Nile tilapia (Rosas et al. 2019; Van Vo et al. 2020). Also, up to 30% replacement of fish meal by *Arthrospira maxima* without negative impact on the performance of red tilapia has been reported (Rincón et al. 2012). Badawy et al. (2008) reported that the substitution of up to 50% FM with *Chlorella* sp. and *Scenedesmus* sp. enhanced the growth of Nile tilapia. Replacement of fish meal by *Spirulina platensis* at up to 100% did not cause negative effects on growth in various fish species Nandeesha et al. (2001).

The use of seaweed as an aquaculture feed ingredient has demonstrated positive responses to enhance growth performance, feed utilization, enhance serum parameters, immune responses, hematological parameters, and disease resistance of many fish species; gray mullet (*Mugil cephalus)*, rainbow trout, Asian sea bass (*Lates calcarifer*), Nile tilapia, rabbitfish, red tilapia, and Atlantic salmon (Zeraatpisheh et al. 2018; Akbary and Aminikhoei 2018; Akbary et al. 2018; Yangthong et al. 2016; Meurer et al. 2009; Hussein 2017; Ashour et al. 2020; Doan et al. 2017; Yengkhom et al. 2018; Hosseinifer et al. 2020; Yengkhom et al. 2018; Abdel-Aziz and Ragab 2017; El-Tawil 2010; Collins et al. 2019), without any negative effect on fish. Pratiwy et al. (2018) found that the inclusion of 8% *Sargassum* meal enhanced the growth of Nile tilapia. Younis et al. (2018) reported that up to 20% of red algae inclusion in the diet improved the performance, feed utilization, and body composition of tilapia.

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Chapter 9
Sustainable Tilapia Farming, the Role of Culture Systems

Hien Van Doan

Abstract Considering environmental sustainability and vulnerability to the effects of climate change on fish production, sustainable and adaptable aquaculture systems are necessary". Biofloc technology (BFT) and recirculating aquaculture systems (RAS) are eco-friendly, water efficient, highly productive intensive farming systems, which are not associated with adverse environmental impacts, such as habitat destruction, water pollution and eutrophication, biotic depletion, ecological effects on biodiversity due to captive fish and exotic species escape, disease outbreaks, and parasite transmission. Moreover, BFT and RAS operate in an indoor controlled environment, and thus, are only minimally affected by climatic factors, including rainfall variation, flood, drought, global warming, cyclone, salinity fluctuation, ocean acidification, and sea level rise. This chapter provides into insight the application of these techniques for sustainable tilapia production, which focuses on their effects on growth performance, immune response, and disease resistance.

Keywords Tilapia · Sustainable · Biofloc technology · RAS

9.1 Introduction

Aquaculture, is one of the fastest food-producing sectors, with an average annual growth rate of 5.3% during the period 2001–2018, and production has increased by over 600% since 1990 (FAO 2020b). Global aquaculture production achieved 82.1 million tons in 2018, of which inland aquaculture produced 51.3 million tons (62%), while coastal and marine aquaculture1 reached 30.8 million tons (38%) (FAO 2020b). Aquaculture is performed in various environments and regions, employing various technologies, and cultured systems, and raising many species (Ahmad et al. 2022). Asia accounts for around 90% of global aquaculture production (FAO 2020a, b), and aquaculture, with its expansion outpacing global population growth,

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is playing an important role in boosting food production for contribution to food security and human nutrition (Pradeepkiran 2019). Although the expansion of aquaculture, the challenge of feeding a growing global population, which is expected to reach 9.7 billion by 2050 (Bahar et al. 2020), is a vivid reality, which is central in global political and scientific debates (Berners-Lee et al. 2018). Because of population growth and stagnation of capture fisheries, global aquaculture production is challenged to achieve remarkable targets, estimated to possibly reach 109 million tons in 2030 (FAO 2020a, b), and 140 million tons in 2050 (Waite et al. 2014).

To achieve the required further increase in global seafood production, aquaculture is envisaged as the only available solution, but it could bring additional adverse environmental effects if its expansion is not based on sustainable farming systems (Ahmed and Turchini 2021). Accordingly, the rapid growth of aquaculture has been linked to raising concerns about its environmental sustainability (Ahmad et al. 2022; Tom et al. 2021). Broadly, aquaculture has already been increasingly associated with a great variety of negative environmental impacts, including habitat destruction, water pollution and eutrophication, biotic depletion, disease and parasite transmission, and greenhouse gas (GHG) emissions (Ahmed et al. 2019; (Carballeira Braña et al. 2021; Adegbeye et al. 2019; Kosten et al. 2020). For example, some intensive aquaculture practices have been reported to cause antibiotic pollution, eutrophication, land occupation, and other environmental hazards (Dauda et al. 2019). The invasion of exotic fish species in some aquaculture systems has been reported to have potentially negative effects on biodiversity and the ecosystem; (Banha et al. 2022; Kiruba-Sankar et al. 2018). Freshwater aquaculture, particularly tilapia, has been reported to cause adverse effects on water resources with a broad range of impacts on the biodiversity, ecosystems, and societies (Bashir et al. 2020; Kaleem and Bio Singou Sabi 2021; Moyo and Rapatsa 2021). Tilapia aquaculture has also been censured for a variety of environmental issues, including water pollution and pathogen transmission to wild fish (Shaaban et al. 2021). The environment, on the other hand, has a series of impacts and imposes certain limitations on aquaculture, with climate change posing a significant threat to increasing global fish production (Baag and Mandal 2022; Maulu et al. 2021). A wide range of climatic factors, such as rainfall, flood, drought, cyclone, global warming, sea surface temperature change, salinity fluctuation, ocean acidification, and sea level rise have a significant impact on aquaculture practices (Ahmed et al. 2019). In essence, future climate change will certainly have adverse impacts on sustainable increasing aquaculture production (Ahmed et al. 2019; Boyd et al. 2020). It is therefore necessary to develop and implement adaptation strategies to cope with these challenges.

Considering the environmental concerns and impacts, as well as the vulnerability to the effects of climate change and other environmental variables of fish production in aquaculture, one of the potential and increasingly proposed adaptation strategies is the implementation of sustainable aquaculture systems, such as BFT and RAS. These systems allow for rising fish in a land-based, indoor, and controlled environment to minimize the direct interactions between the production processes and the environment (Ahmed and Turchini 2021; Khanjani et al. 2022). They offer benefits in improving aquaculture production that could contribute to the achievement of sustainable development goals (Bossier and Ekasari 2017; Nguyen et al. 2021). This technology could result in higher productivity with less impact on the environment. Furthermore, biofloc systems may be developed and performed in integration with other food production, thus promoting productive integrated systems, aiming at producing more food and feed from the same area of land with less input (Nisar et al. 2022). The chapter aims to address different aspects of BFT and RAS as alternatives for sustainable tilapia production.

9.2 Biofloc Technology

9.2.1 History of Biofloc Technology (BFT)

The biofloc system (BFT) evolved as an alternative to the conventional aquaculture system that is used in shrimp and tilapia productions (Ulloa Walker et al. 2020). It originated in the 1970s at the French Research Institute for Exploitation of the Sea (IFREMER) with Gerard Cuzon as the pioneer (Emerenciano et al. 2012; Devi and Kurup 2015). The BFT was then widely applied in commercial shrimp farming (Samocha et al. 2019). In the 1990s, different studies at pilot and commercial scales were conducted in the USA with penaeid shrimp led by J. Stephen Hopkins and with finfish at the Technion-Israel Institute of Technology led by Yoram Avnimelech (Emerenciano et al. 2013). In the mid-2000s, several studies on penaeid shrimp were conducted at the Federal University of Rio Grande-FURG (Brazil) led by Wilson Wasielesky and the Texas A&M University (Corpus Christi Campus, USA) led by Tzachi Samocha. After that, there was a significant increase in the number of scientific publications about biofloc technology worldwide. The number has increased from less than 10 in 2009 to more than 100 publications in 2018, with studies conducted mainly in Brazil, China, the United States of America, Mexico, and India (Ulloa Walker et al. 2020), helping to strengthen the technology and boost the industry. Another important factor for such progress was the wide range of courses and lectures offered in both scientific and commercial events for the scientific community, academia, and aquaculturists. However, despite the progress and benefits of BFT as reported by the scientific community and academia, there is still room for its commercial expansion. For example, in Indonesia, it is estimated that only 20–25% of shrimp production has occurred using biofloc technology (Thong 2014). Among the reasons behind such a scenario are the higher implementation and production costs compared to traditional land-based systems, and the complexities in the management and implementation of the technology, which requires greater technical knowledge and permanent monitoring of water quality (Avnimelech 2015).

9.2.2 Principles of Biofloc Technology

The BFT operates on the principle of nutrient recycling by maintaining a higher carbon: nitrogen (C: N) ratio above 15 to stimulate the mass growth of heterotrophic bacteria (Guo et al. 2020). Higher C: N ratio is maintained when more carbon sources, such as molasses, cassava, hay, sugarcane, starch, wheat bran, cellulose, etc., are sprayed on the surface of pond water with continuous aeration (Ogello et al. 2021). Under favorable BFT conditions, up to 0.5 g of heterotrophic bacterial biomass g^{-1} substrate of carbon can be produced (Eding et al. 2006). With the information that 1 g of carbon produces 0.5 g of bacteria, farmers can estimate quantities of floc in the culture systems (Nisar et al. 2022). The biofloc process stimulates the natural growth of macro-aggregates of organisms that enhance self-nitrification in the culture water (Jamal et al. 2020).

In outdoor BFT systems, the photosynthetic pathway that produces algae normally precedes the bio-flocking process (Ogello et al. 2021). The algae provide a substrate to which the bioflocs attach and are usually referred to as green bioflocs (Ahmad et al. 2017; Khanjani and Sharifinia 2020). Under indoor conditions, bioflocs are mainly bacteria and are referred to as brown bioflocs (El-Sayed 2021; Emerenciano et al. 2021). With the addition of an adequate carbon source bacterial floc stimulates a second production line that involves the degradation of organic wastes by bacteria to produce billions of bacterial cells under optimum aeration conditions (Khanjani et al. 2022). During this process, autotrophic and heterotrophic bacteria proliferate and attract billions of other cells including diatoms, fungi, algae, protozoans, and various types of plankton (Avnimelech 2007; Bossier and Ekasari 2017). The traditional aquaculture ponds lack injection of carbon source, and aeration mechanisms and thus harbor fewer and less diverse bacterial communities, as opposed to BFT (Felix and Menaga 2021). Small quantities of bacteria cannot form substantial flocs in the culture system. The sediment of traditional ponds accumulates higher quantities (49%) of nitrogenous waste while the BFT pond sediments have less (5%) nitrogenous waste (Ogello et al. 2021).

9.2.3 Biofloc in Tilapia

Tilapia has played an important role in global aquaculture in recent decades (FAO 2020a). It ranks the second most cultured fish species worldwide due to its fast growth, resistance to various environmental conditions, and capability of being produced in dense and ultra-dense forms (Menaga et al. 2019; Avnimelech 2007; Avnimelech 2015; Khanjani et al. 2021). Moreover, tilapia is an omnivore species that filter food particles and easily feeds on a rich natural food source and biofloc-dependent microorganisms (Durigon et al. 2020; Prabu et al. 2019). Considering the above considerations, tilapia is a suitable candidate for the biofloc system (Khanjani et al. 2022). The biofloc technique has been accepted in many countries over the past decade (Vyas 2020). Currently, the biofloc technique has been successfully developed in large-scale farms in Asia (China, South Korea), Latin America, and Central America, as well as on a small scale in the United States, Italy, and other countries. In addition, many research centers and academic institutions are expanding BFT, mainly in key areas such as growth, nutrition, reproduction, microbial ecology, biotechnology, and economics (Khanjani and Sharifinia 2020).

9.2.3.1 Biofloc as Growth Promoters

In situ utilization of microbial flocs generated in biofloc systems by some aquaculture organisms as well as the utilization of processed biofloc as a feed ingredient has been well documented (Kuhn et al. 2009; Kuhn et al. 2010; Anand et al. 2014). It has been demonstrated that the concentrations of free amino acids such as alanine, glutamate, arginine, and glycine, which are known attractants in the shrimp diet, are present in biofloc (Vyas 2020; Ahmad et al. 2017). Levels in biofloc were found to be comparable to that of the shrimp commercial diet suggesting that biofloc are likely to be recognized as food particles by some aquaculture organisms. Furthermore, biofloc technology application in larviculture may provide an easily accessible food source for the larvae outside the regular feeding moments, thus minimizing possible negative social interaction during feeding (Ekasari et al. 2015b).

Studies by various researchers have reported that the biofloc system improves the growth performance of the Nile tilapia (Azim and Little 2008; Luo et al. 2013; Mirzakhani et al. 2019; Khanjani et al. 2021; Table 9.1). The improved growth performance was attributed to optimum water quality and continuous production of biofloc. Biofloc contains poly beta-hydroxybutyrate (De Schryver and Verstraete 2009) and bioactive compounds, such as carotenoids, chlorophylls, and phytosteroids that promote the growth of cultured aquatic organisms. The adaptability of tilapia to biofloc consumption and digestion of microbial protein has been demonstrated in several studies (Azim and Little 2008). Avnimelech (2007) reported that the production of biofloc in fish ponds can meet 50% of tilapia's protein requirements. Khanjani et al. (2021) found that tilapia feed better on the biofloc impacted by molasses daily, resulting in the highest biomass increase and the lowest feed conversion ratio. Based on their results, the highest feed conversion ratio and the lowest protein efficiency were observed in the control treatment without biofloc. Researchers have found that a combination of biofloc and artificial foods improves feed conversion ratio and feed efficiency (Khanjani et al. 2021; Mirzakhani et al. 2019).

Biofloc includes bacterial proteins, polyhydroxy butyrate, and bacteria containing peptidoglycan or lipopolysaccharides, which can promote growth performance (Khanjani and Sharifinia 2020). In addition, biofloc have probiotic properties that help fish in digestion and absorption of artificial diets. In the study using orange peel-derived pectin (OPDP) added to the biofloc system, Doan et al. (2018) indicated that the inclusion of 10 g kg^{-1} OPDP significantly improved the growth performance and feed utilization of Nile tilapia. Similarly, a significant increase in growth

Table 9.1 Weight gain (WG), Specific growth rate (SGR), Food conversion efficiency (FCE), Food conversion ratio (FCR), Protein efficiency ratio (PER), Survival rate (SR), and digestive enzyme of Nile tilapia in biofloc and RAS systems

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	Orange peels derived pectin (OPDP)	Nile tilapia (*Oreochromis niloticus*) (9.09 ± 0.05 g)	0 (control in clear water), 0 (control in biofloc system), 5, 10, and 20 g kg^{-1} OPDP for 8 weeks	SGR, WG, and FW ↑ FCR ↓	Doan et al. (2018)
BFT	Stocking density	Tilapias (0.51 ± 0.05 g)	166 orgs m^{-3} (LD, low density), 333 orgs m^{-3} (MD, middle density) and 600 orgs m^{-3} (HD, high density) 120 days	Growth performance ↓ FCR ↑	Liu et al. (2018)
BFT	Carbon sources	Nile tilapia (*O. niloticus*) 2.7 ± 0.4 g	100% of MO, 75% of MO+ 25% of WF, 50% of MO+ 50% of WF, 25% of MO+ 75% of WF,and 100% of WF	Growth performance ↑	Mirzakhani et al. (2019)
BFT	Pizzeria by-product	Nile tilapia (*O. niloticus*) (2.90 ± 0.02 g)	0, 20, 40, 60, 80 and 100% 38 days	Growth performance ↑ Economic benefits ↑	de Sousa et al. (2019)
BFT	Dietary digestible protein and digestible energy	Nile tilapia (*O. niloticus*) (1.25 ± 0.15 g)	Digestible protein (22, 26 and 30% DP) and digestible energy (3000, 3150 and 3300 kcal kg^{-1}) 42 days	Pepsine activity ↑ Trypsine activity ↑	Durigon et al. (2019)
BFT	Carbon sources	Fingerlings	Corn flour (CF), wheat flour (WF), sugar (SU) and a control (C)	FCR and water ↓ Survival rate ↑	García-Ríos et al. (2019)
BFT	OPDP and *L. plantarum*	Nile tilapia (*O. niloticus*) (1.25 ± 0.15 g) 5.92 ± 0.08 g	Diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (108 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP +108 CFU g^{-1} *L. plantarum*)	Growth performance ↑ FCR ↓	Van Doan et al. (2019)

BFT	*in-situ* and *ex-situ* biofloc	Nile tilapia (*O. niloticus*) (5.1 ± 0.05 g/fish)	T1-biofloc developed within the culture systems (*insitu*), T2-biofloc supplementation in fish feed (*exsitu*) and C- Control without biofloc	Growth performance ↑	Menaga et al. (2019)
BFT	Biochar	Nile tilapia (*O. niloticus*) (36.2 g/fish)	B; only biochar, GB; biochar + glucose, while for the control (G) only glucose	Growth performance →	Abakari et al. (2020)
BFT	Jaggery-based BFT	Nile tilapia (*O. niloticus*) (32.2 ± 10.1 g/fish)	22.5%, 27.7%, and 32.3% digestible protein (DP) and 6% lipid	Growth performance ↑	Elayaraja et al. (2020)
BFT	Chestnut polyphenols (CSP)	Nile tilapia (*O. niloticus*) (12.77 ± 0.17 g)	0, 1, 2, 4, and 8 g kg^{-1} of CSP	Growth performance ↑	H. Van Doan et al. (2020a)
BFT	Watermelon rind powder (WMRP)	Nile tilapia (*Oreochromis niloticus*) (17.14 ± 0.12 g)	0 (Diet 1- control), 20 g kg^{-1} WMRP (Diet 2), 40 g kg^{-1} WMRP (Diet 3), 80 g kg^{-1} WMRP (Diet 4), and 160 g kg^{-1} WMRP (Diet 5)	Growth performance ↑ FCR ↓	Hien Van Doan et al. (2020b)
BFT	Phosphatidylcholine	Nile tilapia (*O. niloticus*) 8.03 ± 0.03 g	0, 400, 800 and 1200 mg/kg of feed 40 days	Growth performance → Energy metabolism ↑	Sousa et al. (2020)
BFT	*Tenebrio molitor* meal (TM)	Nile tilapia (*O. niloticus*) (2.08 ± 0.19 g)	0% or control, 5, 10, 15 and 20% of TM	Productivity and survival rate ↑	Tubin et al. (2020)
BFT	Density and dietary carbon sources	Nile tilapia (*O. niloticus*) (50.47 ± 0.05 g)	Stocking densities [20, 40 and 60 fish per m^3] and fed the basal diet without carbon sources or with broken rice flour (BRF) or broken wheat grain flour (BWGF) 84 days	Growth performance ↑ Feed utilization ↑	Zaki et al. (2020)

(continued)

Table 9.1 (continued)

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	Pineapple peel powder (PAPP)	Nile tilapia (*O. niloticus*) (20.91 ± 0.11 g)	0, 10, 20, 30 and 40 g kg^{-1} PAPP 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2021a)
BFT	Watermelon rind powder (WMRP) + *L. plantarum*	Nile tilapia (*O. niloticus*) (16.57 ± 0.14 g)	Diet 1 (0 g kg^{-1} WMRP and 0 CFU g^{-1} *L. plantarum*) (control), Diet 2 (40 g kg^{-1} WMRP), Diet 3 (10^8 CFU g^{-1} LP), and Diet 4 (40 g kg^{-1} WMRP + 10^8 CFU g^{-1} LP) 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2021b)
BFT	Pineapple peel powder (PAPP) + *L. plantarum*	Nile tilapia (*O. niloticus*) (20.91 ± 0.11 g)	Diet 1 (0 g kg^{-1} PAPP and 0 CFU g^{-1} *L. plantarum*) (control), Diet 2 (10 g kg^{-1} PAPP), Diet 3 (10^8 CFU g^{-1} LP), and Diet 4 (10 g kg^{-1} PAPP + 10^8 CFU g^{-1} LP) 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2021d)
BFT	Amla (*Phyllanthus emblica*) fruit extract	Nile tilapia (*O. niloticus*) (10.48 ± 0.56 g)	0, 5, 10, 20, and 40 mg kg^{-1} AFE 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2022b)
BFT	Light levels	Nile tilapia (*O. niloticus*) (1.73 ± 0.16 g)	24 h of light (24hL), 12 h of light/12 h of darkness (12hL/12hD) and 24 h of darkness (24hD)	Carcass quality ↑ Digestive and hepatic enzymes ↑	Khanjani and Sharifinia (2021)
BFT	Symbiotics	Nile tilapia (*O. niloticus*) (30–35 g)	BFT with and without symbiotics 40 days	Growth performance ↑	Laice et al. (2021)
BFT	Stocking densities	Nile tilapia (*O. niloticus*) (133.91 g)	18.75, 37.50, 56.25, and 75.00 fish · m^{-3} 260 days	Growth performance ↑	Manduca et al. (2021)

	Beneficial bacteria	Nile tilapia (*O. niloticus*) (8.63 ± 3.35g)	T1, SR control; T2, SR + SSP; T3, SR + MSP; T4, BF + MSP; T5, BF + SSP; T6, BF control 112 days	Growth performance ↑	Mohammadi et al. (2021)
BFT	Coffee silverskin (CSS)	Nile tilapia (*O. niloticus*) (15.54 ± 0.21 g)	CSS1 (Control), CSS2 (10 g kg−1), CSS3 (20 g kg−1), CSS4 (40 g kg−1), and CSS5 (80 g kg−1) 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2021c)
BFT	Carbon sources and stocking densities	Nile tilapia (*O. niloticus*) (5.15 ± 1.12 g)	Low stocking density (LSD), 140 fish /m^3 and high stocking density, (HSD), 280/m^3 98 days	Growth performance ↑	El-Hawarry et al. (2021)
BFT	Salinities	Nile tilapia (*O. niloticus*) (13.78 ± 0.62 g)	T1 (5 ppt), T2 (10 ppt), T3 (15 ppt), T4 (20 ppt) and control (0 ppt) 90 days	Growth performance ↑ Survival rate ↑ Carcass quality ↑	Kumari et al. (2021)
BFT	Dietary phytase	Nile tilapia (*O. niloticus*) (29.8 g)	6-phytase (3000 FTU/kg; Quantum Blue™; P-F + Phy), and positive (C) and negative (P-F) control 154–156 days	Growth performance →	Green et al. (2021)
BFT	Spent coffee grounds (SCG)	Nile tilapia (*O. niloticus*) (15.25 ± 0.07 g)	SCG1 (control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}) 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2022a)
BFT	Host-associated probiotic *Bacillus altitudinis* B61-34b	Nile tilapia (*O. niloticus*) (25.50 ± 0.52 g)	0 (BAA1—Control), 10^6 (BAA2), 10^7 (BAA3), 10^8 (BAA4) and 10^9 (BAA5) CFU ml^{-1} 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2021e)
BFT	Host-associated probiotic *Lactobacillus paracasei* l61-27b	Nile tilapia (*O. niloticus*) (25.40 ± 0.52 g)	LP1 = 0 (Control), LP2 = 10^6 CFU mL^{-1}, LP3 = 10^7 CFU mL^{-1}, LP4 = 10^8 CFU mL^{-1}, and LP5 = 10^9 CFU mL^{-1}) 8 weeks	Growth performance ↑ FCR ↓	Van Doan et al. (2021c)
BFT	Grade feeding rates	Nile tilapia (*O. niloticus*) (3.1 ± 0.1 g)	0%, 2.5%, 5.0%, 7.5%, and 10% of body weight per day 70 days	Growth performance ↑	Oliveira et al. (2021)

(continued)

Table 9.1 (continued)

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	17-α-methyltestosterone	Nile tilapia (*O. niloticus*) (Fry)	60, 90, 120, 150 and 180 mg kg^{-1}	Masculinization rates ↑	Costa e Silva et al. (2022)
BFT	Feeding levels and stocking densities	Nile tilapia (*O. niloticus*) (3.2 ± 0.05 g)	Feeding levels (0, 15, 30, 45 and 100) and 2 stocking densities (500 fish/m^3 and 1000fish/m^3)	Growth performance ↑	Sarsangi Aliabad et al. (2022)
BFT	Longan seed powder (LS)	Nile tilapia (*O. niloticus*) (13.82 ± 0.06 g)	Control (LS0), 10 (LS10), 20 (LS20), 40 (LS40), and 80 (LS80) g kg^{-1} LS 8 weeks	Growth performance ↑ FCR ↓	Wannavijit et al. (2022)
BFT	Rambutan seed (RS)	Nile tilapia (*O. niloticus*) (14.77 ± 0.80 g)	0, 5, 10, 20, and 40 g kg^{-1} of RS 8 weeks	Growth performance ↑ FCR ↓	Xuan et al. (2022)
BFT	Rambutan peel (RP)	Nile tilapia (*O. niloticus*) (17.14 ± 0.12 g)	0 g kg^{-1} (control – RP0); 10 g kg^{-1} (RP10); 20 g kg^{-1} (RP20); 40 g kg^{-1} (RP40), and 80 g kg^{-1} (RP80) 8 weeks	Growth performance ↑ FCR ↓	Le Xuan et al. (2022)
BFT	Chitosan	Nile tilapia (*O. niloticus*) (1.70 ± 0.36 g)	30, 60 and 90 ppm and 10, 20 and 30 ppm of chitosan	Growth performance ↑ Digestive enzymes ↑ FCR ↓	Chutia et al. (2022)
RAS	Light intensity and photoperiod	Nile tilapia (*O. niloticus*) (5 ± 0.9 g)	(1000, 2000, and 3000 lx) and photoperiods (12L:12D, 18L:6D, 24L:0D) 160 days	Growth performance ↑ FCR ↓ Stress →	Wang et al. (2020)

RAS	Magnetic field	Nile tilapia (*O. niloticus*) (7.16 ± 0.05 g)	0.00, 0.10, 0.15 and 0.20 T 70 days	Growth performance ↑ FCR ↓	Hassan et al. (2018)
RAS	Rearing systems and dietary probiotic	Nile tilapia (*O. niloticus*) (embryos)	FTS + control diet, RAS + control diet and RAS + *B. subtilis* coated diet (RASB) 33 days	Survival rate ↑ Beneficial bacteria in gut ↑	Deng et al. (2022)
BFT-RAS	Culture systems	Nile tilapia (*O. niloticus*) (0.17 ± 0.00 g)	Clear-water (CW), biofloc (BF), or hybrid (HY) 9 weeks	Growth performance ↑ FCR ↓	Fleckenstein et al. (2018)
RAS + BFT	Nitrogen and phosphorus budgets	Nile tilapia (*O. niloticus*) (3.54 ± 2.82 g)	(BFT) aquaculture system and a recirculation aquaculture system (RAS) during over-wintering of tilapia 64 days	Recovery rate of N in BFTSs ↑ P recovery rate →	Cao et al. (2020)
BFT-RAS	Protein levels	Nile tilapia (*O. niloticus*) (39.1 ± 2.5 g)	23, 27, 31 or 35% crude protein 9 weeks	Growth performance ↑ FCR ↓	Nguyen et al. (2021)

performance and feed utilization were observed in Nile tilapia fed dietary inclusion of OPDP and *Lactobacillus plantarum*, chestnut polyphenols (CSP), watermelon rind powder (WMRP), pineapple peel powder (PAPP), watermelon rind powder (WMRP) + *L. plantarum*, pineapple peel powder (PAPP) + *L. plantarum*, amla (*Phyllanthus emblica*) fruit extract, coffee silverskin (CSS), spent coffee grounds (SCG) (Van Doan et al. 2019; 2020a, b; 2021a, b, c, d; 2022a, b). Significant improvement in growth performance and FCR may be attributable to the bioactive compounds of these supplementations, which act not only as a nutrient source for fish but also are carbon sources for microbial protein production in biofloc systems. In studies using host-associated probiotics (*Bacillus altitudinis* B61-34b and *Lactobacillus paracasei* l61-27b), the authors indicated that supplementation of host-associated probiotics in indoor biofloc system resulted in better growth performance and feed utilization compared to the control group. This may be due to the complementary roles of biofloc and *B. altitudinis*. Numerous investigations have demonstrated that biofloc offers an essential nutrient source for tilapia (Ekasari et al. 2014a; Green et al. 2019). The addition of *Bacillus* spp. in cultured water or diets lowers ammonium levels in fish culture systems (Dash et al. 2018; Elsabagh et al. 2018). Furthermore, the presence of favorable microbial flocs and external probiotics will likely boost the number of valuable microbiota in the tilapia's digestive system (Rohani et al. 2022; de Sousa et al. 2019). Increased secretion of digestive enzymes through the colonization of bacteria facilitates the absorption of nutrients by the intestinal epithelial cells (Liu et al. 2017). *Bacillus* produces many biological substances, including cellulase, phytase, tannase, chitinase, xylanase, protease, amylase, and lipase (Ringø 2020). Favorable bacteria also release several nutrients, in particular vitamins, amino acids, and fatty acids, and diminish lethal feedstuffs and infectious bacteria (Zaineldin et al. 2021). Recently, the dietary inclusion of rambutan and long seed powder or rambutan peel in the Nile tilapia diet led to an increase in growth rate and feed utilization (Xuan et al. 2022; Wannavijit et al. 2022). It has been reported that these seeds are known as carbon sources (Yang et al. 2015; Lawtae and Tangsathitkulchai 2021) and has hence been used in biofloc system (Liu et al. 2019). It has been observed that adding carbon to a biofloc system causes heterotrophic bacteria to utilize the inorganic nitrogen by changing the water C: N ratio, resulting in a higher microbic protein source for the host and improved water quality (Guo et al. 2020). In addition, incorporating a carbon source leads to the formation of biofloc, a new protein source for fish (Krummenauer et al. 2020; Tinh et al. 2021). Additionally, these products also act as potential prebiotics or carbohydrate (Estrada-Gil et al. 2022; Jahurul et al. 2020). Similarly, supplementation of pizzeria by-product de Sousa et al. (2019); dietary digestible protein and digestible energy (Durigon et al. 2019); phosphatidylcholine (Sousa et al. 2020); *Tenebrio molitor* meal (TM) (Tubin et al. 2020); symbiotics (Laice et al. 2021); beneficial bacteria (Mohammadi et al. 2021); dietary phytase (Green et al. 2021), and chitosan (Chutia et al. 2022) led to higher growth rate and feed utilization.

Another common study aspect using biofloc technology is the application of different carbon sources and their effects on tilapia growth and feed conversion ratio. (Mirzakhani et al. (2019) reported that fish in a biofloc system with 100% of wheat flour at a C:N ratio of 15:1 showed the highest growth performance with improved intestine histoarchitecture. Wheat flour as a major source of starch and energy can also provide substantial amounts of other nutrients such as protein, vitamins, and phytochemicals and especially high fiber content (ca. 12%) compared to the lower fiber content in molasses (ca. 0.5%) (Shewry and Hey 2015). These nutrients might enhance the biochemical composition and bioactive compounds of biofloc. In addition, dispersed particles of wheat flour in water may provide a good substrate for the development and growth of microorganisms and bacteria because of which the nutrition value of the produced biofloc increases, ultimately influencing the fish growth and immune response (Mirzakhani et al. (2019). García-Ríos et al. (2019) indicated that the fingerlings obtained in BFT, with corn and sugar as C sources, had a similar growth rate to the control. However, the BFT promotes significant savings in feed (41.1 to 58.9%) and water (67.4 to 75.5%) compared to the traditional method. Similar results were in the study of Zaki et al. (2020), where the authors indicated that Increased growth and feed utilization were recorded in 40 fish per m^3 fed with broken rice flour. El-Hawarry et al. (2021) also found that the growth rate was improved in the groups of fish under low stocking density with molasses and glycerol as carbon sources. Carbon source affects the cultured species' growth depending on the formatted biofloc characteristics, such as its "volume, chemical composition, and ability to store bioactive compounds (Wang et al. 2015; Zhao et al. 2016). Additionally, microbial flocs which are formed from different carbon sources act as a supplemental food source that constantly provides additional essential amino acids profile (microbial protein), polyunsaturated fatty acids, minerals, vitamins, and an external source of digestive enzymes (Avnimelech 2007; Azim and Little 2008; Bakhshi et al. 2018; De Schryver and Verstraete 2009). In contrast, the use of biochar as an alternative carbon source for biofloc technology did not affect the growth rate of Nile tilapia (Abakari et al. 2020). Although biochar is regarded as a recalcitrant carbon source, the utilization of biochar-derived carbon by heterotrophic bacteria has been described (Farrell et al. 2013).

Effects of stocking density on Nile tilapia growth raised in biofloc systems have been conducted by several researchers. Liu et al. (2018) showed that low stocking density (166 fish/m^{-3}) improved growth performance and FCR of Nile tilapia raised in the biofloc system. Similarly, Manduca et al. (2021) reported that tilapia stocking density in BFT around 33 fish m^{-3} had higher profitability since it produces a large proportion of harvested fish that reach high body weights, and possibly high selling prices, combined with desirable biomass. Recently, Sarsangi Aliabad et al. (2022) also suggested that the stocking density of 1000/m^3 for larviculture of tilapia in BFT uses water and equipment more efficiently. Biofloc acts as the natural food that contributes significantly to the nutrition of tilapia fingerlings, allowing the reduction of the feeding rations. Biofloc consumption corresponds to 50% of the daily food of tilapia (Avnimelech 2007). Another study revealed that 25% of the protein requirement of tilapia could be provided by floc consumption (Avnimelech 2015). Besides, significantly improved growth performance, FCR, and digestive enzymes were observed in Nile tilapia raised in biofloc combined with different conditions, such as in-situ and ex-situ biofloc (Menaga et al. 2019), jaggery-based BFT (Elayaraja et al. 2020); light levels (Khanjani and Sharifinia 2021); salinities (Kumari et al. 2021); grade feeding rates (Oliveira et al. 2021), and 17-α-methyltestosterone (Costa e Silva et al. 2022).

9.2.3.2 Biofloc as Immunostimulants

Bioflocs also offer a lot of MAMPs (microbial-associated molecular patterns), which may be recognized as immunostimulants, resulting in higher resistance to diseases (Ekasari et al. 2014b, 2015a). Additionally, it consists of a wide range of organic compounds, such as carotenoids, chlorophylls, bromophenols, phytosterols, and antibacterials that have a positive effect on immune factors of cultivated aquatic species (Crab et al. 2010; Najdegerami et al. 2016; Bakhshi et al. 2018; Mirzakhani et al. 2019).

The effects of biofloc in combination with different functional feed additives on the immune response of Nile tilapia have been reported in previous studies (Doan et al. 2018; Van Doan et al. 2019, 2020a, b; 2021a, b, c, d; 2022a, b; Xuan et al. 2022; Le Xuan et al. 2022; Wannavijit et al. 2022; Table 9.2). Similar findings were observed in Nile tilapia fed in-situ and ex-situ biofloc (Menaga et al. 2019); phosphatidylcholine (Sousa et al. 2020); symbiotics (Laice et al. 2021); beneficial bacteria (Mohammadi et al. 2021), and probiotics (Bañuelos-Vargas et al. 2021). These substances act as immunostimulants and/or carbon sources for the proliferation of microbial proteins in the biofloc system.

Carbon source applications in the biofloc system could result in better immune response in Nile tilapia. Mirzakhani et al. (2019) indicated that fish reared in a biofloc system based on 100% wheat flour and a C/N ratio of 15 demonstrated the humoral immune response. Similarly, a significant increase in innate and specific immune responses was observed in Nile tilapia raised in biofloc with biochar (Abakari et al. 2020) and jaggery-based BFT (Elayaraja et al. 2020) as carbon sources. Carbon sources play a vital role in the proliferation of microbial protein in biofloc systems, which in turn act as immunostimulants for culture species (Panigrahi et al. 2019). Carbon sources and stocking densities also have a great impact on Nile tilapia's immune response raised under the biofloc system. El-Hawarry et al. (2021) indicated that the growth rate and growth-related genes were improved in the groups of fish under low stocking density (LSD) with molasses and glycerol as carbon sources. Recently, (Sarsangi Aliabad et al. 2022) also reported that the BFT system improved water quality, growth performance, and immune function of Nile tilapia fry.

9.2.3.3 Biofloc as Disease Prevention Techniques

In biofloc systems, aquaculture animals may also benefit from reduced pathogen pressure (Bossier and Ekasari 2017). Some studies demonstrated that the presence of potentially pathogenic bacteria might be reduced in biofloc systems (Gustilatov et al. 2022; de Lima Vieira et al. 2021; Khanjani et al. 2022; Table 9.3). Increase disease

Table 9.2 Immune responses of tilapia cultured biofloc system

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	Stocking density	Tilapias (0.51 ± 0.05 g)	166 orgs m^{-3} (LD, low density), 333 orgs m^{-3} (MD, middle density) and 600 orgs m^{-3} (HD, high density) 120 days	Lysozyme activity ↓ Complement 3 activity ↓ Glutathione level ↓	Liu et al. (2018)
BFT	Orange peels derived pectin	Nile tilapia (9.09 ± 0.05 g)	0 (control in clear water), 0 (control in biofloc system), 5, 10, and 20 g kg^{-1} OPDP for 8 weeks	Skin mucus immunity ↑ Serum immunity ↑	Doan et al. (2018)
BFT	*in-situ* and *ex-situ* biofloc	Nile tilapia (*O. niloticus*) (5.1 ± 0.05 g/fish)	T1-biofloc developed within the culture systems (*insitu*), T2-biofloc supplementation in fish feed (*exsitu*) and C- Control without biofloc	Immune gene expressions ↑	Menaga et al. (2019)
BFT	Carbon sources	Nile tilapia (*O. niloticus*) 2.7 ± 0.4 g	100% of MO, 75% of MO+ 25% of WF, 50% of MO+ 50% of WF, 25% of MO+ 75% of WF,and 100% of WF	Humoral immune responses ↑	Mirzakhani et al. (2019)
BFT	OPDP and *L. plantarum*	Nile tilapia (*O. niloticus*) (1.25 ± 0.15 g) 5.92 ± 0.08 g	Diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (108 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP +108 CFU g^{-1} *L. plantarum*)	Skin mucus immunity ↑ Serum immunity ↑	Van Doan et al., (2019)
BFT	Biochar	Nile tilapia (*O. niloticus*) (36.2 g/fish)	B; only biochar, GB; biochar + glucose, while for the control (G) only glucose	Immune parameters ↑	Abakari et al. (2020)
BFT	Jaggery-based BFT	Nile tilapia (*O. niloticus*) (32.2 ± 10.1 g/fish)	22.5%, 27.7%, and 32.3% digestible protein (DP) and 6% lipid	Innate immunity ↑ Immune gene expressions ↑	Elayaraja et al. (2020)

(continued)

Table 9.2 (continued)

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	Chestnut polyphenols (CSP)	Nile tilapia (*O. niloticus*) (12.77 ± 0.17 g)	0, 1, 2, 4, and 8 g kg^{-1} of CSP	Skin mucus immunity ↑ Serum immunity ↑	Van Doan et al. (2020a)
BFT	Watermelon rind powder (WMRP)	Nile tilapia (*O. niloticus*) (17.14 ± 0.12 g)	0 (Diet 1- control), 20 g kg^{-1} WMRP (Diet 2), 40 g kg^{-1} WMRP (Diet 3), 80 g kg^{-1} WMRP (Diet 4), and 160 g kg^{-1} WMRP (Diet 5)	Immune responses ↑	Van Doan et al. (2020b)
BFT	Phosphatidylcholine	Nile tilapia (*O. niloticus*)	0, 400, 800 and 1200 mg/kg of feed	Antioxidant enzymes Lipoperoxidation in liver ↓	Sousa et al. (2020)
BFT	Pineapple peel powder	Nile tilapia (*O. niloticus*) (20.91 ± 0.11 g)	0, 10, 20, 30 and 40 g kg^{-1} PAPP 8 weeks	Skin mucus immunity ↑ Serum immunity ↑	Van Doan et al. (2021a)
BFT	Watermelon rind powder (WMRP) + *L. plantarum*	Nile tilapia (*O. niloticus*) (16.57 ± 0.14 g)	Diet 1 (0 g kg^{-1} WMRP and 0 CFU g^{-1} L. *plantarum*) (control), Diet 2 (40 g kg^{-1} WMRP), Diet 3 (10^8 CFU g^{-1} LP), and Diet 4 (40 g kg^{-1} WMRP + 10^8 CFU g^{-1} LP) 8 weeks	Skin mucus immunity ↑ Serum immunity ↑	Van Doan, Seyed Hossein Hoseinifar, et al. (2021b)
BFT	Pineapple peel powder (PAPP) + *L. plantarum*	Nile tilapia (*O. niloticus*) (20.91 ± 0.11 g)	Diet 1 (0 g kg^{-1} PAPP and 0 CFU g^{-1} L. *plantarum*) (control), Diet 2 (10 g kg^{-1} PAPP), Diet 3 (10^8 CFU g^{-1} LP), and Diet 4 (10 g kg^{-1} PAPP + 10^8 CFU g^{-1} LP) 8 weeks	Skin mucus immunity ↑ Serum immunity ↑	Van Doan et al. (2021d)
BFT	Amla (*Phyllanthus emblica*) fruit extract	Nile tilapia (*O. niloticus*) (10.48 ± 0.56 g)	0, 5, 10, 20, and 40 mg kg^{-1} AFE 8 weeks	Skin mucus immunity ↑ Serum immunity ↑	Van Doan et al. (2022b)

BFT	Symbiotics	Nile tilapia (*O. niloticus*) (30–35 g)	BFT with and without symbiotics 40 days	Hematological parameters ↑	Laice et al. (2021)
BFT	Beneficial bacteria	Nile tilapia (*O. niloticus*) (8.63 ± 3.35g)	T1, SR control; T2, SR + SSP; T3, SR + MSP; T4, BF + MSP; T5, BF + SSP; T6, BF control 112 days	Innate immune response ↑	Mohammadi et al. (2021)
BFT	Coffee silverskin (CSS)	Nile tilapia (*O. niloticus*) (15.54 ± 0.21 g)	CSS1 (Control), CSS2 (10 g kg−1), CSS3 (20 g kg−1), CSS4 (40 g kg−1), and CSS5 (80 g kg−1) 8 weeks	Skin mucus immunity ↑ Serum immunity ↑	Hien Van Doan et al. (2021c)
BFT	Carbon source and stocking density	Nile tilapia (*O. niloticus*) (5.15 ± 1.12 g)	Low stocking density (LSD), 140 fish /m^3 and high stocking density, (HSD), 280/m^3 98 days	Growth-related genes ↑	El-Hawarry et al. (2021)
BFT	Probiotics	Nile tilapia (*O. niloticus*) (6.7 ± 0.2 g)	Stocking densities (D1, 120 fish/m^3; D2, 240 fish/m^3) with biofloc plus probiotics	Immune response ↑	Bañuelos-Vargas et al. (2021)
BFT	Spent coffee grounds (SCG)	Nile tilapia (*O. niloticus*) (15.25 ± 0.07 g)	SCG1 (control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}) 8 weeks	Skin mucus immunity ↑ Serum immunity ↑	Van Doan et al. (2022a)
BFT	Feeding levels and stocking densities	Nile tilapia (*O. niloticus*) (3.2 ± 0.05 g)	Feeding levels (0, 15, 30, 45 and 100) and 2 stocking densities (500 fish/m3 and 1000fish/m3)	Innate immune response ↑	Sarsangi Aliabad et al. (2022)
BFT	Rambutan seed (RS)	Nile tilapia (*O. niloticus*) (14.77 ± 0.80 g)	0, 5, 10, 20, and 40 g kg^{-1} of RS 8 weeks	Skin mucus immunity ↑ Serum immunity ↑ Gene expressions ↑	Xuan et al. (2022)

(continued)

Table 9.2 (continued)

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	Longan seed powder (LS)	Nile tilapia (*O. niloticus*) (13.82 ± 0.06 g)	Control (LS0), 10 (LS10), 20 (LS20), 40 (LS40), and 80 (LS80) g kg^{-1} LS 8 weeks	Skin mucus immunity ↑ Serum immunity ↑ Gene expressions ↑	Wannavijit et al. (2022)
BFT	Rambutan peel (RP)	Nile tilapia (*O. niloticus*) (17.14 ± 0.12 g)	0 g kg^{-1} (control – RP0); 10 g kg^{-1} (RP10); 20 g kg^{-1} (RP20); 40 g kg^{-1} (RP40), and 80 g kg^{-1} (RP80) 8 weeks	Skin mucus immunity ↑ Serum immunity ↑ Gene expressions ↑	Le Xuan et al. (2022)

Table 9.3 Increase disease resistance of tilapia cultured under biofloc system

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	Orange peels derived pectin	Nile tilapia (9.09 ± 0.05 g)	0 (control in clear water), 0 (control in biofloc system), 5, 10, and 20 g kg^{-1} OPDP for 8 weeks	Resistance to *S. agalactiae* ↑	Doan et al. (2018)
BFT	Dietary digestible protein and digestible energy	Nile tilapia (*Oreochromis niloticus*) (1.25 ± 0.15 g)	Digestible protein (22, 26 and 30% DP) and digestible energy (3000, 3150 and 3300 kcal kg^{-1}) 42 days	Ectoparasite spread ↓	Durigon et al. (2019)
BFT	*in-situ* and *ex-situ* biofloc	Nile tilapia (*O. niloticus*) (5.1 ± 0.05 g/fish)	T1-biofloc developed within the culture systems (*insitu*), T2-biofloc supplementation in fish feed (*exsitu*) and C- Control without biofloc	Resistance to *A. hydrophila* ↑	Menaga et al. (2019)
BFT	OPDP and *L. plantarum*	Nile tilapia (*O. niloticus*) (1.25 ± 0.15 g) 5.92 ± 0.08 g	Diet 1 (0 g kg^{-1} OPDP and 0 CFU g^{-1} *L. plantarum*), diet 2 (10 g kg^{-1} OPDP), diet 3 (108 CFU g^{-1} *L. plantarum*), and diet 4 (10 g kg^{-1} OPDP +108 CFU g^{-1} *L. plantarum*)	Resistance to *S. agalactiae* ↑	Van Doan et al. (2019)
BFT	Jaggery-based BFT	Nile tilapia (*O. niloticus*) (32.2 ± 10.1 g/fish)	22.5%, 27.7%, and 32.3% digestible protein (DP) and 6% lipid	Resistance to *A. hydrophila* ↑	Elayaraja et al. (2020)
BFT	Chestnut polyphenols (CSP)	Nile tilapia (*O. niloticus*) (12.77 ± 0.17 g)	0, 1, 2, 4, and 8 g kg^{-1} of CSP	Resistance to *S. agalactiae* ↑	Van Doan et al. (2020a)
BFT	Watermelon rind powder (WMRP)	Nile tilapia (*Oreochromis niloticus*) (17.14 ± 0.12 g)	0 (Diet 1- control), 20 g kg^{-1} WMRP (Diet 2), 40 g kg^{-1} WMRP (Diet 3), 80 g kg^{-1} WMRP (Diet 4), and 160 g kg^{-1} WMRP (Diet 5)	Resistance to *S. agalactiae* ↑	Van Doan et al. (2020b)
BFT	Pineapple peel powder (PAPP)	Nile tilapia (*O. niloticus*) (20.91 ± 0.11 g)	0, 10, 20, 30 and 40 g kg^{-1} PAPP 8 weeks	Resistance to *S. agalactiae* ↑	Van Doan et al. (2021a)

(continued)

Table 9.3 (continued)

Studied systems	Studies parameters	Fish species	Doses and supplementations duration	Results	References
BFT	Pineapple peel powder (PAPP) + *L. plantarum*	Nile tilapia (*O. niloticus*) (20.91 ± 0.11 g)	Diet 1 (0 g kg^{-1} PAPP and 0 CFU g^{-1} L. *plantarum*) (control), Diet 2 (10 g kg^{-1} PAPP), Diet 3 (10^{8} CFU g^{-1} LP), and Diet 4 (10 g kg^{-1} PAPP + 10^{8} CFU g^{-1} LP) 8 weeks	Resistance to *S. agalactiae* ↑	Van Doan et al. (2021d)
BFT	Coffee silverskin (CSS)	Nile tilapia (*O. niloticus*) (15.54 ± 0.21 g)	CSS1 (Control), CSS2 (10 g kg−1), CSS3 (20 g kg−1), CSS4 (40 g kg−1), and CSS5 (80 g kg−1) 8 weeks	Resistance to *S. agalactiae* ↑	Van Doan et al. (2021c)
BFT	Amla (*Phyllanthus emblica*) fruit extract	Nile tilapia (*O. niloticus*) (10.48 ± 0.56 g)	0, 5, 10, 20, and 40 mg kg^{-1} AFE 8 weeks	Resistance to *S. agalactiae* ↑	Van Doan et al. (2022b)
BFT	Beneficial bacteria	Nile tilapia (*O. niloticus*) (8.63 ± 3.35g)	T1, SR control; T2, SR + SSP; T3, SR + MSP; T4, BF + MSP; T5, BF + SSP; T6, BF control 112 days	Resistance to *A. hydrophila* ↑	Mohammadi et al. (2021)
BFT	Spent coffee grounds (SCG)	Nile tilapia (*O. niloticus*) (15.25 ± 0.07 g)	SCG1 (control), SCG2 (10 g kg^{-1}), SCG3 (20 g kg^{-1}), SCG4 (40 g kg^{-1}), and SCG5 (80 g kg^{-1}) 8 weeks	Resistance to *S. agalactiae* ↑	Van Doan et al. (2022a)

resistance against *Streptococcus agalactiae* and *Aeromonas hydrophila* have been reported in Nile tilapia-fed orange peels derived pectin (OPDP) (Doan et al. 2018); OPDP and *L. plantarum* (Van Doan et al. 2019); in-situ and ex-situ biofloc (Menaga et al. 2019); jaggery-based BFT (Elayaraja et al. 2020); chestnut polyphenols (CSP) (Van Doan et al. 2020a); watermelon rind powder (WMRP) (Van Doan et al. 2020b); pineapple peel powder (PAPP) (Van Doan et al. 2021a); pineapple peel powder (PAPP) + *L. plantarum* (Van Doan et al. 2021d); coffee silverskin (CSS) (Van Doan et al. 2021c); amla (*Phyllanthus emblica*) fruit extract (Van Doan et al. 2022b); beneficial bacteria (Mohammadi et al. 2021); spent coffee grounds (SCG) (Van Doan et al. 2022a). It has been also reported that a biofloc system could reduce ectoparasite spread Durigon et al. (2019). A significant increase in disease resistance may be attributable to the presence of MAMPs in the biofloc system, which may be recognized as immunostimulants, resulting in higher resistance to diseases (Ekasari et al. 2014b). In addition, it may be due to the prebiotic and probiotic properties of feed additives, which are known to enhance the immune response and disease resistance of Nile tilapia (Cavalcante et al. 2020; Cano-Lozano et al. 2022).

9.3 Recirculating Aquaculture Systems (RAS)

9.3.1 Brief History of Development

Though in its infancy and still considered to be a recent innovation, the basic technology of RAS has existed for over 65 years, with the first, pioneering RAS research activity being conducted in Japan in the 1950s (Murray et al. 2014; Saeki 1958). According to Espinal and Matulić (2019), the technology of RAS including aquaponics has been developed over the past 40 years. In the 1970s, a German program demonstrated the feasibility of intensive carp production in RAS, and subsequently, the Danish Aquaculture Institute undertook an innovative effort to develop further technical aspects of RAS (Goldman 2016). The idea for commercial fish production in RAS was first fostered in Denmark in the mid-1970s, and the first commercial RAS was then built in 1980 (Warrer-Hansen 2015). The Danish efforts supported the development of one of the initial commercial RAS industries, specifically for the production of European eel (Anguilla Anguilla) (Goldman 2016). This work inspired the subsequent further development and uptake of RAS in other European countries in the late 1980s and 1990s (Martins et al. 2010). Over the last 25–35 years, a significant and growing experience in designing, building, and operating RAS, particularly in Nordic countries, has been reported (Dalsgaard et al. 2013). The initial success of the RAS-based European eel industry also inspired to development of RAS in North America (Goldman 2016). In China, marine RAS was initiated in the 1980s, and since then China has made considerable progress in RAS (Ying et al. 2015). Since the 2000s, further development of RAS has occurred in Europe, North America, Australia, and other aquaculture-producing countries (Espinal and Matulić 2019; Martins et al. 2010).

A significant acceleration in the development of RAS technology has been observed over the last two decades (Espinal and Matulić 2019), and RAS have become popular in recent years. RAS has been developed to grow fish where inadequate biophysical conditions, water scarcity, poor water quality, and unfavorable environment exist (Murray et al. 2014). According to Malone (2013), RAS provides an alternative production method when environmental regulations, disease, land availability, salinity, temperature, and water supply prevent more cost-effective alternatives. However, other factors stimulated the development and implementation of RAS. For example, RAS are increasingly being used for Mediterranean marine fish and salmonid production cycle, particularly for juvenile stages, before being transferred into outdoor grow-out systems, such as cages or flow-through raceways (Bostock et al. 2016; Clarke and Bostock 2017; Terjesen et al. 2013). In fact, RAS can be used for broodstock and seedstock production, which can support cage and net-pen aquaculture (Malone 2013). In Europe and North America, RAS was developed as an alternative to the cage culture of salmon (Murray et al. 2014). RAS has also been developed to culture exotic fish species, to avoid adverse effects on native species and biodiversity (Malone 2013; Murray et al. 2014).

9.3.2 Basic Principles of RAS Operation and Production

RAS are land-based, indoor fish-rearing facilities, where fish are stocked in tanks within a controlled environment, and where filtration is applied to purify water by removing metabolic wastes of stock, before being recirculated into the system itself. Water purification is achieved through mechanical and/or biological filtration, sterilization, and oxygenation. Different levels of sophistication and efficiency can be achieved, but generally, all RAS have a high degree (>90%) of water reuse (Murray et al. 2014; Badiola et al. 2012). RAS provides opportunities to enhance waste management, reduce water usage, and nutrient recycling (Martins et al. 2010; Murray et al. 2014; van Rijn 2013).

Although RAS have been initially developed and are ideally suited to produce freshwater as well as warm water fish species, RAS is flexible and can be modified and adapted to be operated with brackish and marine water as well as cold water species (Helfrich and Libey 1991). Therefore, by decoupling fish production from the marine environment, RAS may offer an alternative to traditional and net pen aquaculture (O'Shea et al. 2019). RAS can also provide suitable environmental conditions for fish species that are sensitive to water quality (Zhang et al. 2011). Despite RAS the potential to produce diverse seafood products, RAS is generally utilized to culture high-value fish, with high stocking densities and year-round production to offset high operational costs (Dalsgaard et al. 2013; Martins et al. 2010; Murray et al. 2014). Nevertheless, other fish species, including arctic char, clarias, halibut, pangasius, tilapia, and turbot are also commonly produced in RAS (Badiola et al. 2018; Ngoc et al. 2016a; Ngoc et al. 2016b; Summerfelt and Vinci 2008). The selection of fish species can be "market-driven" due to a high return on investment to keep the RAS profitable (Badiola et al. 2017). The choice of fish also depends on the fast-growing and hardy fish in RAS (Badiola et al. 2018).

RAS can be categorized into five types: (1) hatchery and grow-out, (2) breeding, (3) long-term holding, (4) short-term holding, and (5) display (Yanong 2012). Moreover, RAS can be incorporated into an "integrated agriculture-aquaculture" system, which is known as aquaponics (Martins et al. 2010). Aquaponics is considered a particular type of RAS, where vegetable plants are included with fish to provide water filtration and crop diversification (Goddek et al. 2019). RAS have greater control over production outcomes, and the productivity of RAS depends on culture species, stocking densities, feeding rate, duration of the production cycle, and other management aspects. According to available scientific literature, the stocking densities of RAS range from 70 to 120 kg/m^3 with feed conversion ratio (FCR) values from 0.8 to 1.1. RAS can be of various sizes including small, medium, and large (Helfrich and Libey 1991), with a large-scale RAS typically being able to produce 400e500 tons of fish per annum (Murray et al. 2014). However, even higher stocking densities and total production values are currently reported by some commercial producers. According to (Bregnballe 2015), RAS are highly productive intensive farming, which generates vast quantities of fish (500 tons/ha/year) in a comparatively small volume of water. Because of higher production, RAS is often referred to as "hyper" or "super" intensive farming (O'Shea et al. 2019).

9.3.3 RAS in Tilapia Culture

There is limited information regarding the application of RAS in Nile tilapia farming. It has been reported that RAS is a costly engineering approach, with a high initial investment in installation and operation (Murray et al. 2014). The reported annual production cost of RAS (US$2250e8800 per ton) is considerably higher than conventional pond aquaculture (US$2000 per ton) (Waite et al. 2014). Additionally, the Economic viability of RAS requires a long payback period, on average 8 years (Badiola et al. 2012). Wang et al. (2020) indicated that light intensity and photoperiod manipulation did not cause a significant chronic stress response in tilapia. This study demonstrated that light intensity, especially at 2000 lx, and photoperiod manipulation could stimulate the growth of tilapia in the RAS and significantly affect economic profitability. Another study using a different magnetic field (Hassan et al. 2018) showed that based on the growth, water properties, and serum biochemistry, it was concluded that magnetized water at 0.15 T intensity may improve tilapia growth in recirculating aquaculture systems. Recently, Deng et al. (2022) indicated that rearing fish larvae in RAS supports better survival compared to the flow-through system, while dietary probiotic supplementation further modulates the gut bacterial composition and stimulates the presence of beneficial bacteria during early life. It has been reported that RAS has a more stable and diverse microbial community composition, which could result in better growth performance compared to other culture systems (Deng et al. 2022). In addition, RAS could reduce the ammonium and toxic gas in the culture system (Villar-Navarro et al. 2021; Nguyen et al. 2021).

A comparison between RAS, clear water, and biofloc system has been conducted in Nile tilapia. (Fleckenstein et al. 2018) indicated that clear water or hybrid systems may be a better choice for tilapia nurseries than chemoautotrophic biofloc systems due to the short-term periods in which nurseries operate and the volatility of nitrification in biofloc systems. In another study, Cao et al. (2020) indicated that There was no significant difference between the RASs and BFT aquaculture systems in terms of P recovery rate. The regular backwashing of the drum filter and biological filter in RAS accounted for 41 ± 2% of input N and 39 ± 2% of input P. Approximately 54% of unassimilated nitrogen N was removed by nitrification in the BFT aquaculture systems. The results from the present study suggest that nitrification may be the dominant pathway for ammonia removal in a BFT aquaculture system rather than by heterotrophic bacterial assimilation. RAS is characterized as a closed aquaculture and water reuse system; however, without an efficient and effective system for the treatment of discharged water and solids, this characterization only seems to indicate potential. Significantly, the treatment of the solids and water discharged from RASs has been suggested by (Luo et al. 2013). In BFT systems, most unused N and P are retained in biofloc and nitrate in tanks. The biofloc can be used for shrimp feed (Ray et al. 2017) or for feeding Artemia (Luo et al. 2017). Nitrate may be reduced by denitrification and dissimilatory nitrate reduction to ammonium (DNRA) activities in the BFT systems (Chutivisut et al. 2014). In these respects, the production activity in BFT systems may be more closed than that of RAS (Cao et al. 2020).

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