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Probiotic Aquaculture Techniques for Fish Production in Jericho (Palestine)

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Probiotic Aquaculture Techniques for Fish Production in Jericho (Palestine)

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Probiotic Aquaculture Techniques for Fish Production in Jericho (Palestine)

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Dedication

This work is dedicated to those who gave me life and grew me up, my parents who were always my supportive. I owe them each moment of my life.

To my beloved brothers and sisters.

To my valuable treasure my wife, for her endless love and support. To my children.

To my great professors who were always a constant source of knowledge and inspiration.

To my friend, Imad Aljabali, for his efforts

إهداء

هذا العمل مقدم لأولئك الذين أعطوني الحياة ورعوني حتى كبرت، والداي الذين كانوا داعمين لي دائماً، أنا مدين لهم بكل لحظة من حياتي.

لإخوتي وأخواتي.

إلى كنزي الثمين زوجتي، لحبها ودعمها اللامتناهي، الى اطفالي فلذاتي قلبي.

لأساتذتي العظماء الذين كانوا دائماً مصادر ثابتة للمعرفة والإلهام.

إلى صديقي عماد الجبالي على جهوده

Declaration

I Certify that this thesis submitted for the degree of Master of Science in environmental studies is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

Name: Samer Munawar Mohammad Jebreen

Signed: ……………Samer………………..

Date: 11/5/2022

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List of Abbreviations

mg/L	Milligram per Liter
T	Temperature centigrade
cm	centimeter
mg/kg	Milligram per Kilogram
^oC	Degree Celsius
(K) factor	Condition factor
L	Length
WHO	World Health Organization
g	Gram
m	Metter
pH	Potential Hydrogen
ppm	Parts-per-million, 10^{-6}
BW	Body Weight
CP	Crud Protein
USAID	United States Agency for International Development
PLO	Palestine Liberation Organization
FAO	Food and Agricultural Organization
ADS	Arab Development Society
mm	Mille Metter
ft.	Feet
OCHA	Office for the Coordination of Humanitarian Affairs
ARIJ	Applied Research Institute - Jerusalem
EM	Effective Microorganisms
DO	Dissolved Oxygen
BFT	Bio floc technology
UIA	unionized ammonia
NH3	Ammonia
NH_4^+	Ammonium
$NO2^-$	Nitrite
$NO3^-$	Nitrate
CFU	colony forming units
RAS	recirculating aquaculture systems
UK	United Kingdom
TAN	total ammonium nitrogen
NGOs	non-government organizations
ADG	Average daily gain
TADG	Total average daily gain
TW	Total weight
SD	Standard deviation
Min	Minimum
Max	Maximum
Crude protein	Cp

تقنيات استزراع الخمائر لإنتاج الاسماك في أريحا (فلسطين)

اعداد: سامر منور محمد جبرين

إشراف: البروفيسور معتز علي القطب

الملخص

خلال السنوات الأخيرة ومنذ أكثر من ستين عاما كانت هناك محاولات عديدة لتربية الأسماك في الضفة الغربية وغالبيتها لم يكتب لها النجاح بسبب تحكم الاحتلال بمصادر المياه ومصادر الأسماك بشكل عام وكان لذلك اثرا سلبيا كبيرا على الثروة المائية والحياة الزراعية وبالأخص الثروة السمكية التي تعتبر شبه معدومة في الضفة الغربية ويعود ذلك الى شح المياه، نقص الخبرة، تكاليف الاستثمار في هذا المجال والمنافسة الشرسة لمنتجات المستوطنات والاحتلال. الأسماك التي تم دراستها بتاريخ 2020/3/20 م، من الأسماك المهمة والتي تحتل المرتبة الثالثة من حيث جودتها، تربيتها، تحملها للظروف البيئية واحتوائها على مصدر جيد من البروتين والعناصر الغذائية. من الضروري ان يتم تنشيط القطاع السمكي في الضفة الغربية لما لذلك من أهمية كبيرة على مختلف الأصعدة السياسية، الاقتصادية، الثقافية والتغذية والتي تخص كل مزارع ومواطن. أجريت هذه الدراسة لإيجاد تقنيات جديدة وغير مكلفة تستخدم في تربية الأسماك بحيث تصل نسبة التغيير اليومي للمياه للصفر، تقلل استهلاك العلف، حيث تم استهلاك 211 كغم من العلف الطافي (بروتين 35%) خلال فترة التجربة (125) يوما وتزيد الكثافة السمكية ضمن المتر المكعب الواحد حيث تم وضع 200 سمكة في 5 متر مكعب من المياه في حوض الخمائر مقارنة بالأحواض الأخرى (150) سمكة فقط وتم تغذية الاحواض الثلاثة باستخدام نفس كمية العلف والذي نتج عنه استهلاك كمية علف اقل في حوض الخمائر.

تم تجهيز ثلاثة احواض اسمنتية بسعة ستة متر مكعب لكل حوض لأجراء هذه التجربة في منطقة اريحا حيث تم استخدام الخمائر لتغذية البكتيريا التي تساهم في التخلص من المركبات النيتروجينية السامة وتوفير بيئة ملائمة. تم استخدام سمك البلطي (المشط) في التجربة لتوفره وملائمته للظروف المتوفرة في المنطقة دون الاخذ بعين الاعتبار الجنس. من خلال مراقبة الأسماك والبيئة المائية أظهرت التجربة نتائج إيجابية وفعالية جيدة لهذه الخمائر وكانت تراكيز الامونيوم والنترات في حوض الخمائر اقل من الحدود المسموح بها وكذلك باقي الاحواض. تقنية الخمائر توفر بيئة ملائمة لتربية الأسماك وكان اقبال السمك على العلف مناسبا حيث، تمكنت الأسماك من التعايش وحققت نموا جيدا ولم تحدث أي علامات على الاجهاد. استخدام هذه التقنية في منطقة الضفة الغربية كفيل بان يساعد على الاستثمار في مجال الثروة السمكية الذي بدوره يساعد في توفير اسماك محلية للمستهلك الفلسطيني وبالتالي تطوير ثقافة التغذية ليرفع بذلك كمية استهلاك الفرد الفلسطيني من الأسماك. ومن خلال هذه الدراسة، نستنتج أن هناك تقنيات عديدة لتربية الأسماك دون استنزاف الموارد المحلية وخاصة تقنية الخمائر التي تقلص استهلاك المياه الى حدود قصوى، تقلل استهلاك العلف وتزيد الكثافة السمكية ضمن الحوض الواحد مما اضاف قيمة اقتصادية جيدة قد تساعد في تطوير الاستزراع السمكي في الضفة الغربية. توضح هذه الورقة المعرفة الحالية باستخدام الخمائر في تربية الأحياء المائية، وسوابقها، وإجراءات السلامة التي يجب تنفيذها، وتناقش آفاق الدراسة في هذا المجال.

Abstract

The increasing demand for an environmentally friendly aquaculture technique has certainly developed to serve intensive farming and water quality. Probiotics is a new technique that can be adapted to fish farming in order to minimize both water consumption and food supply.

This study was conducted to find new and inexpensive techniques that can be used in fish farming so that the daily change of water is reduced near to zero, reduces feed consumption, and increases fish density within one cubic meter. The fish consumed 121 kg of floating feed in 125 days which resulted in low feed consumption in the probiotics pond which contained 200 fish compared with the other pond which contained 150 fish per each. The experiment started on March 20,2020 and ended on July 25,2020.

Three cement ponds with a capacity of six cubic meters per pond were prepared for this experiment in the Jericho area, where probiotics were used to feed bacteria that contribute to the disposal of toxic nitrogenous compounds and provide an appropriate environment for fish growth. Tilapia fish mix (male and female) was used in the experiment due to their suitability for area conditions. Through monitoring fish and the aquatic environment, the experiment showed positive results and good efficacy for these probiotics. The concentrations of ammonium and nitrate in the probiotics pond were less than the harm limits as well as the rest of the basins. The probiotics technology provides a favorable environment for fish farming. However, the fish managed to cope with the temperatures and nutrients in the water, as they achieved good growth and there were no signs of stress. The use of probiotics technology in the West Bank region is enough to help in investing in the field of fish sector, which in turn helps in providing local fish to the Palestinian consumer and thus develops a culture of nutrition to raise the amount of Palestinian fish consumption per person. Through this study, it was concluded that to produce one kg of tilapia fish need 1.77 kg of feed. Overall, probiotics created an improved water quality environment within low and near-zero water exchange that generally resulted in enhanced growth rates, survival, feed conversion, and condition factor. The cost result for tilapia production when using probiotics is 1.55 USA dollars compared with control pond and the mix pond cost 2 USA dollars. Probiotics are, therefore, recommended to the fish farmers to increase the profitability of the aquaculture business.

Chapter One

Introduction

Fish are very important in the development of countries. Apart from being a cheap source of a highly nutritive protein, it also contains other essential nutrients required by the body (Famoofo *et al.*, 2020). Fish acts as a medicinal food due to its supplementation of the human body with essential vitamins A and D; calcium, phosphorus, and important elements. Nile tilapia (Oreochromis niloticus) is the second or third most farmed fresh fish species globally after carps (Naiel *et al.*, 2019). It can live in wide culture and have the ability to feed on various feed ration, rapid growth to reach the market weight, tolerance to a wide range of culture conditions and environment like (T, salinity and DO), popularity with consumers, ease of breeding in captivity, and wide availability to farmers. Nile tilapia is the second most farmed fish in the world after carps that in general are very famous and popular.

Tilapia aquaculture production was spread to many additional countries in more than 140 countries. China is the largest aquaculture producer, consumer, and exporter (Li *et al.*, 2011). The Aquaculture in the West Bank – Palestine Authority is very limited due to the occupation of Israel. The most limiting factor for fish aquaculture in the West Bank is the availability of water resources and fish availability. There is a limited production of freshwater fish distributed in different cities in the West Bank with only one hatchery located in the Arab Development Society – Jericho where Tilapia fish are raised and bred in different ponds. The average fish consumption per capita per year, in the West Bank, is very low (3 kg) compared with (20.9 kg) for the average world consumption per capita per year (Obiero *et al.*, 2019).

Many types of fish can be adapted in the West Bank area like (Tilapia, Mullet, Bass, Carp, and Catfish). In order to encourage farmers to work in this sector we should find new pioneer ways to grow fish with the least amount of water exchange and also reduce the amount of feed. We need to increase the density of breeding and achieve good productivity and quality that are acceptable to the consumer. Searching for cheap organic and inorganic resources that can enhance the environment by enhancing the beneficial bacteria is a great goal. The striped bass is adapted to live in both saltwater and freshwater. Culturing the bass fish gives beneficial characteristics because they grow faster and are trained easily to a pellet feed. They are more disease-resistant and can grow in a wide range of environments.

In recent years, intensive ponds have been developed. They are based on the idea that well-aerated mixed pond water can contribute to its purification. Blowers or buddle wheels had been used. Water is mixed to keep particles continuously in suspension, and water exchange is greatly reduced (to about 10% per day). This method saves about 90% of the water used. Under these conditions, microbial flora was developed, functioning both as a bioreactor controlling water quality by controlling the nitrogen cycle, and as a protein food source for the fish. The intensive ponds are used in occupied Palestine mostly to grow tilapia (Oreochromis niloticus x O. aureus) using filter feeders that are known to ingest suspended particles (Milstein *et al.*, 2001). Elsewhere, the same principle is used for intensive Carp fish culture, Bass fish, and Nile tilapia in the west bank of Palestine (Jebreen, 2020).

The fisheries were divided into the marine fisheries along the east coast of the Mediterranean Sea with operations carried out from the main fishing ports of Acre, Haifa, Jaffa, Askalan, Gaza as well as from the Gulf of Aqaba in the south and the fisheries activities in fresh and brackish waters and pond culture. There was a high demand for fish in the local markets but despite the length of the coast of Palestine along the Mediterranean; landings did not exceed 900 tons of fish per year (Zaqoot *et al.,* 2012). During the period from 1921 to 1952 there are more than 91 boats of different sizes, equipment and the number of fishermen was more than 534.

All the fishermen were Arab inhabitants which numbered reaching about more than 190 fishermen. The number of fishing boats in the lake increased, and the amount of fish caught increased to nearly 1000 tons in 1951/1952. The main fishing centers were in north-western coasts and the eastern shores of the Lake. As for the Dead Sea, its waters physical and chemical conditions do not permit the existence of a fish's wealth and are free from any life. The waters of some of these rivers run fast with some fish species of tilapia, mullet and Carp.

As aquaculture develops, its environmental impact and need increases. Rotating biological contactors, trickling filters, bead filters, and fluidized sand biofilters are conventionally used in intensive aquaculture systems to remove nitrogen from culture water. Besides the water treatment systems, there are other possible ways to recycle aquaculture water to produce fish feed. probiotics technology, which can be used in extensive as well as in intensive systems and semi-intensive culture. To maintain good water quality, this technique provides a feed source with a higher efficiency of nutrient conversion of feed. The probiotics technology has the advantage over the other techniques in that it is relatively inexpensive; this makes it an economically viable approach for sustainable aquaculture (Crab *et al.*, 2007).

Wide range of beneficial bacteria have been evaluated as probiotics such as Aspergillus, Lactobacillus, Bacillus, Micrococcus, Carnobacterium, Enterococcus, Streptococcus, and Saccharomyces species. The products of probiotics could be added through water as solutions or adding in feed daily.

Condition factor (K) is the parameter that reflects the state of well-being of the fish and its health. The K value is based on the role of a given length that reflects the physiological condition of the fish and can be used as an index to assess the status of the aquatic ecosystem (Dan-Kishiya *et al.*, 2013).

Probiotics, defined by the Food and Agricultural Organization and World Health Organization, are live microorganisms that when administered in adequate amounts give a health benefit. The word "probiotic" was first described by Parker (1974), as organisms and substances that contribute to the intestinal microbial balance in the water and body. Probiotics are a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Rollo *et al.*, 2006). There are various strategies to enhance the composition of the gut microbiota for better growth, digestion, immunity and disease resistance have been investigated in various kinds of livestock and humans.

1.1 Background

This study aims to find inexpensive pioneer alternatives to fish farming in the West Bank of Palestine. The study aims to develop the fisheries sector in the region and to solve the problem of water scarcity and feed cost in Palestine to encourage the farmers to invest in this field despite the importance of the fish sector in all aspects.

This study is considered as a cornerstone for fish farming in the West Bank and supports the hypothesis that despite the occupation and control over agricultural resources, we can raise fish and promote this sector in cooperation between the public and private sectors. We can find positive solutions that contribute to the development of fish wealth. The West Bank region suffers from limited water sources, in which all sectors depend on well water and springs, a large part of the Palestinian water resources is exposed to pollution by settlements and wastewater, accordingly there is a need for scientific research and studies to develop fish resources that are almost non-existent except for some farms. The Palestinian consumer have the right to buy fish alive Palestine has multiple water resources that support a diverse marine and aquatic animals.

The study area is distinguished because it is an agricultural area with diverse plants and animals. It contains agricultural pools and ponds for fish farming, especially the Arab Development Society. The Jericho and Jordan valley region are considered a warm and suitable area for fish farming in the West Bank, but the availability of water and the cost of fish farming remains an obstacle and a problem that the Palestinian farmer suffers from.

1.2 Problem Statement

Agriculture is one of the most important sectors especially in developed countries. It is an ideal way to achieve self-sufficiency and provide resources, especially fishery resources, which are considered a culture that has originated in Palestine. The Mediterranean Sea which unfortunately has very poor natural fish resources is the primary local resources for fishery products here in Palestine and it has accommodated a thriving fishing industry over the years. For many reasons including the occupation control on the Palestinian coastal region, Palestinian fishers have faced many obstacles resulting in a steady decline in fishery catch. Due to low catches and the need to import, fish prices are extremely high. Few Palestinians can afford to purchase seafood in spite of increasing need for this vital source of healthy animal protein. Aquaculture may be a viable alternative to overcome the shortage of fish in Palestine. Although Palestinians realize the exigency of introducing such an industry, the lack of knowledge, scientific research and expertise, and the absence of governmental support and infrastructure, have hindered any serious achievements in this field.

1.3 Project Objectives

The main goal of this thesis is to introduce new techniques for fish farming in Palestine. New techniques that reduce water consumption and feed supply by using probiotic materials in well-aerated water. This new technique will enhance fish aquaculture in poor water regions on the west bank of Palestine.

The Specific Objectives are:

1. To determine the nature and composition of probiotic material that gives the maximum production in brackish and freshwater.
2. To determine the physical and chemical water parameters that give the maximum production in brackish and freshwater.
3. Study the possibility of reducing feed consumption (up to 25 % or more) without affecting fish quality and production.

4 To develop environmentally friendly aquaculture systems.

5 To build up systems providing an equitable cost/benefit ratio to support economic and social sustainability in the poor water region of Palestine.

6 Intensive fish farming by increasing the density of fish per cubic meter using the probiotic technique.

1.4 Project Significance

This project is important for citizens and the local community in Palestine, especially those who own vegetable and fruit farms, as they have untapped agricultural ponds for fish farming as well as those who have ponds and fishponds. Through this study, decision-makers can rely on data related to fish farming in the region to support fish wealth and guide them to invest in this sector for economic importance.

This research project will provide rich information and data on the reality of fish in the West Bank of Palestine and through this study, the Palestinian farmer can control fish farming and achieve reasonable profitability through this technology a deeper understanding of the management and operation of the farm and avoid the occurrence of stresses for fish and thus obtain a local fish product to reduce the size of import from foreign markets, which represents 99% of the volume of fish consumption in the region.

This technique uses simple materials and is available at the lowest prices. It can be used for more than one type of freshwater fish such as carp, Bass, Tilapia, Mullet, some ornamental fish when providing a good source of oxygen and temperature monitoring. This technology is expected to be used in all governorates of Palestine.

Chapter Two

Fresh water aquaculture particularly in Palestine

As a result of the population explosion and the increase in the demand for protein in general and marine products and fish in particular, fish farming systems have been developed to provide food that has a great demand. Aquaculture is a system for raising marine organisms and fish in very large quantities within the least possible space, based on providing a tight environment, providing a permanent oxygen source, and controlling the chemical and physical properties of water. Aquaculture is the industrial development of animal farming and the fish sector in all its aspects. The more technological development in the aquaculture sector increases, the higher the risks of causing pollution in the environment, which requires studying the preservation of the environment and taking it into account. (Pillay, 2008)

Aquaculture represents a complete circle of aquatic production represented in the establishment of fish hatcheries, nurseries, breeding ponds, production, and marketing in various appropriate ways. Aquaculture is the tool to fill in the gap of the seafood supply. Farming fish is the solution to providing future generations with access to healthy and environmentally friendly protein types. Not only is aquaculture necessary, but it is also a sustainable option for consumers, especially in comparison to other farmed proteins. Seafood has the highest protein source compared to chicken, sheep, and beef. It has the lowest feed conversion ratio among the same forms of animal's protein.

Marine aquaculture refers to the culturing of marine species in ponds or intensive tanks (Salayo *et al.*, 2012), while freshwater aquaculture is the culturing of freshwater species. According to the United Nations Food and Agriculture Organization (FAO), the world caught or produced about 171 million tons of fish in 2016—with more than 80 million tons coming from fish farms, including fresh-water ponds and ocean farming inside or outside. Interestingly, 20 million tons came from wild stocks mainly to be processed as feedstocks for fish farms, which is a reduction from previous years. Palestine is among the countries that contain a great diversity of animal production, especially marine life and various water resources. They are located in the Mediterranean Sea of Palestine, the Red Sea, the Sea of Galilee, Hula Lake, and some of the major rivers and springs, which gives them a great diversity of fish and marine biology resources. Fish production from ponds in the West Bank does not exceed 8 tons for the year 2019 and most of it (6 tons) were produced from the Arab development society - Jericho and only for certain types of fish (Tilapia, Basses, Mullet, Carp). Not much research and studies have been done in the field concerning aquaculture, fish and algae. There is no significant development in this field, except for some fish farms and laboratories in the West Bank.

2.1 Freshwater fish in Palestine

Despite its small area, Palestine is distinguished by a great biological diversity at the side of aquatic organisms, as it provides many different water sources (the Red Sea, the Mediterranean Sea, Jordan River, and Lake Tiberias). Lake Tiberias is considered an ancient original home for freshwater fish (Borovski *et al.*, 2018), especially tilapia, which is called al-Tabarani concerning Lake Tiberias and the Jordan River as well and lives there and raised in ponds adjacent to the lake area in addition to some other species that have adapted to water conditions such as mullet, bass, and carp (Borovski *et al.*, 2018).

2.1.1 Nile Tilapia fish

Tilapia aquaculture is known in more than 140 countries. Its production continues to spread worldwide. (Webster *et al.*, 2006) China is the largest aquaculture producer, consumer, and exporter while Egypt and Indonesia come second in the amount of tilapia production/ton. The Aquaculture in the West Bank – Palestine Authority is very limited due to the occupation of Israel. There is a limited production of freshwater fish distributed in different cities in the West Bank with only one hatchery located in the Arab Development Society – Jericho in which Tilapia fish were raised and bred in different ponds. All types of Tilapia can grow, breeding and farming in fresh and brackish water, the most important one is Nile tilapia.

Figure 2.1: Nile tilapia fish (ADS.2021).

Sexual maturity is reached at 12-20 cm TL, (20-40 gm/fish) and is related to the maximum size attained in a given population and condition, which in turn is determined by food availability, oxygen and temperature. Reproduction occurs only when the temperature exceeds 20 °C and more. The best temperature for spawning is (26-28) °C. The female incubates the eggs for 7-10 days when they hatch depending on the water temperature and the early juveniles remain in the mouth until after yolk sac absorption. Depending on size, females of 200 gm can carry up to (350-1200) eggs each cycle. The eggs are large and diameter varied between 2mm and 3 mm and at hatching the fish are around 4mm in length (Komolafe *et al.*, 2007).

Early juveniles and fry fish are omnivorous, feeding mainly on zooplankton and zoo benthos but also ingest detritus and feed on phytoplankton this is in natural life but in industrial and intensive culture are fed with high source protein (50-53) % to grow very fast. At around 10 gm the fingerlings become herbivorous feeding mainly on phytoplankton, using the mucus trap mechanism and its pharyngeal teeth. The digestive tract of Nile tilapia is at least five - six times the total length of the fish (Chaudhary, 2018). Tilapia can be alive and grow without feeding for many years depending just on the microorganism's, algae and bacteria species from the water content.

Nile tilapia requires the same ten essential amino acids as other fishes. Fish in aquaculture need a good and high source of protein, up to 50-53% for newly hatched young fingerlings, 30-35 percent for fish weighing 50-350 g, 25-30% for fish weighing 350 g and more.

The best protein benefit and digestibility occurs at 25 °C (Chae *et al.*, 2008) and the optimum dietary protein to energy ratio was estimated in the region of 100 to 120 mg per kcal digestible energy respectively for fry and fingerling. Tilapia brood fish or mothers need high crude protein about 30-45 % for highly reproduction, spawning efficiency, and larval growth and survival. Tilapia fish needs a good level of lipids, up to 5-8%, to obtain the necessary fatty acids.

Carbohydrates are included in tilapia feeds to provide a cheap source of energy and for improving pellet feed properties. Tilapia can efficiently utilize as much as 35-40 percent digestible carbohydrate. Carbohydrate utilization by tilapia is affected by carbohydrate source, dietary ingredients, fish species, age and size, and feeding frequency per day (Ogello *et al.*, 2014). Nile tilapia can be utilizing high levels of various carbohydrates more than 30 % of the diet.

Vitamin is not necessary for tilapia in semi-intensive farming systems, while vitamins are necessary for optimum growth and health of tilapia in intensive culture systems where limited natural foods are available that can be added in the feed ration. Vitamin requirements of tilapia are known to be affected by other dietary factors and these must be taken into consideration in diet and feed rations.

The diet of the Nile tilapia comparing with fish size are as follow for many countries with some differentials:

Table 2.1: The diet of the Nile tilapia and fish size.

Fish size	Fry (<10g)	Fingerling(10–30g)	Grow out (>30g)	>300g
Moisture	10	10	10	10
Crude protein	50-53	35-40	35	30
Crude lipid	6–13	6–13	4–12	4–12
Crude fiber	4	8	8	10
Ash	16	16	16	16
Carbohydrate	25	25	25	25

Table2.2: Nile tilapia feeding rates and feeding frequency.

Fish size (g)	Feed type	Feed size	Feeding rate	Feeding frequency
		(mm)	(% body weight)	(no./day)
0–1	Powder/crumble	0.2–1	30–10	8 to satiation
1–5	Crumble	1–1.5	10–6	5–6
5–20	Extruded, floating	1.5–2	6–4	4
20–100	Extruded, floating	2	4–3	3
100–250	Extruded, floating	3	3–2	3
>250	Extruded, floating	4	3–1.5	3

2.1.2 Striped bass fish

Striped bass has many names like stripers, lineside, or rockfish. They are silvery in color. They can live in freshwater, brackish water and saltwater. Spawning begins in the spring and running water is necessary to allow eggs in motion until hatching. Striped bass don't have eyelids so when the sun shines and comes up, they will stay in deeper water to avoid the bright light. A mature female of striped bass fish can lay a lot of eggs, more than 2,500,000 eggs and the female will grow larger than the males (Boyd, 2011).

Figure 2.2: Striped bass fish (ADS.2021).

Ideal temperatures range from 18 to 22 °C, and evidence suggests a lower temperature limit of 10 °C. It can be adapted to 30 °C with sensitivity to oxygen deficiency. The physiological changes during stress were greatest and the recovery from these changes was slowest at 6 and more than 30°C. Striped Bass can tolerate the stress of low DO at 5 mg/l, high T, low T, fluctuating pH, high nitrite, and a wide range of salinity. (Davis *et al.*, 1990)

In the table below the optimum water quality parameters for striped bass are shown:

Table 2.3: Optimum water quality parameters for striped bass culture (Turano *et al.*, 2008)

Water Temperature	19-27°C
Dissolved Oxygen	>5 mg/l
pH	7-9
Ammonia	>0.02 mg/l
Hardness (CaCO3)	>60 mg/L

2.1.3 Mullets fish

Mullets are related to the Order Mugiliformes, Family Mugilidae. Mullets are ray-finned fish found worldwide in coastal temperate, tropical waters and in freshwater. Most species commonly reach about 20 cm in total length, but some may reach more than 70 cm. The head is small and flattened in most species. The mouth is small and gills of many species are specialized and have an expanded use for filtration of some materials. In many species of mullet, the tiny teeth are positioned on the lips (Schultz, 1946).

Figure 2.3: Mullet fish at ADS. Picture taken in 2019

Mullet are usually grayish-green or blue dorsally, and their flanks are silvery, often with dark longitudinal stripes Mullet are usually farmed in polyculture with other fish species (Saleh, 2008). Most mullets are found in coastal marine and brackish waters.

Mullet are cultured in a large number of countries usually in intensive, extensive and semi-intensive pond systems. Egypt has a long history of mullet aquaculture, which was traditionally practiced in the "hosha" system in the Nile Delta region for centuries (Saleh, 2008). Egypt is a leading country in mullet aquaculture with a record production of more than 150000 tons in 2005. The production of mullet fry has been reported in many countries like Italy, Israel and Egypt (Saleh, 2008). In Palestine (west bank) just one experiment about growing mullet fish in the ADS hatchery resulted in the fish reaching 900 gm in two years. Mullet fish are found in Palestine and their main source is the occupied interior areas. They can be reared in ponds all over the West Bank. Mullet is one of the fishes that copes with the climatic conditions of the West Bank region and was tried in Jericho. These fish tolerate a salinity of 3.5 parts per thousand, dissolved oxygen 6 mg/liter, temperatures of 30 degrees Celsius, $NH3^{-}$(0.02 mg/l) and ammonium 2 mg /Liter.

2.1.4 Carp fish (Cyprinus carpio)

Cyprinus carpio fish of the family Cyprinidae is native to Asia but has been introduced into Europe and North America and other countries. It is a large-scaled fish with two barbells on each side of its upper jaw, the carp lives alone or in small groups in quiet, weedy, mud-bottomed ponds, lakes, and rivers of freshwater or brackish water. It is omnivorous, and can be feed on many plants and animals.

Common carp are native to Europe but have been widely introduced and are now found in many countries except for the poles and northern Asia (Dwivedi *et al.*, 2013).

Figure 2.4: Carp fish in ADS. Picture taken in 2020

2.2 Fish in Arab Development Society –Jericho

The ADS is considered one of the oldest private agricultural societies in Palestine. It was established in 1945 on an area of 8300 dunums of land in the city of Jericho, near the Jordanian-Palestinian border. This association includes palm plantations, cow's farms, dairy factories, blacksmithing and carpentry department, alfalfa farms, and the fish aquaculture farms.

The fish hatchery was established in 2011 with funding from the Danish government from a hotbed for the hatchery of freshwater fish, especially Nile tilapia and accessories.

The idea of the hatchery is based on providing small fingerlings to farmers interested in raising fish in the West Bank, considering the limited fish sources and the Palestinians' reliance only on Israeli sources.

Figure 2.5: Hatchery in ADS and fishponds. Picture taken in 2020

Besides, ponds were established to breed fish in various shapes, octagonal cement, round, plastic and longitudinal cement, where fish are raised in them from 20 gm weight until they reach a marketing weight of approximately 300 g and more, the Palestinian consumer prefers larger weights at a rate of not less than 400 g / fish For tilapia fish, 400 g for mullet, at least 1 kg for carp and 500 g or more for bass, and these types are all raised in the ADS and the main dependence on tilapia that is spawned and raised at the same time.

The ADS is the only source in Palestine for fish farming and spawning in ponds for the West Bank in addition to some ponds within the Brazilian project that was completed in the year 2012 with the support of the Brazilian government by doing 29 ponds distributed in the West Bank with a capacity of 100 cubic per ponds and some unused agricultural ponds, as a result, The political circumstances and the cost of such projects in light of the decline in the Palestinian economic situation due to the Israeli occupation and the decline of the livestock sector in general.

Periodic tests such as (ammonium, nitrates, nitrites, salinity, heat, and oxygen) are used to evaluate the water quality in the ponds and direct dependence on changing the water, as 20% of the available water is changed daily so that this water is irrigated by the alfalfa, vegetable gardens, palm trees, and fields. The oxygen exchanges and pumps are used to supply fish with oxygen continuously in the ponds using blowers to maintain the dissolved oxygen in the ponds more than 6 mg per liter. The cost of removing water from wells and providing oxygen sources is one of the most important difficulties facing the fisheries sector in the region. Therefore, the private sector seeks with the public sector to find solutions to these problems to develop the fisheries sector in Palestine.

2.3 Fish farming methods

Culture types can be divided into three main categories: intensive, semi-intensive, and extensive, which represent low, medium, and high stocking densities, respectively.

2.3.1 Intensive farms

Intensive farms are commonly located in most aquaculture areas of Palestine. The ponds can be completely drained, dried, and prepared before each stocking. The bottom of the pond is covered with cement or plastic film.

Culture water must be pumped from groundwater or the water source. It should be very convenient to drain water easily. In this mode, each pond surface is about 1000 cubic (more intensive is 100 cubic) with a depth of (1-2) m and covered by a plastic film shed. Pond water is strongly aerated with paddlewheels (a paddlewheel per 300cubic) and a bottom aeration facility.

Figure 2.6: Intensive farm design in ADS. Picture taken in 2021

The intensive culture model has the advantages of strong controllability, high stocking density with 40-100 fish/cubic, high output range from 20kg to 40kg per cubic, and high profits. But it also has some disadvantages like huge-investment and high risk and so on. Intensive culture mode can be also classified into high-place pond intensive culture, industrial culture with smaller pond areas about 100~300 cubic, and circulating water culture.

2.3.2 Intensive culture characters

Cement bottom, earth film, greenhouse, pot-shape, pollution discharge in pond center, pollution absorption; height: 1.5 m above, coverage: shade (35-70) %, water inlet/outlet anytime. Equipment: paddlewheel per 300cubic, water quality test device. good quality land underground water or control water quality by probiotics, bio floc, or other techniques.

2.3.3 Tank culture of tilapia

Tank culture of tilapia is a good alternative to pond or cage culture if sufficient water or land is not available and the economics are favorable. Tilapia grow well at high densities in the conferment of tanks when good water quality is maintained. This is accomplished by aeration and frequent or continuous water exchange to renew dissolved oxygen (DO) supplies and remove wastes. Culture systems that discard water after use are called flow through systems while those that filter and recycle water are referred to as recirculating systems. Intensive tank culture offers several advantages over pond culture. High fish density in tanks disrupts breeding behavior and allows male and female tilapia to be grown together to marketable size with no big difference in weight (Rakocy *et al.*, 1990). In ponds, mixed sex populations breed so much that parents and offspring compete for food and become stunted. Tanks allow the fish culturist to easily manage stocks and to exert a relatively high degree of environmental control over parameters (e.g., water temperature, DO, pH, NH_4^+) that can be adjusted for maximum production. With tanks, feeding and harvesting operations require much less time and labor compared to ponds.

2.3.4 Semi-intensive culture

There is natural mud or sediment at the bottom of this culture pond. Only paddle wheels (one per 1000cubic) are applied to increase dissolved oxygen, and a plastic film shed is an optional facility. Although the controllability of this mode is not as good as that of intensive culture mode, the mud and sediment have a certain function to adjust the pond ecosystem. Supplemented feed is used to feed the fish like maize meal, soybean meal that can be used if there is no source of protein meal and rice (Nguyen, 2008). The stocking densities range from 10-30 fish per cubic. The production yield is around 8kg per cubic for tilapia fish. The ponds range from (2000-10000) cubic.

Figure 2.7: Semi-intensive culture at ADS. Picture taken in 2020

2.3.5 Extensive culture

Muddy or sandy bottom, difficult in water inlet per outlet, Ponds surface is usually 1–5 ha with (1–5) m in-depth, with only a few paddle wheels. The stocking densities are low (5 fish per cubic), and the output is (2-3) kg per cubic,1500- 3000 kg per ha, with or without supplementary feeding, no oxygen source and risk free.

The application of combined intensive-extensive (CIE) systems offers new opportunities to increase growth of aquaculture. In such systems a smaller intensive unit is linked to a larger extensive pond making possible this way the sustainable intensification and contributing to the economy. The extensive fish ponds will continue to provide valuable environmental and ecological services by maintaining the aquatic habitat for wild species, trapping and processing the organic wastes from the surrounding areas and contributing to the improvement of water quality in the ponds (Laszlo, 2013).

Chapter Three

Water quality and Treatment technique

Water chemistry requirements and optimal water quality is essential to a healthy, balanced, functioning aquaculture system and best farming. The growth of different fish species is also influenced by a different range of factors, among them water quality parameters. Fish growth is generally greater in ponds with optimal levels of DO, temperature among other parameters. Nile tilapia is ideal for culture due to its high growth rates, adaptability to a wide range of environmental conditions, ability to grow and reproduce in captivity, and ability to feed at low trophic levels. The most preferred temperature range for optimal growth of tilapia is 25 to 27 °C, while the ideal pH ranges between (6.5 – 9) (Makori *et al.*, 2017).

Water quality exerts a direct impact on the growth and survival of fish. Good water quality is the most fundamental condition for aquaculture. There are many water quality indexes, such as water temperature, salinity, dissolved organic matter, ammonia, nitrite and nitrate/nitrogen levels, hydrogen sulfide levels, phosphate levels, etc. It is difficult to conduct real-time monitoring on these indexes, and it is also difficult to handle when an exceptional situation occurs. Therefore, the best way to keep the water quality stable is ecological techniques.

3.1 Water Resources in Palestine

Water resources are a vital element of Palestinian ecosystems. Sources consist mainly of surface and groundwater resources (springs and wells). The most stable surface water resource is the Jordan River, however, a central feature of the Israeli occupation of the Palestinian territories is the denial of access to water and over-extraction of sources like the Jordan River, this has left Palestinians dependent on groundwater resources. Groundwater resources in the West Bank are more than 590 wells and 300 springs.

Two main springs in Jericho governorate, 'Ein al Sultan and Al'Auja, Jericho city has 99 private artesian wells used for agricultural purposes. The high levels of salinity limit the utilization of groundwater for both domestic and agriculture applications especially banana and vegetables.

3.2 Effective Microorganisms (EM)

Effective Microorganisms is a complex microorganism, which consists of more than 80 species such as photosynthesis, lactic acid bacteria, yeast, etc. EM can transform the dissolved organic matter into nutrients through a series of nitrogen fixation and photosynthesis, which benefits itself and other organisms. It also can increase the dissolved oxygen and reduce the content of harmful substances such as ammonia, ammonium and nitrite. The effective microorganisms (EM) as a probiotic is important in enhancing biological features and growth performance of cultured fish like Nile tilapia; O. niloticus (Omar *et al.*, 2017).

3.3 Watercolor

The color of pond water can be classified into brown (tawny)and green, of which the green is more stable, and the brown is the best. Maintaining a good water environment is the most important way to prevent the spread of diseases. For maintaining good watercolor, renewing water when needed, fertilizing, oxygenating, the introduction of other algae species, and keep the transparency of 30~40cm by using Secchi disk test (Garaba *et al.*, 2015).

3.4 Salinity

There are many classifications of salinity depending on the type of aquaculture. Tilapia fish can tolerate a wide range of salinity up to 10 ppt but can grow better in a range of salinity less than 2 ppt. The figure and table below show the salinity of water in many units and fish species with salinity tolerance.

The classification of water salinity as the table below:

Table 3.1: The water salinity units.

Classification	EC dS/m	EC µS/CM	Mm NaCl	ppm
Fresh water	0.8	800	8	500
Slightly brackish	1.7	1700	17	1000
Medium brackish	1.7-8	1700-8000	17-80	1000-5000
brackish	8-25	8000-25000	80-250	5000-15000
Strong brackish	25-58	25000-58000	250-580	15000-35000
Sea water	58	58000	580	35000

3.5 Ecological culture

Ecological farming is based on some ecological theories, such as the ecological balance, species coexistence, and multi-level use of the material. By mutualism and complementary principles between different organisms, they can live together in the same environment, maintaining biological balance, declining disease, and improving benefits. Besides fish, shrimp, shellfish, and algae, microorganisms, such as microalgae, bacteria, and small swimming animals also play an important role in the ecology system.

phytoplankton plays an important role in the ecosystem of fish ponds. Phytoplankton are not only directly or indirectly fed for the farming organism, but also the main oxygen provider for the entire ecosystem. If the ecosystem of fish ponds has many kinds of red tide algae, fish would easily be infected by the disease.

Zooplankton is an important live feed for fish fingerlings in the farming ecosystem as. It is also one of the key secondary producers in the pond ecosystem and plays an important role in the material cycle and energy flow.

Bacteria are an important part of the aquatic ecosystem biomes. Its production and function on debris decomposition play an important role in the material cycle and energy flow. The relationship of the three bio-populations: phytoplankton is the food supply for the zooplankton, but if phytoplankton overproduction (like red tide), it can change the water quality, which will inhibit the growth of zooplankton. Bacteria decompose organic matter, provide nutrients for phytoplankton, reduce the content of ammonia and nitrogen in the water (Park *et al.*, 2007).

3.6 Bio floc technology (BFT)

Bio floc technology (BFT) is a modern aquaculture farming technique that used to reduce toxic nitrogen compounds concentration, act as food source and eradicate pollutants using carbon and therefore to control C:N ratio in an aquaculture system (Panigrahi *et al.*, 2018).

Bio floc technology (BFT), also named bio flocculation technology, is achieved by regulating the C:N ratio of water and lead heterotrophic bacteria to transform nitrogen to protein as a kind of biological food (bio floc) supply for aquaculture. (a flocculating constituent). Ecological functions of bio floc by drive ecological nutrient circulation, nitrogen assimilation to save feed, increase C:N ratio to replace nutrient resource (sugar-protein), change water into nutrient(circulation) establish ecological nutrient chain and improve animal nutrient and improve growth rate and quality. Eliminate environmental pollution by removing ammonia and nitrogen, zero-water change, reduce water requirement, decrease eutrophication, restrain pathogenic microorganism, reduce culture risk, reduce medicine dosage, enhance digestion and immune system, improve growth and health. The composition of bio-floc are bacteria, algae, protozoa, rotifer, nematode, and gastrotrich. suspending bio floc size：0.5-5mm and suspending bio floc volume：>7mg/L.

Technically, the wastewater can't be discharged into natural waters without processing. Set up purification pools and sump pits to dispose of sewage by filtration, precipitation, and disinfection. In case there is enough space, it is possible to set up a wetland to handle and clean wastewater.

Figure 3.1: Bio-floc tube test in the experiment in ADS. Picture taken in 2021

3.7 Nitrogen

The source of nitrogen compounds is molecular nitrogen (N_2) in the air. It already referred to the composition of air and the solubility of atmospheric gasses in water. The nitrogen cycle involves ammonia fixing and nitrifying reactions in organizing and denitrification, which is the same process in reverse. The nitrification process involves oxidation of ammonia to nitrite and nitrite to nitrate which is an energy-yielding process utilized by nitrifying bacteria (Schmidt *et al.*, 1983). The reduction of nitrate to N_2 is brought about by denitrifying bacteria. The different forms of nitrogen present in natural waters include molecular nitrogen (N_2) in solution, organic compounds, protein and their breakdown products (amino-acids, urea, and methylamines); ammonia as NH_3, NH_4^+, NH_4OH; nitrite as NO_2^- mainly and fractions as nitrate NO_3^-.

3.8 Ammonia

Unlike nitrogen and oxygen ammonia is highly soluble in water. In natural waters where the decomposition of organic matter takes place ammonia levels will be high. Ammonia increase is often concomitant with a decrease in DO and an increase in CO_2. The level of ammonia will also depend on the activity of the nitrifying and denitrifying bacteria, referred to earlier. Ammonia is important as the predominant excretory product of aquatic animals and in high-density culture, high ammonia levels can develop, NH_3 is excreted directly and also by the degradation of fecal matter and uneaten feed.

Ammonia is highly toxic at levels of 0.02 mg/l even though they cause toxic effects in several fishes. The toxicity of ammonia is mainly caused by the unionized ammonia (UIA) (NH_3). Mead (1985) observes that NH_3 (UIA) is 300 – 400 times more toxic than NH_4^+.

The effect of ammonia toxicity is high, at higher pH, the proportion of unionized ammonia being higher at higher pH.

3.9 Probiotics technique

Probiotics are living microorganisms such as bacteria that resemble microorganisms that are naturally found in the human body and may be beneficial to health in general. Also referred to as "good bacteria" probiotics are available to consumers in oral products such as nutritional supplements and yogurt, as well as other products such as suppositories and creams.

The lower gastrointestinal tract (the gut) of the body, contains a complex and diverse community of bacteria (In the body of a healthy adult, cells of microorganisms are estimated to outnumber human cells by a factor of ten to one). Although it tends to think of bacteria as harmful "germs," many bacteria help the body function properly. Most probiotics are bacteria similar to the beneficial bacteria found naturally in the gut. Probiotics work by acting as food sources, providing enzymes to improve digestion, modify the immune system, and increase the immune response against disease-causing bacteria.

The most common probiotics used in aquaculture include different species as lactic acid bacteria such as Lactobacillus-sp., Bacillus-sp., Enterococcus-sp., and yeast, Saccharomyces.

Probiotics are measured in colony-forming units (CFU) (Ng *et al.*, 2014). CFUs are measured in the millions or billions per serving or work. Probiotics are most commonly beneficial bacteria but can also be friendly to fungal or other organisms that are typically freeze-dried to stabilize them in an inert state during storage and production. Their continued stability and viability, as measured by the number of CFUs when bred, depends on reducing their exposure to stimulating environmental conditions such as warmth and humidity. Besides refrigerating all the probiotics after opening, this protection can be done by adding freshness packs that help absorb and reduce moisture in the package.

Different routes of probiotics administration in an aquatic environment are delivery via feeding supplemented pellet food with probiotic, delivery via feeding supplemented live food with probiotics, direct addition to the water, and delivery via injection. Temperature plays a role in stabilizing probiotics. Cold air retains less moisture and does not fall within the ideal temperature range for bacteria to commonly grow and thrive, thus preventing the reactivation of dormant organisms by depriving them of the warmth and moisture that represent their ideal growing conditions. High heat can also degrade the viability of these organisms in probiotics. Under ideal storage conditions, the number of cells will slowly decrease over time. Cooling down will extend the potency and viability of most probiotics to maintain a higher population over a longer period.

Figure 3.2: Probiotics technique solution of the experiment in ADS. Picture taken in 2021

Chapter Four

Literature Review

4.1 Introduction

This chapter presents an approach to the concept of the study. The discussion presents reviews on probiotics, which includes an overview of the definition of probiotics as well as its various uses, followed by approaches for the perspective of reuse and preservation of the environmental resource. Furthermore, this chapter provides a range of previous studies and their results for the same concept. These studies will contribute to a general understanding of the research problem and to the adoption of relevant methods to answer the research questions. This chapter provides an overview of the use of probiotics in diets and their effects on growth performance, feed utilization efficiency, gut microbiota, immune responses, and disease resistance of tilapia.

4.2 History of Probiotics in Aquaculture Industry:

According to Morrarty (1966), microbial feed is an integral part of aquaculture ponds and has a direct benefit on productivity. Parker in 1974 described Probiotics for the first time as "organisms and substances that contribute to intestinal microbial balance and growth. Bacteria belonging to the genera Lactobacillus, Enterococci, Pediococcus, Bacillus, microscopic fungi, and Saccharomyces yeast are the main probiotics used in animal nutrition (Fuller, 1997). In 1998, Guarner and Schaafsma assumed that probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits to the host (Martínez Cruz *et al.*, 2012). Gatesoupe in 1999, defined them as "microbial cells administered in a certain way, which reaches the gastrointestinal tract and remain alive to improve health. In the same year, studies were carried out on the inhibition of pathogens using probiotics, this expanded the definition to live microbial supplement which benefits the host by improving its microbial balance. The traditional use of probiotics as feed additives, they are used in aquaculture and production systems to modify microbial populations in the environment leading to better growth and survival of many fish species. (Vijayakumaran, 2001)

Probiotics is "live microbial food supplement that benefits the host (human or animal) by improving the microbial balance of the body" and said that it would be effective in a range of extreme temperatures and salinity variations (Isolauri *et al.*, 2004). Probiotics (bacteria or yeasts) were defined by the Food Agricultural Organization (FAO) and the World Health Organization (WHO) joint report as live microorganisms which when administered in adequate amounts (in food or as a dietary supplement) confer a health benefit on the host (Miniello *et al.*, 2010). Probiotics these days are used to control fish disease and are very essential for the digestive process for fish and animals (Sihag *et al.*, 2012). Afterwards, it was suggested that probiotics were "monocultures or mixed cultures of microorganisms applied to animals or humans, that benefit the host by improving properties of indigenous microflora (Fijan *et al.*, 2014)

The term probiotics were used originally to describe the organisms and substances that contribute to intestinal microbial balance. Such substances prevented colonization of the gut by pathogenic organisms and promoted the utilization of feed. The use of probiotics is well documented in human and animal nutrition. Contrary to its traditional use as food additives, probiotics are used in aquaculture to mainly modify and manipulate the microbial population of the environment and to reduce or eliminate selected pathogenic species of microorganisms leading to better growth and survival. In aquaculture, the use of probiotics is confined to hatcheries of shrimps and fishes. However, the use of probiotics in the growth systems of fish have many benefits.

4.3 Urban probiotics in aquaculture

Toxic nitrogen forms must be removed from aquaculture systems since those high concentrations of nitrite and non-ionized ammonia can drastically reduce the growth rate, due to damage to gills and other internal organs (Lee *et al.*, 2000). Biological nitrification is the most common method used to eliminate toxic metabolites in high-density semi-closed and closed aquaculture systems. Ammonia oxidation into relatively harmless nitrate forms by biological oxidation must be monitored closely in the system. In a recirculating aquaculture system (RAS), ammonium excreted by the fish is typically transformed to less toxic nitrate by microbial activity in bioreactors. Nitrate-nitrogen, the nitrate-nitrogen load could be detrimental to the receptor aqueous body when released from the RAS facility (Lindholm-Lehto *et al.*, 2020).

Ammonium and nitrite, toxic metabolites originating in the feces, underused feed, and waste in aquatic systems can result in an enormous economic loss and they can affect the physiology, immunity, survival, and growth of animals (Jahangiri *et al.*, 2018). Traditionally, toxic metabolites have been controlled by bio filters and daily water exchange to remove any source of contaminants that causes any accumulation of ammonia. Specifically, in recirculating aquaculture systems (RAS), parameters of water quality need to be regularly controlled and monitored daily. Recirculating aquaculture systems (RASs) provide opportunities to reduce water usage and to improve waste management and nutrient recycling (Martins *et al.*, 2010). The zero-water exchange (under sufficient management of carbon: nitrogen ratio) leads to an accumulation of organic matter and nutrients in aquaculture systems. Usually the microbial community develops, and the diversity promotes microorganism's stability by way of nitrogen compounds that generate the microbial protein in situ. Organic carbon sources in the bio floc system can decrease ammonia-nitrogen concentration and improve the community diversities of overall and ammonia-oxidizing bacteria (Deng *et al.*, 2018). Probiotics are beneficial as they can increase microbial species' composition in the water and modify its quality (Zorriehzahra *et al.*, 2016). The temperature, pH, dissolved oxygen, and NH3 in rearing water were found to be of higher quality when probiotics were added, hence maintaining a positive healthy environment for fish in aquatic systems.

Supplementary feeding assumes a vital part in intensive and semi-intensive aquaculture systems and for example, the utilization of supplementary feed in carp culture turned out to be significant for successful fish culture (Abdel-Wahed *et al.*, 2018). Knowledge of probiotics has increased, currently, it is known that these microorganisms have an antimicrobial effect through modifying the intestinal microbiota, secreting antibacterial substances (organic acids), competing with pathogens to prevent their adhesion to the intestine, competing for nutrients necessary for pathogen survival, and producing an antitoxin effect. Probiotics are also capable of modulating the immune system, regulating the allergic response of the body, and reducing the proliferation of cancer in mammals (Yousefi *et al.*, 2019).

4.4 Feed probiotics

The concept behind the composition of feed probiotics is to apply the beneficial bacterial strains in feed using binders such as eggs, cod liver oil, molasses and flour to obtain the beneficial microbial effects with more efficiency and at a less environmental cost. Several alternative suggestions for disease prevention have been on probiotics for its efficacy, low cost, less side effects and accessible to farmers. Probiotics are getting a high priority in developed countries with the aim of replacing traditional medicines. The main bacterial groups tested as probiotic bacteria in the culture of shrimp, crab, shellfish, fish and humans are Vibrio, Pseudomonas, Bacillus, Bifidobacteria and several Lactobacilli (Kolndadacha *et al.*, 2011). The Feed quality and feeding methods, therefore, need to be thoroughly considered to improve growth performance and feed efficiency of the cultured animals. Several previous reports indicated that probiotic supplementation can reduce disease outbreaks by boosting the immune systems of fish and shrimp and can reduce farming costs by improving the growth and feeding efficiency of fish. (Tuan *et al.*, 2013)

4.5 Probiotics in aquaculture systems

Use of probiotics is one of such methods that is gaining importance in controlling potential pathogens. This review provides a summary of the criteria for the selection of the potential probiotics, their importance and future perspectives in the aquaculture industry (Sahu *et al.*, 2008). Commercial products are available in liquid or powder presentations, and various techniques have been developed for improvement. In the case of fermentation processes, the focus has been on optimizing fermentation conditions to increase the effectiveness and functions of probiotics, and to improve performance (Martínez Cruz *et al.*, 2012). The probiotic organism has good action in fish culture and safety evaluation (Pandiyan *et al.*, 2013). The necessity of using the probiotic *Bacillus* in sustainable aquaculture as a good alternative to improve feed utilization, stress response, immune response and disease resistance, maintenance of tissue integrity, and as well improvement of water quality for sustainable aquaculture are be summarized (Kuebutornye *et al.*, 2019). Several ways probiotics work in boosting immune responses, improving growth and survival rates for tilapia are presented, while the effects of others are not yet understood to the same degree as they are for other fish species (Van Hai *et al.*, 2015). For probiotic treatments, the 40% protein diet supplemented with yeast produced the best growth performance and feed efficiency, suggesting that yeast is an appropriate growth-stimulating additive in tilapia cultivation (Lara-Flores *et al.*, 2003).

4.6 Applications of Probiotics in Aquaculture

The need for sustainable aquaculture has promoted research into the use of probiotics on aquatic organisms (Dawood *et al.*, 2019). Primary attention focused on their use as stimulants for growth and to improve animal health; However, new areas have been found, such as their effect on reproduction or stress tolerance, although this requires further scientific development. Probiotics have been used in aquaculture to increase the growth of cultured species, in fact, it is not known whether these products increase appetite, or if they naturally improve digestibility (Yeh *et al.*, 2020). The effect of probiotics has been tested on phytoplankton (microalgae), which forms the basis of aquatic food chains, due to its nutrient-producing photosynthetic machinery that in most cases, higher organisms are unable to synthesize such is the case of polyunsaturated fatty acids and vitamins. The limitations and prospects of probiotics in sustainable and eco-friendly shrimp culture to augment the total shrimp aquaculture production

(Kumar *et al.*, 2016). Probiotics may also detoxify the potentially harmful compounds in feeds, by denaturing the potentially indigestible components in the diet by hydrolytic enzymes such as amylase and protease. They can also improve feed utilization, that is, probiotics can decrease the amount of feed necessary for animal growth, and thereby reduce production cost (Ige, 2013).

The use of probiotics as growth promoters of edible fishes has been reported. The diet of Nile tilapia (Oreochromis niloticus) was modified with a series of bacteria that significantly increased protein and oval food industries in fish, as well as weight from 0.150 g to 6.16 g at 9 weeks of culture. A 15-day study of Oreochromis niloticus fry with the aim of evaluating the outcome on the performance of the Lactobacillus strain as a probiotic separation (Sahraoui *et al.*, 2021). Inclusion of probiotics in the diets of fish species such as hybrid striped bass (Morone chrysops XM saxatilis), Oreochromis niloticus, catfish, and carp could improve the growth performance, body length, weight gain, and feed conversion ratio (FCR) of fish species.

Antibiotics were used for a long time in aquaculture to prevent diseases in the crop. However, this has caused various problems such as the presence of antibiotic residues in the tissues of the animals, the generation of bacterial resistance mechanisms, as well as an imbalance of the gastrointestinal microorganisms of the aquatic species, which affected their health (Sekizawa *et al.*, 2007) The European Union has regulated the use of antibiotics in organisms for human consumption (Ronsón *et al.*, 2002).

In several studies, water quality was measured during addition of probiotic strains, especially of the gram-positive genus Bacillus. Probably since this bacterial group is more efficient than gram-negative in transforming organic matter to CO_2. It is suggested that by maintaining high levels of probiotics in production ponds, fish farmers can minimize the accumulation of dissolved and particulate organic carbon during the growing season. At present, there are several commercial preparations of probiotics that contain one or more live microorganisms, which have been introduced to improve the cultivation of aquatic organisms. Probiotics can be used as a food additive added directly to the culture tank or mixed with food (Martínez Cruz *et al.*, 2012) Isolated several strains of Bacillus from Cyprinus carpio and carried out tests to improve water quality in ornamental fish culture and to inhibit the growth of Aeromonas hydrophila. Natural isolates obtained from mud sediment and Cyprinus carpio were purified and assessed in vitro for efficacy based on the inhibition of growth of pathogenic Aeromonas hydrophila and the decrease in concentrations of ammonium, nitrite, nitrate, and phosphate ions (Lalloo *et al.*, 2007). Commercial probiotic in catfish (Ictalurus punctatus), noting a survival and net fish production significantly higher when the probiotic was applied.

4.7 The source of probiotics

The selection of probiotic bacteria is extremely important because there is so little evidence from researchers that is being used in practice. It may create a negative host situation due to inappropriate microorganism selection. It is very important to apply the correct and quality probiotics to your culture farm. High quality probiotics can ensure proper growth and provide biological safety in agriculture. But if there is a problem with choosing and using probiotics, this can have adverse effects, and the dangerous species may grow and make them more resistant to the host. This depends on the choice of probiotics. While choosing probiotics, a farmer must know the molecular function. The production of probiotics should be increased through active research. This is a very interesting approach that FAO is now concerned with about the use of probiotics for policies to improve the quality of the aquatic environment. Because there was no international consensus to ensure efficiency and safety of probiotics, FAO and WHO recognized the need to create guidelines for a systematic approach for the evaluation of probiotics in food, to substantiate their health claims.

As a result, the "Guide for the Evaluation of Probiotics in Food" was presented, providing guidelines on the evaluation of health and nutrition properties of probiotics in food. The working group stated that no pathogenic or virulent properties were found in lactobacilli, or lactococci, although they acknowledged that under certain conditions, some strains of lactobacilli have been associated with rare cases of bacteremia. However, its occurrence does not increase with the increased use of lactobacilli in probiotics. It was also mentioned that enterococci may possess virulence characteristics; therefore, it is not recommended as a probiotic for human consumption (Morelli *et al.*, 2012).

Chapter Five

Materials and Methods

5.1 Experimental Locations

The fieldwork was conducted in the Jericho city in Palestinian territories, precisely in the Association of the Arab development society on the Jordanian-Palestinian borders and 4 km from the city center. Coordinates of Jericho in degrees and decimal minutes Latitude: 31°52.0002′ N, Longitude: 35°27′ E, Jericho is located at an altitude of 273m below sea level with a mean annual rainfall of 133mm. The average annual temperature is 24°C, and the average annual humidity is approximately 49.3% (Isaac *et al.*, 2010).

Jericho is located at the borders near the Jordan River in the West Bank. It has been held under Israeli occupation since 1967; It is one of the oldest cities in the world, also the first of which dates back 10,000 years (9000 BCE), almost to the very beginning of the Holocene epoch of the Earth's history (Ighbareyeh *et al.*, 2015).

The Arab Development Society is in the eastern part of Jericho. The ADS is considered one of the oldest NGOs in Palestine, and its land area is 8000 dunums along the Jordanian-Palestinian borders.

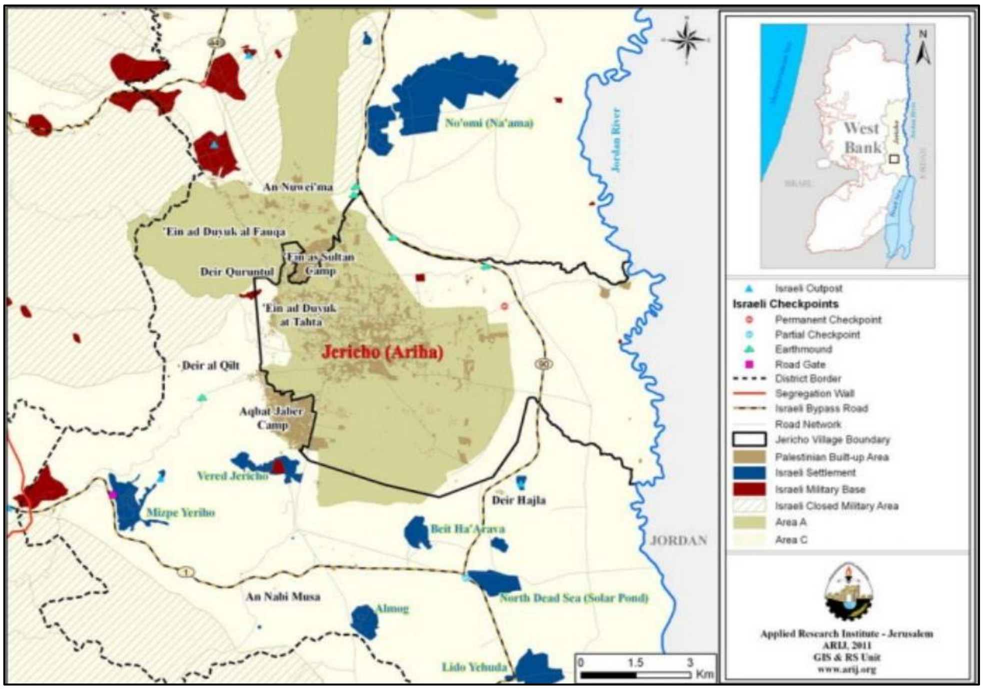

Figure 5.1: Location of ADS in Jericho city, West Bank, Palestine.

Figure 5.2: Map of West Bank which in the study area ADS and Jericho location.

For this research project, Experiments were carried out using the aquaculture and spawning facilities in the ADS. Currently these facilities are used for spawning and raising fish, In the ADS area, there are agricultural lands, multiple water wells, a cow's farm, a dairy factory, a section for general maintenance and marketing of products in general. The ADS is shown in figure 4.3:


Figure 5.3: The area of ADS and study area

5.2 Methodology

In this research project, a scientific experiment was used for four consecutive months in the study area (Jericho), through which data related to probiotics was collected by conducting periodic tests on pond water. The following parameters were monitored: ammonium, nitrates, nitrites, and some physical properties of water that are directly related to water quality as temperature, turbidity, and salinity. Fish growth was monitored, records and data for fish growth and behavior during the experimental period was recorded as a base information data that will help investors in understanding the status of this field.

The research methodology consists of three phases: preparing the experiment ponds, probiotics, and fish used in the experiment for recording and samples. The second stage is to start the experiment during the beginning of the temperature rise period to adjust the physiological characteristics of the fish and provide all the appropriate requirements in the period (March. 2020- July. 2020). The last stage involves summarizing and analyzing the expected results of the experiment and making it public for the local community. Scientific experiments always try to find a solution for aquaculture without daily water exchange and reduce the feed consumption. In this study different equipment and tools are needed to initiate and complete the study. The media of probiotics are made of a limited amount of (Molasses, rice, cola, flour, turmeric, yeast, water, and yogurt).

The research was started at the beginning of 20 March.2020 because during these months the temperature is suitable for the growth of fish and their environment. During March water temperature in the Jericho region increases to 20 ° C and more. The temperature starts to increase during the summer. This period is ideal to raise fish in Jericho region.

Water samples and fish samples were collected during different stages of this period. Water samples were taken before being introduced to the experiment ponds to ensure their suitability for fish farming. Water samples were tested for their physical and chemical parameters. These parameters include salinity, temperature, Nitrate, Nitrite, Ammonia, dissolved oxygen, and suspended matter by using simple kits.

Figure 5.4: Nitrate, Nitrite, Ammonia Kits.

Fish samples were taken before and during the experimental period to do some measurements such as weight and length using the way by choosing three specimens with twenty fishes per each randomly and calculate the average fish weight.

5.3 Preparation

In this research, three concrete pans with a capacity of six cups each were used to conduct the probiotic experiment during this project. These ponds are equipped with a water source, emptying valves and a siphon in the deepest part of the ponds; the dimensions of these ponds are one-meter depth, two meters wide, and three meters' long.

The ponds were prepared at the end of February, one week before the experiment started the fish were transported to these ponds and have been supplied with water and an oxygen source. Feed was added to encourage the growth of bacteria and algae.

Experimental fish were prepared, many fish samples were taken to calculate the fish average weight. In the first pond (control pond) 150 fish were stocked at a weight of 151.2 g, water was changed by 20% per day, feeding naturally and according to the fish's appetite and needs each period, opening the traps daily to extract the residue for about 20 seconds, conducting periodic and important checks (ammonium, nitrate, Nitrite, oxygen, and temperatures) and monthly weight - tracking samples. In the second pond, 150 fish were used, at a weight of 150.8 g, where samples were taken for comparison (weight and number), a daily change of water by 10%, the addition of probiotics (2 ml/day), normal feeding and according to appetite as in the control pond, opening the valves daily to extract the remaining, conducting daily water tests, taking monthly samples to compare weight. For the third and most important pond, 200 fish were placed at a weight of 150.7 g so that the density of fish exceeds that of other ponds. No water change was done for this pond. Probiotics was added daily (average of 2 ml). Fodder was added in the same amount that was added to the other two ponds and at a rate of at least 25% In general, taking into consideration the number of fishes in each pond. Water was added only to compensate for the lack of water from evaporation and disposal of residues, doing daily water checks, taking monthly samples to compare weight and recording readings.

5.4 Pre-experiments

Small scale experiments were carried out before the main experiment described above in order to prepare the most suitable organic Probiotic that is suitable to our environment and also to choose the ideal environmental factor as water salinity and temperature. The ideas came from my long experience in Aquaculture practice and national and international training courses and workshops. Corn flour was first used in our preliminary investigations as a source of carbon and nitrogen for the bacteria that could enhance the water quality.

The experiments extended to three large scale tanks. The same number of fishes were stocked in each tank. The fish were fed on 35% protein with a slight difference in the percentages of materials used in the fermented solution using (molasses, rice and yogurt) in the first tank of one cubic, In the second tank (cola, flour and molasses) while in the third tank using (rice, yogurt and cola). The experiments were run for two consecutive months to examine the possibility of these materials to reduce the % of ammonium and nitrite in fish water and the ability of tilapia fish to grow and survive. The results were very promising in the three tanks Ammonium concentration was less than one mg/l NH_4^+.

Table 5.1: Average concentration of ammonium and nitrite in mg/l.

Tank	Ammonium, mg/l	Nitrite, mg/l
Tank 1	0.5	1.5
Tank 2	0.5	2
Tank 3	0.5	1.5

Figure 5.5 Flour used as a source of carbon and nitrogen for the bacteria (ADS 2019).

The second experiment: After analyzing the results of the first experiment, the best materials that could give a good growth of bacteria were selected. Three tanks of one cubic meter were used for each tank. The same number of fishes was placed so that 2 ml, 3 ml, and 4 ml of the probiotic fermented solution were added respectively. The percentage of ammonium and the growth of fish in it without a daily change of water, and the experiment proved the effectiveness of probiotics in the three tanks, thus overcoming the danger stage of the accumulation of toxic nitrogenous substances in the water as a result of metabolism and feed residues, adding probiotics can be used daily in the beginning and then can be added every 3 days.

Figure 5.6 Probiotics media used in pre- experiment (ADS 2019).

5.5 Probiotics experiment preparation

In this study, work was done on preparing yeasts to maintain water quality in ponds and fishponds, reduce the amount of feed consumed and increase fish density in the ponds.

Probiotics were prepared using many simple materials and in specific quantities that were fermented for at least 48 hours, where they were used as a solution to be added to the feed, mixed well, and used for two days of preparation. The yeasts were added directly to the water, and the results were similar and effective. After its preparation, the yeasts were placed in a freezer and used daily as needed. They can also be stored in a refrigerator at 4 ° C and used for a week in divided quantities in appropriate packages.

Table 5.2: Tools and Reagents used in the experiment.

Tool	Component	Number
Probiotics media	Molasses	0.5 L
	rice	1.5 kg
	cola	1 L
	flour	1 kg
	turmeric	20 gm
	yeast	20 gm
	water	9 L
	yogurt	2.5 L
Concrete ponds	-	3
Hand net	-	3
Aerator	-	1
Air stone	-	12

Table 5.2.1: Continue: Tools and Reagents used in the experiment.

Tool	Component	Number
pH kit	-	1
Ammonium kit	-	1
Nitrate kit	-	1
Nitrite kit	-	1
Salinity test	-	1
Oxy meter	-	1
Water exchange	-	250 cubic/125 days
Bio floc test	-	1 beaker

Probiotics media are made of nutritional material which are rich in carbohydrates and carbon sources for growing the beneficial bacteria in general. It's recommended that a 10 g baker's yeast/kg diet should be supplemented to a practical diet to produce stronger and healthier Galilee tilapia. Baker's yeast, Saccharomyces cerevisiae, contains various immune stimulating compounds such as β-glucans, nucleic acids, and oligosaccharides, source of carbohydrates and it has the capability to enhance the growth of various fish species. The sources of carbohydrates used (molasses powder, liquid molasses and sugar) were efficient in controlling ammonia in the fish ponds. Molasses was the best carbon source for bio floc production in tilapia culture. The dietary turmeric powder (TP) in fish diet may enhance the diet digestion and nutrient digestibility, leading to improved nutrient utilization, which in turn would improve fish growth and feed utilization which enhances the water quality.

5.6 Statistical Analysis

For the statistical analysis, the results of the experiment were evaluated and the tests analyzed using Excel 2016.

The results and data were interpreted through mathematical equations to understand the probiotics experiment by comparing the results and readings for each of the experiment ponds. To connect and explain these numbers to the results of the experiment, many graphs and shapes were drawn to understand the effectiveness of probiotics and their important role in fish farming and aquatic life, link the results with their impact on water quality and fish growth and compare that with natural and international standards. This material was chosen for its availability in the region, the ease of preparation at simple prices, its values in the results and the main objective of this research.

Chapter Six

Results and discussion

Water samples were analyzed from the experiment ponds during four consecutive months to compare some of the water chemical properties, which play a major role in determining the processes of breeding and growth of fish, depending on the conditions suitable for fish to carry out biological and physiological processes.

6.1 Physical and chemical water parameters

Water quality for aquaculture is defined as the combination of chemical, physical and biological characteristics of water which have direct or indirect influence on fish growth and survival. Water quality factors should be primarily considered in evaluating the adequacy of water resources for aquaculture. Ammonium, nitrite, nitrate, and pH levels were monitored in the experimental ponds. The values did not exceed the permissible limit of the FAO and EUROFISH standards.

Table (6.1): Average concentration of nitrogen compounds (mg/l) in studied ponds from the experiment in comparison to the FAO and EUROFISH, 2015.

ponds	**NH_4^+**	**NO_3^-**	**NO_2^-**	**pH**
probiotics	0.11	1.22	0.33	7.36
control	0.21	1.27	0.3	7.24
mix	0.19	1.13	0.2	7.35
FAO and EUROFISH, Safe limit	0 - 2.5	100	0 - 0.5	6.5-8

Nitrogen in the form of free ammonia (NH_3) is toxic and needs to be transformed into the less harmless nitrate. The breakdown of organic matter and ammonia is a biological process carried out by bacteria in the probiotics. In probiotics and heterotrophic bacteria oxidize the organic matter by consuming oxygen and producing carbon dioxide, ammonia, and sludge. Nitrifying bacteria convert ammonia into nitrite and finally to nitrate. The low ammonium values indicate that nitrifying bacteria are working in great effeminacy with the probiotic media.

The optimum temperature and pH to achieve high nitrification rate is 15 to 33 °C (best around 27 °C) and pH levels between 6.5 and 8 for tilapia. The water temperature will most often depend on the species reared and is as such not adjusted to reach the most optimal nitrification rate, but to give optimal levels for fish growth. The pH should be kept above 6.5 to reach a high rate of bacterial nitrifying. On the other hand, increasing pH will result in an increasing amount of free ammonia (NH_3), which will increase the toxic effect. The aim is therefore to find the balance between these two opposites aims of adjusting the pH. A recommended adjustment point is between pH 7.0 and pH 7.5. Fish excretes a mixture of ammonia and ammonium (Total Ammonia Nitrogen TAN) = ammonium (NH_4^+) + ammonia (NH_3) where ammonia constitutes the main part of the excretion.

Toxic ammonia is absent at pH below 7 but rises fast as pH is increased as shown figure 6.1:

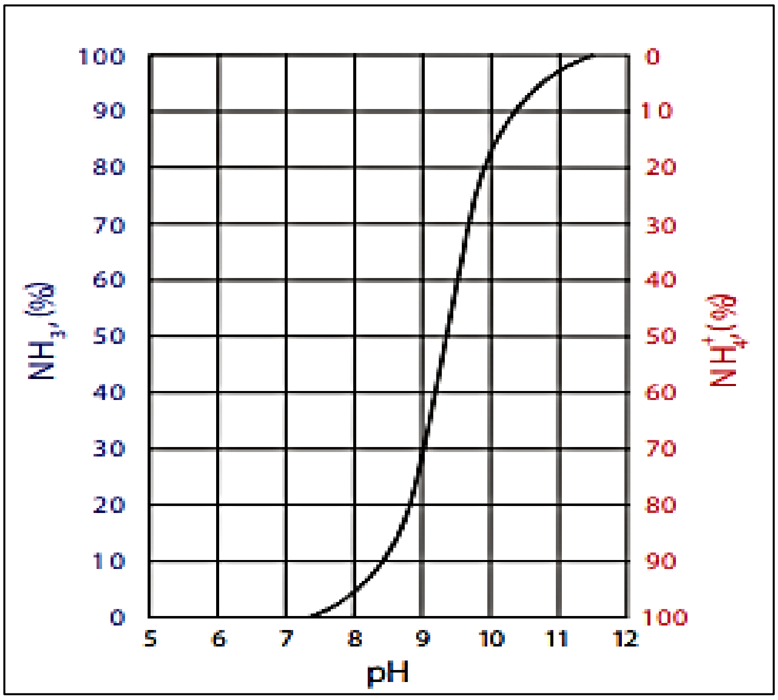

Figure 6.1: The equilibrium between ammonia (NH_3) and ammonium (NH_4^+) at 20 °C. Balasubramanian C. P.

6.1.1 Ammonium NH_4^+

Ammonia is the end product of the protein catabolism by living organisms in the water. The total ammonia nitrogen (TAN) can be in forms: NH_3 or non-ionized ammonia and NH_4^+ or ionized ammonia. Only the NH_3 form goes freely through fish gills and is considered the most toxic form for fish (Lemarie *et al.*, 2004). Myrick *et al.* (1983) reported that NH_3 is 300 - 400 times more toxic than NH_4^+ to fish. The pH and temperature of the water are the main factors that affect the proportion between NH_3 and NH_4^+ (Yúfera *et al.*, 1999). Excretion of ammonia to the water by fish is carried out mainly through their gills by simple diffusion as NH_3. Therefore, high concentrations of NH_3 in water can interfere with NH_3 excretion by fish and cause toxicity. Hence, the ideal water quality for an efficient NH_3 excretion is low TAN and low pH (< 8). This could be achieved by daily water exchange or any different water treatment technique like biofilters or probiotics.

The average concentration of NH_4^+ (mg/l) in the probiotics pond is shown in Tables (6.2) and Figure (6.2) compared with control and mix ponds.

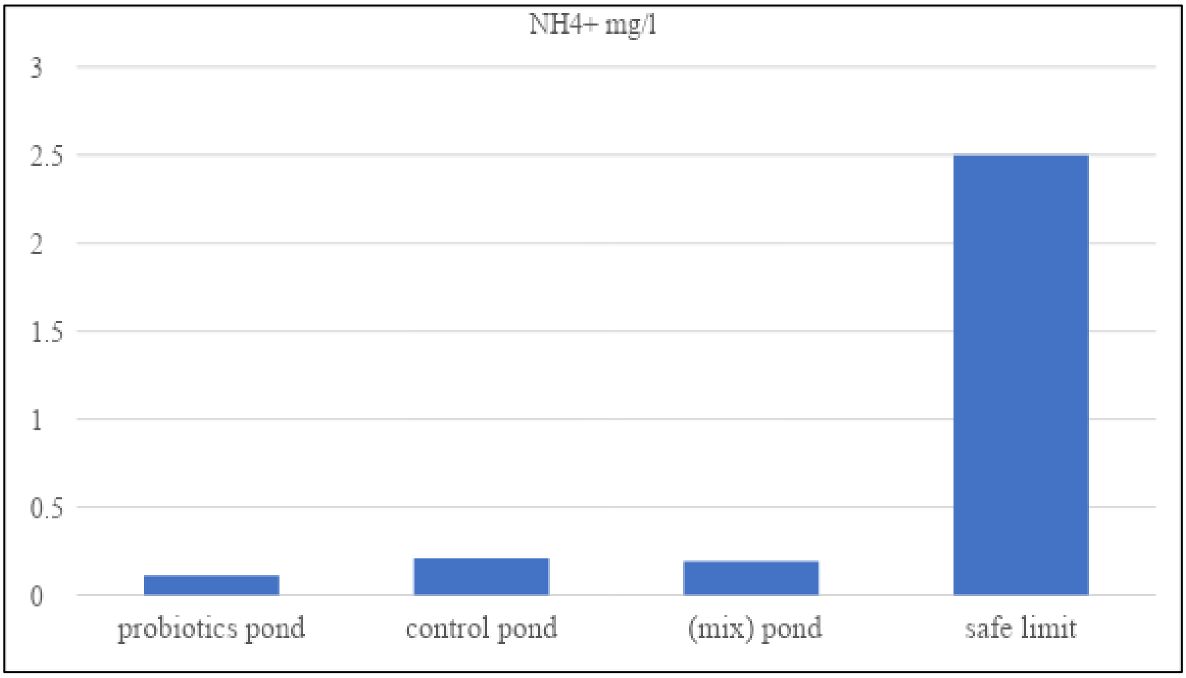

Figure 6.2: Ammonium concentration (mg/l) in the probiotics, mix, and control ponds.

As indicated from the results of the analysis, the concentration of NH_4^+ is below the safe limit which indicates a safe and suitable amount for the fish. The result indicates the efficiency of probiotics to achieve good water quality as indicated from the low concentration of NH_3 as compared to other ponds and keeps red to the other ponds. The water quality is good even with no water exchange. Ammonium concentration NH_4^+ < 2.5 mg/L is toxic for fish. Ammonia is toxic to fish at levels above 0.02 mg/L.

Table 6.2: Ammonium concentration (mg/l) in the probiotics, mix, and control ponds.

	probiotics pond	**control pond**	**mix pond**
mean	0.11	0.21	0.19
max	1.20	0.80	1.00
min	0.00	0.00	0.00
Standard deviation	0.23	0.18	0.19

6.1.2 Nitrite

Nitrite is formed intermediate step in the nitrification process and is toxic to fish at levels above 2.0 mg/L. Good nitrification process as with our experiment indicates the efficacy of probiotics technique in both the first and second stages of nitrification, ammonia oxidation to Nitrite and Nitrite oxidation to Nitrate). The average concentration of Nitrite (mg/l) in the probiotics pond is shown in table (6.3) and Figure (6.3) compared with control and mix ponds.

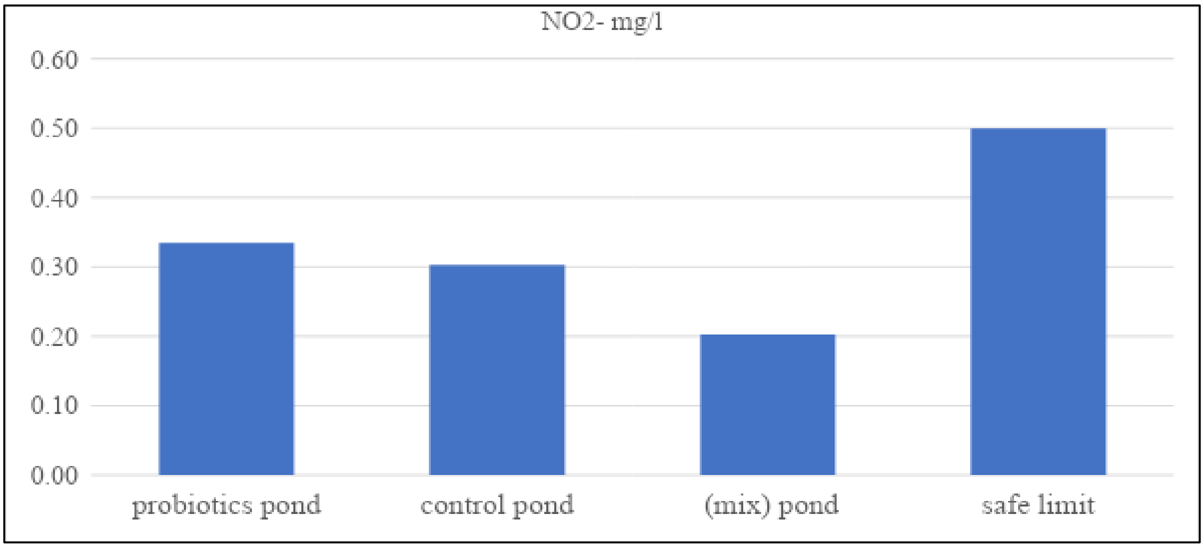

Figure 6.3: Concentration of NO_2^- (mg/l) in the probiotics, control, and mix ponds compared with the save limit (FAO).

The figure indicates that in all ponds during the experimental time the amount of $NO2^-$ in the water is below the safe limit and better. Low nitrite concentration means that the effect of probiotics on nitrification efficiency in the nitrogen cycle is high. Probiotics prevent the accumulation of nitrite as well as ammonia in the systems. The control pond has the lowest amount of NO_2^- due to the amount of water exchanged daily.

Table 6.3: Concentration of NO_2^- (mg/l) in the probiotics, control, and mix ponds.

	probiotics pond	**control pond**	**mix pond**
mean	0.33	0.30	0.20
max	1.00	1.00	2.00
min	0.00	0.00	0.00
Standard deviation	0.26	0.17	0.26

6.1.3 pH

The pH of water can significantly affect the physiology of fish. The degree of acidity and basicity of water can stress and disrupt the normal growth of fish in tanks or ponds. In general, the suitable range of water pH for aquaculture is 6.5 - 9, the optimal water pH for the culture of Nile tilapia, Oreochromis niloticus is (7 – 8). Probiotics technique is used to maintain water quality in the experiment ponds to keep a suitable environment in the water ponds for best fish growth.

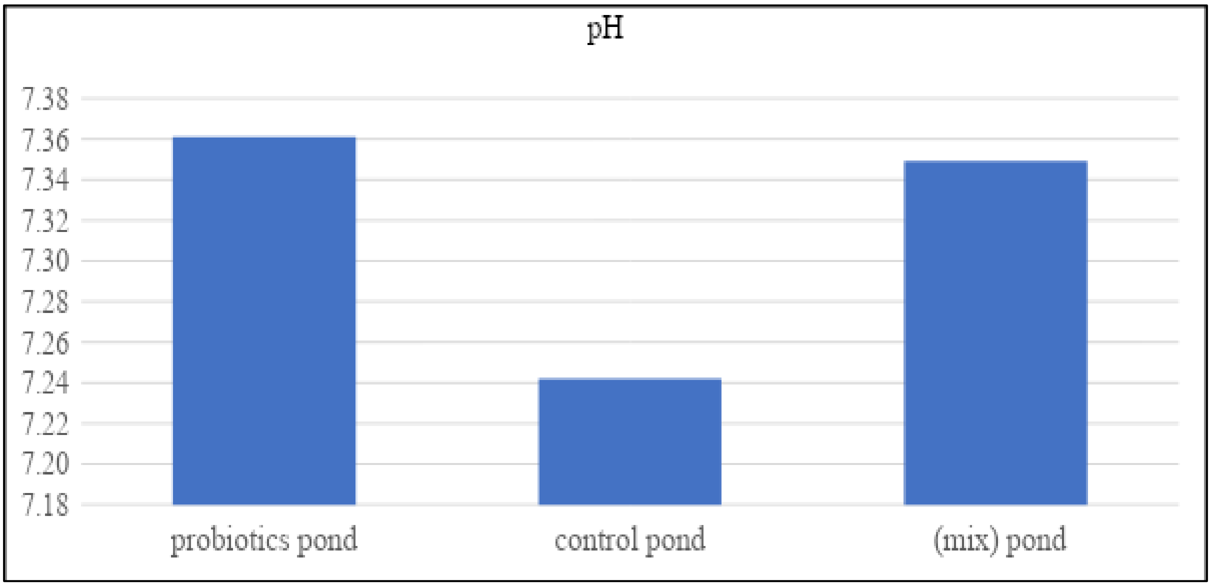

Figure 6.4: Average concentration of pH in the probiotics, control, and mix ponds.

The analysis indicates slightly the same pH in all ponds which indicates suitable pH compared with the safe range for Tilapia fish (6.5-9).

Table 6.4: Concentration of pH in the probiotics, control, and mix ponds.

pH	probiotics pond	control pond	mix pond
mean	7.36	7.24	7.35
max	7.50	7.50	7.50
min	7.00	7.00	7.00
SD	0.22	0.25	0.23

6.1.4 Nitrate (NO_3^-)

In the second step of nitrification, nitrite (NO_2^-), which is also highly toxic to fish, is converted to nitrate (NO_3^-) by Nitrobacter bacteria. This second step also requires oxygen and lowers pH. In general, NO_3^- is not harmful to fish but a low concentration of NO_3^- shows the success of the probiotics in the nitrogen cycle and fish farms without any need for water treatment or exchange. The average concentration of NO_3^- (mg/l) in the probiotics, control, and mix ponds is shown in figure 6.5 and table 6.5.

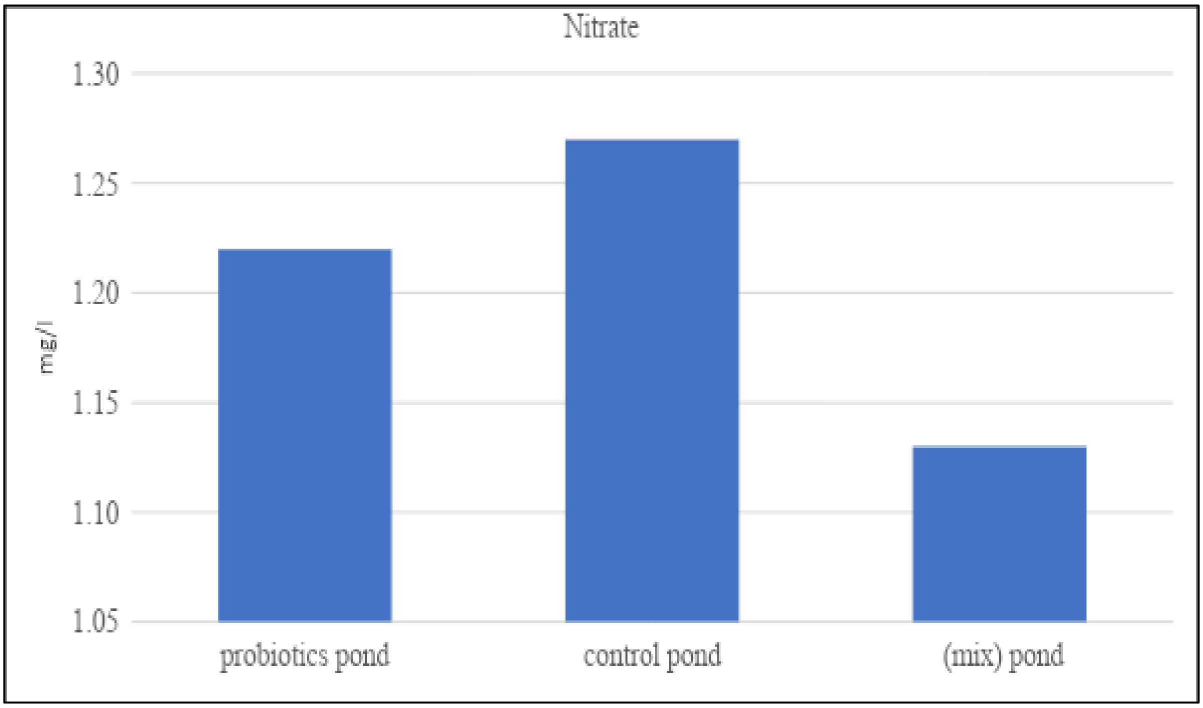

Figure 6.5: concentration of NO_3^- (mg/l) in the probiotics, control, and mix ponds.

The analysis indicates that the concentration of NO_3^- in all ponds are slightly similar and below the safe limit and better, this shows that the use of the probiotics is high efficiency in water quality management and fish growth. Low Nitrate concentration indicates that Nitrogen nitrification is completed to give N2 Gas and also nitrate could be utilized directly during primary productivity of the algae and bacteria. Probiotics enhance the growth of these microorganisms that consume nitrogen during photosynthesis and produce food for the fish culture. The nitrate amount was less in the probiotics pond than in the control pond due to the activity of the microorganism in the probiotics pond.

Table 6.5: Nitrate concentration (mg/l) in the probiotics, control, and mix ponds.

	probiotics pond	**control pond**	**mix pond**
mean	1.22	1.27	1.13
max	3.50	3.00	3.00
min	0.00	0.00	0.00
SD	0.82	0.67	0.61

6.1.5 Dissolved Oxygen

Oxygen is one of the most important characteristics that must be considered in fish farming. The quantities of oxygen are controlled on a continuous daily basis by using appropriate oxygen pumps, especially at night hours when the oxygen consumption by fish and algae is highest. The rate of dissolved oxygen is inversely related to temperatures, at temperatures from one to twenty-eight in which the oxygen levels are highest, and they decrease as the temperatures in the water and air rise.

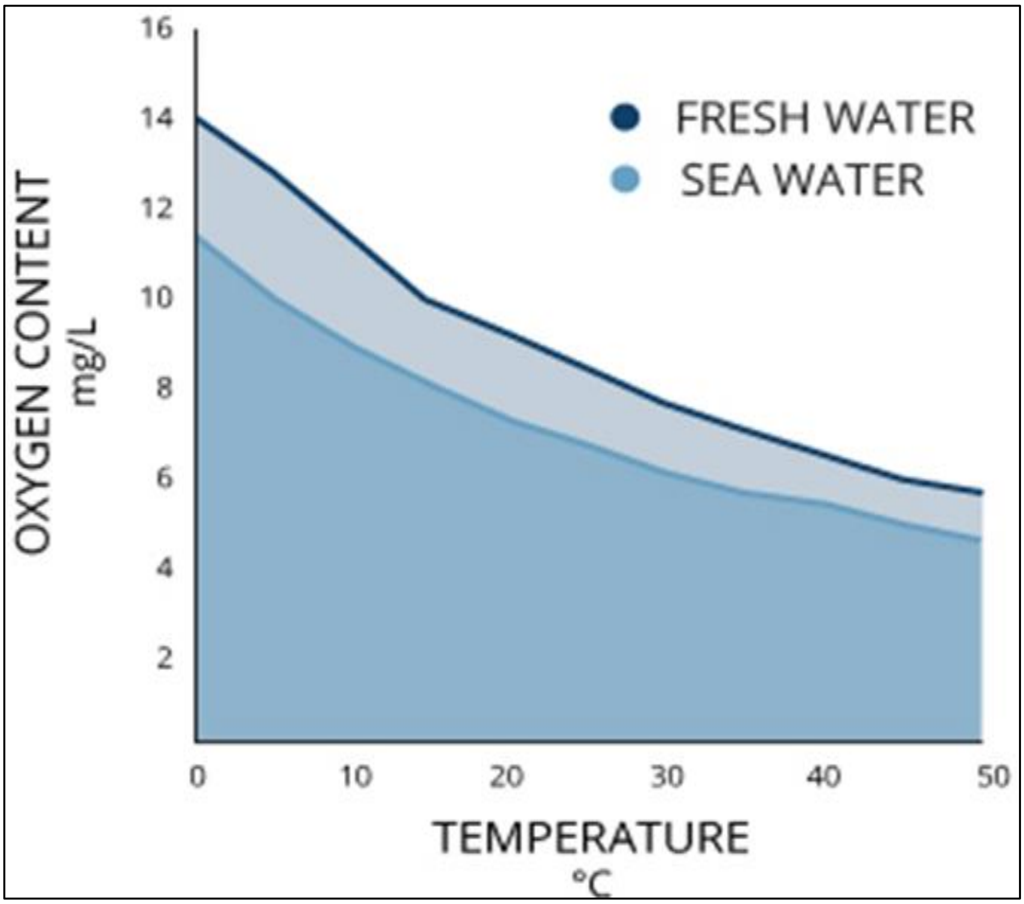

Figure 6.6: DO relation with T, (FONDRIEST environmental learning center 2021).

The amount of DO depends on the water T and salinity. Freshwater can hold more oxygen than saline water. The concentration is higher in cold water than in warm water.

Using a daily oxygen tester (OxyGuard), the amounts of oxygen in the experimental ponds were monitored to avoid the occurrence of oxygen deficiency, which causes stress to the fish, followed by negative effects on the growth processes. The save limit of DO (mg/l) 6 while below 6 mg/l is lethal for fish. The amount of oxygen (mg/l) in the probiotics, mix, and control ponds are shown in the table below:

Table 6.6: Concentration of oxygen (mg/l) in the probiotics, mix, and control ponds.

DO	probiotics pond	control pond	mix pond
mean	7.39	7.50	7.49
max	9.70	9.70	9.40
min	6.30	6.40	6.50
standard deviation	0.77	0.69	0.65

6.1.6 Temperature (°C)

Temperature is an important factor affecting the growth and survival of all organisms. However, water temperature is especially important to the growth and survival of fish, because they are poikilothermic (coldblooded). Poikilothermic animals cannot control body temperature, and they equilibrate with the temperature of the surrounding water. Tilapia, Bass, Mullet, Carp, and catfish can live in the range of (15-30) °C in general with slight differences in breeding, spawning, and growth. The lower and upper lethal temperatures for Nile tilapia are 11-12 °C and 42 °C, respectively, while the preferred temperature ranges from 25 to 35 °C. (Yamamoto, 2006)

In fish farms daily water exchange can control the T in the water ponds while in the probiotics experiment there is no water exchange just with a shadow of 50% over the ponds can maintain the T below 35°C. In ADS the water T from the wells is stable during the winter and the summer seasons 26-28 °C and the salinity is ranged from (3000 – 6000) ppm. in the experimental ponds, the salinity was 3450 ppm. The following table shows the temperature (°C) in the ponds.

Table 6.7: The temperature (°C) in the probiotics, mix, and control ponds.

T	probiotics pond	control pond	mix pond
mean	25.66	24.90	24.92
max	33.50	28.70	28.70
min	18.30	18.30	18.50
standard deviation	3.84	2.86	2.87

The analysis of water temperature indicates the higher T level in probiotics ponds 33.5 °C while the same T in the other ponds. In control and mix ponds where the daily water exchange keeps the water below 29 °C due to the stability of water T source during all the time of the year around the 27.5 °C. Considering the experiment area is a hot region in the summer. This indicates the suitability of using the probiotics technique in the other cities of the West Bank.

6.2 Fish results

Three ponds stocking with Nile tilapia (female and males) to investigate the growth rate, k factor, and the feed conversion ratio.150 fish stocking in the control pond,150 tilapia fish in mix pond and 200 tilapia fish in probiotics pond, and 10 Bass fish in each pond to see how much the fish can tolerate the condition in the probiotics water.

6.2.1 K factor (condition factor), Fulton formula

The relationship between length-weight is very essential for fish management schemes and it is possible to estimate the average weight of fishes and length (Zhai *et al.*, 2019). Furthermore, the length-weight relationships among the fish population indicate their wellness. The difference in height and weight is obtained through biotic and abiotic environmental factors as well as the nutritional status of a particular aquatic ecosystem.

Condition factor is a good parameter that shows the wellbeing of fishes in their natural habitat or aquaculture, and it is represented by the coefficient of body condition. It is an indicator of different biological and ecological factors about fish's feeding habits and growth rate. Better body condition for fish is have a high value of condition factor. Similarly, poor body condition is obtained when the values of the condition factor are less. Although, it is influenced by stress, sex, season, availability of food and the water quality in the environment in which they live, and the daily management in the fish farm.

In 1902, Fulton proposed the use of a mathematical formula that would quantify the condition of fish: K =1000* W/ L3, where: K is the Condition Factor or Coefficient of Condition is the "K factor" while W is the weight of the fish in grams (g) and L is the length of the fish. K value of 2.2 Excellent condition, 2 good,1.8 fair fish,1.5 poor fish, long and thin 1 Extremely poor fish for tilapia. Mahomoud et al. (2011) records of k factor for tilapia ranged between 1.6 to 2.0 and 1.6 to 2.1 (Nehemia *et al.*, 2012). The Fulton's condition factors (K) of fish as expressed by Bagenal and Tesch (1978) were used to estimate the well-being of the fishes, that is, compare the health or fattening of the fishes. They were estimated using the relationship: K=100 * W /L3.


Figure 6.7: K factor (the relationship between length-weight).

Table 6.8: k factor in the control pond.

	Weight in gm	Length in cm	k factor
mean	401.455	28.059	1.810
max	540.000	30.500	2.200
min	244.000	23.500	1.585
standard deviation	58.020	1.377	0.145

Table 6.9: k factor in the mixed pond.

	Weight in gm	Length in cm	k factor
mean	408.068	28.004	1.851
max	562.000	31.000	2.248
min	261.000	23.800	1.602
standard deviation	60.708	1.460	0.155

Table 6.10: k factor in the probiotics pond.

	Weight in gm	Length in cm	k factor
mean	357.104	26.601	1.885
max	520.000	29.800	2.217
min	200.000	22.100	1.381
standard deviation	57.695	1.370	0.143

The tables above indicate good growth for the fish during the life stage and show that the fish live in healthy conditions in all ponds of the experiment (mix, control and probiotics) compared with the standard k factor for tilapia fish.

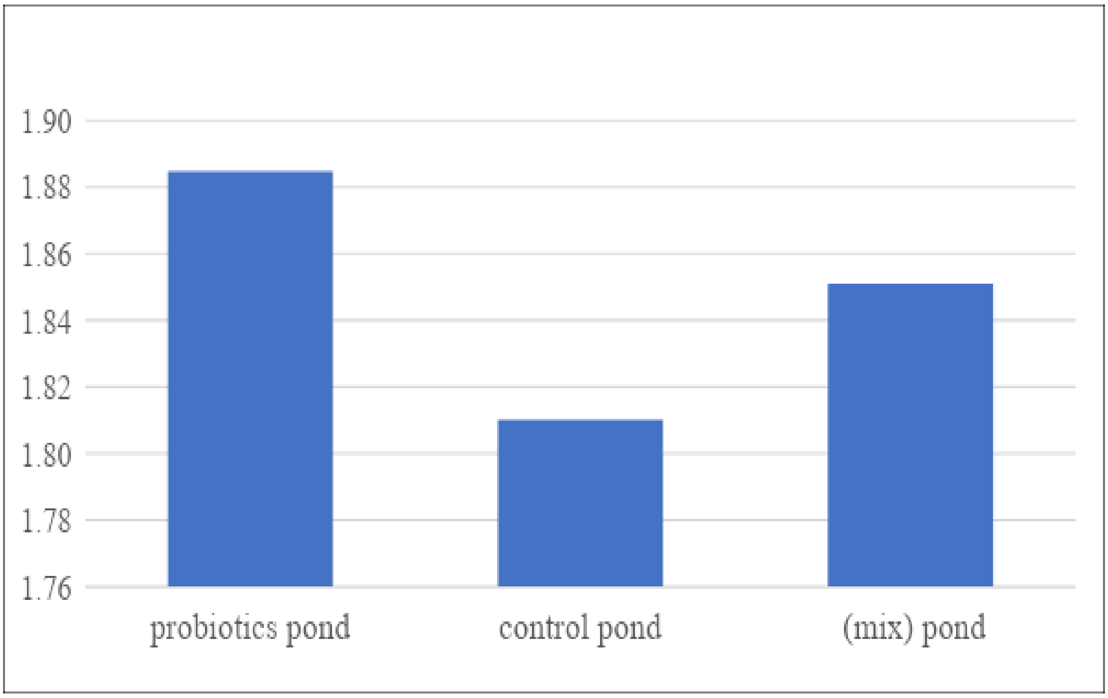

Figure 6. 8: average k factor of the probiotics, mix and control ponds of the experiment.

Condition factor has been used as an indicator of health in fishing biology studies since the beginning of the 20th century, such as growth and feeding intensity. (Ighwela *et al.*, 2011) Condition factors decrease with increase in. The condition factor provides information on the variation. of fish physiological status and may be used for comparing populations living in certain feeding, climate, and other conditions. There was a significant correlation between length and weight, while the condition factor computed for Tilapia (Oreochromis niloticus) were 1.885,1.81 and 1.85, which indicated good health condition during the experiment and it is indicating an isometric growth, which is desirable for fish of fish farms. The k factor in the probiotics pond indicates good growth for the fish without any stress during the experiment.to avoid the stress during the experiment the k factor can tell good information about the fish growth and health.

6.2.2 Feed ration

High quality formulated feeds are used to achieve high yields and large-sized fish (400-600 g) within a short period. In the intensive pond and tank culture systems or cages, tilapia farmers mainly depend on commercial pellet feeds. Table (6.11) Tilapia feed ration example, FAO – (2004).

Table 6.11: feed ration, FAO – (2004).

Ingredient composition (%)	Pre-starter	Starter	Grower	Finisher
Fish meal	15.0	12.0	10.0	5.0
Fish oil	4.0	3.0	3.0	2.0
Corn	0.0	0.0	3.1	14.9
Rice bran	0.0	24.6	35.0	35.0
Wheat bran	10.0	10.0	10.0	10.0
Cassava	6.7	10.0	10.0	10.0
Soybean meal	62.4	38.5	27.3	21.1
Limestone	0.6	0.7	0.7	0.8
Dicalcium phosphate	1.1	1.0	0.7	1.0
Vitamin premix	0.1	0.1	0.1	0.1
Mineral premix	0.1	0.1	0.1	0.1

The table 6.11 shows the ingredient composition in the fish feed used to produce pellet feed which contain different amounts according to the stage of life. In the experiment, pellet feeds are used with high quality CP 35%, Ash 7%, C fat 4%, Ca 1.2%, P 1.2%, salt 0.7%, moisture 10%, C Fiber 5%. The floating feed is used to make sure that all feed is consumed by fish. The amount of daily feed added to fish determined by the appetite of the fish is not more than 20 minutes. This is enough time for all fish to take what they need every time, after that the feed dissolves in the water and affects the quality of the water. The fish fed 3 times on average during the experiment. all ponds of the experiment are feeding the same amount with the difference in fish number, water exchange, and probiotics used.

6.2.3 Feed calculations:

ADG/gm= (end fish weight - start fish weight) divided by number of days.

TADG/gm = ADG * number of fishes.

T W /gm = end weight * number of fishes.

FCR = Total Feed Consumption divided by TADG.

Tables (6.14), (6.15), (6.16) and figures show the fish weights, Feed conversion ratio, and ponds productivity from fish in probiotics, mix and control ponds. Productivity of probiotics, mix and control ponds are in the tables below:

Table (6.12): Feed calculation in control, mix and probiotics ponds.

fish	Start weight /gm	end weight/gm	ADG/gm	TADG/gm	FCR	T W /gm	kg
Control	151.2	401.455	2.002	290.296	1.93	58211	58.211
mix	150.8	408.068	2.058	302.547	1.85	59986	59.986
probiotics	150.7	357.1	1.65	317.03	1.766	68564	68.564

The feed used for feeding the fish is pellet feed, each pond consumes 70 kg in 125 days, the fish number at the end of the experiment are 145 fish in the control pond, 147 fish in the mix pond and 192 fish in the probiotics pond.

Feed expenses are often the main budget item on fish farms and costing like 50-70% of any project costs. Therefore, reliable indicators are needed to determine the level of performance obtained from a feed or an additive. The feed conversion ratio (F.C.R.) is a relevant example of such indicators as it reflects how effective a feed strategy is. The feed conversion ratio is an indicator that is commonly used in all types of farming, as well as in the field of research. It can provide a good indication of how efficient a feed or a feeding strategy can be. In the context of aquaculture, the F.C.R. is calculated as follows:

F.C.R.=Feed given divided by fish weight gain.

In other words, the F.C.R. is the mathematical relationship between the input of the feed that has been fed and the weight gain of a population. For example, if it requires two kilograms of feed to grow one kilogram of fish, the FCR would be two. This means that when a feed has a low FCR, it takes less feed to produce one kilogram of fish than it would if the FCR were higher. A low FCR is a good indication of a high-quality feed. The analysis indicates good FCR in probiotics ponds compared with the mix and control ponds which are 1.8 considering the stage life of the fish. The average fish weight of probiotics pond indicates less weight than control and mix ponds which is 357.1 gm/fish but have more production than the other ponds due to the number of the fish used in probiotics ponds. The average fish start and end weight of tilapia and bass for probiotics, mix, and control ponds are shown in the figures.

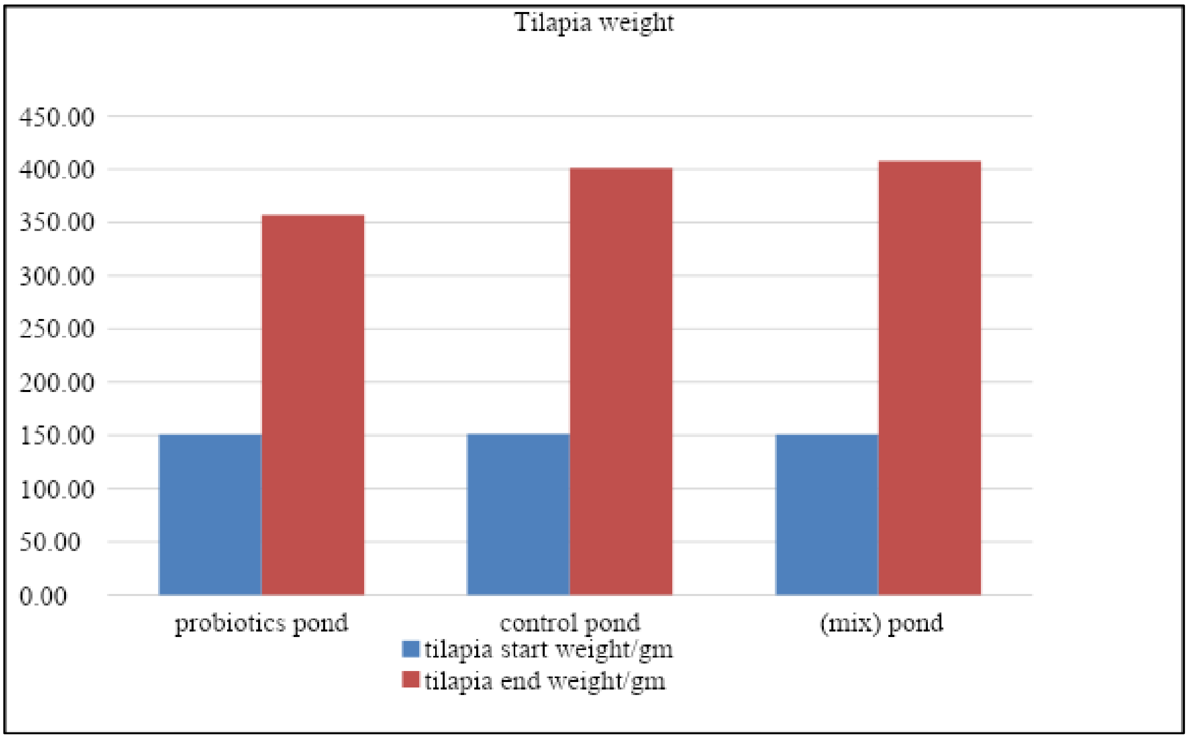

Figure 6.9: The average fish start and end weight for probiotics, mix and control ponds.

Table (6.13) fish weight and length in control pond, three samples, each sample consisted of 20 fish.

weight (g)	length (cm)	weight (g)	length (cm)	weight (g)	length (cm)
415	28.1	375	27	368	27.4
471	29.8	311	25.5	378	27.6
340	25.4	355	26.2	384	28
371	28.3	374	27	415	29
411	29.6	369	26.8	435	29
452	30.5	412	28	432	29.3
485	30	425	28.3	412	27.7
510	29.3	295	24.5	400	28.6
502	29	452	28.9	471	29.8
411	28.8	512	29.2	395	29
422	27.7	540	30.2	375	28.4
298	26.5	356	27.3	374	28.4
312	24.7	379	27.5	415	29
352	26.3	356	27.1	403	29.1
321	25.6	415	29.5	362	25.5
374	28.4	481	30.2	362	27.6
415	27.1	471	29.8	374	27.6
462	29	358	28.2	365	27.2
402	28.3	422	28	356	28.1
400	28.5	433	28.5	380	26.6

Table (6.14) average fish weight and length in the control pond for the three samples of fish.

Control pond	weight (g)	length (cm)
Sample 1	406.3	28.0
Sample 2	392.8	27.9
Sample 3	404.6	28.1
Average	401.2	28.0

Table (6.15) fish weight and length in mix pond, three samples, each sample consisted of 20 fish.

weight (g)	length (cm)	weight (g)	length (cm)	weight (g)	length (cm)
390	27	415	28.1	386	27.4
324	25.5	492	29.8	376	27.6
355	26.2	340	25.9	384	28
406	27	387	28.3	421	29
369	26.8	452	29.6	435	29
412	28	452	29	412	29.3
431	28.3	462	30	412	27.7
350	26.3	542	30.5	408	28.6
452	28.9	486	30.4	476	29.8
509	29.2	452	30	410	29
562	30.2	472	30.2	362	26.3
371	27.3	321	24.8	381	28.4
379	27.5	312	25.4	419	29
382	27.1	352	25.5	418	29.1
431	29.5	321	26	362	25.5
492	30.2	386	28.4	347	27.6
399	27	415	27.1	367	27.6
381	28.2	496	31	381	27.2
422	28	409	26.3	356	28.1
446	28.5	412	28.5	333	26.2

Table (6.16) average fish weight and length in mix pond for the three samples of fish.

Mix pond	weight (g)	length (cm)
Sample 1	413.2	27.7
Sample 2	418.8	28.2
Sample 3	392.3	28.0
Average	408.1	28.0

Table (6.17) fish weight and length in probiotic pond, three samples, each sample consisted of 20 fish.

weight (g)	length (cm)	weight (g)	length (cm)	weight (g)	length (cm)
333	27.4	342	27.1	251	24.8
300	25	321	24.8	421	28.3
352	26.2	258	24.3	416	28
241	24.2	298	25.3	400	27
351	26.8	312	26.7	401	27.6
367	26.8	345	27.4	367	25.5
411	27.9	451	29	394	27.4
255	25	322	25	452	28
422	28.6	345	27	355	26.7
487	29.5	385	27.3	364	26.1
467	29	234	24.4	420	27.6
321	25.8	289	25.5	394	27.5
322	26	387	27.4	421	28
336	27.1	352	26.7	321	27
400	27.7	336	26.5	398	26.5
412	27.1	355	28.6	354	26.3
323	28.6	320	25.3	361	27.3
310	26.8	325	26.9	347	27.2
396	27	365	27.1	412	27
360	26.8	345	27.4	325	26.5

Table (6.18) average fish weight and length in the probiotics pond for the three samples of fish.

Probiotics pond	weight (g)	length (cm)
Sample 1	358.3	27.0
Sample 2	334.4	26.5
Sample 3	378.7	27.0
Average	357.1	26.8

Table (6.13) Table (6.14) Table (6.15) Table (6.16) Table (6.17) Table (6.18) above explain the fish samples were taken at the end of the experiment and the average of fish weight calculation that indicate good growth even the ponds contain different number of fish while consume the same amount of feed. The experiment started at the beginning of march and in Jericho the average daily temperature is around 20 °C. The time of the experiment is four months. The ponds stock with tilapia fish 150 gm on average. The fish weight determines by choosing three specimens contain 20 fish per each weighted and the average will be taken, the analysis indicates less weight in probiotics pond comparing with mix and control ponds due to the intensity of the fish which is 200 fish per 6 cubic comparing with the other ponds which are 150 fish per 6 cubic and the use of probiotics and water exchange.

The figure shows the weight gain during the time of the experiment that the fish in all ponds was grown very well; that in probiotics pond was grown 208 gm, in mix pond 256 gm and in the control pond 250 gm, this indicate suitable adaptation with the probiotic's technique.

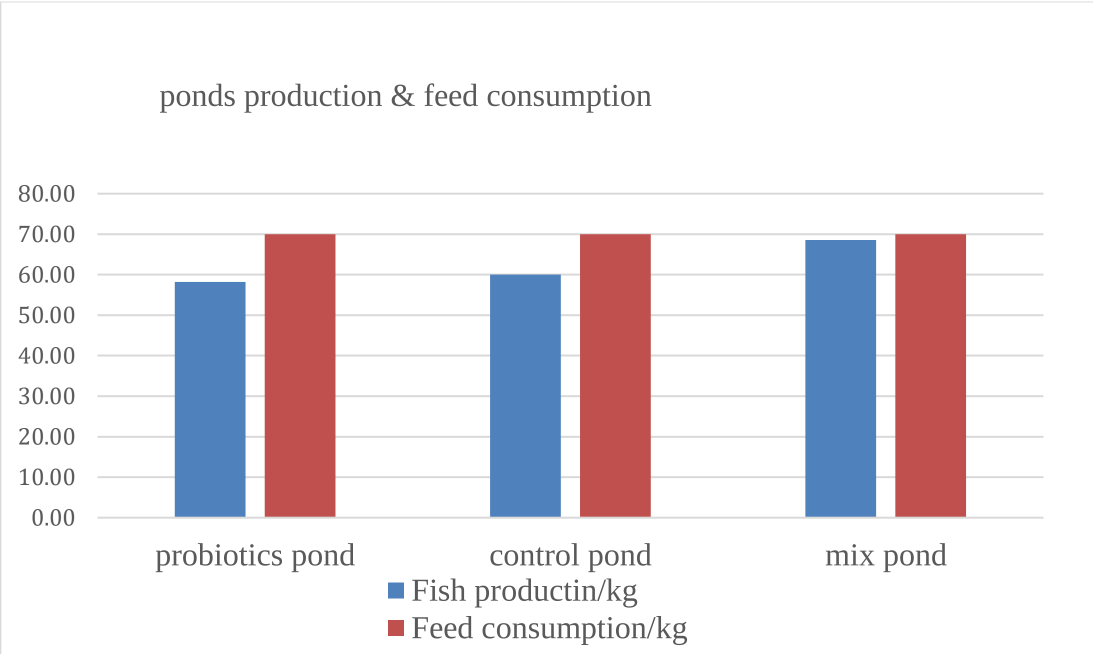

Figure 6.10: Ponds productions & feed consumption in probiotics, mix and control ponds.

Fish stocking in three ponds of the experiment is feeding the same amount of feed. The analysis indicates good production in all ponds (58.2 kg in control pond,59.9 kg in mix pond, and 68.57 kg in probiotics pond). In a probiotics pond, the fish feed on pellet feeds, probiotics, bacteria, and another microorganism in the water. The pond is consuming the same amount of feed, this indicates the effectiveness of probiotics for the fish as feed material.

The table shows that after consuming less feed using probiotics techniques, the cost of production decreases by an average of 0.17 US dollars to produce one kilogram of fish. In addition, reducing water use to control water quality is reduced by an average of 90 % of the basic needs for breeding and this also reduces the cost of production by an average of 0.29 US dollars shekel to produce 1 kg of tilapia, comparing with the control pond and the mix pond the production of 1 kg tilapia fish is 2 US dollars while in the probiotics pond is 1.55 US dollars.

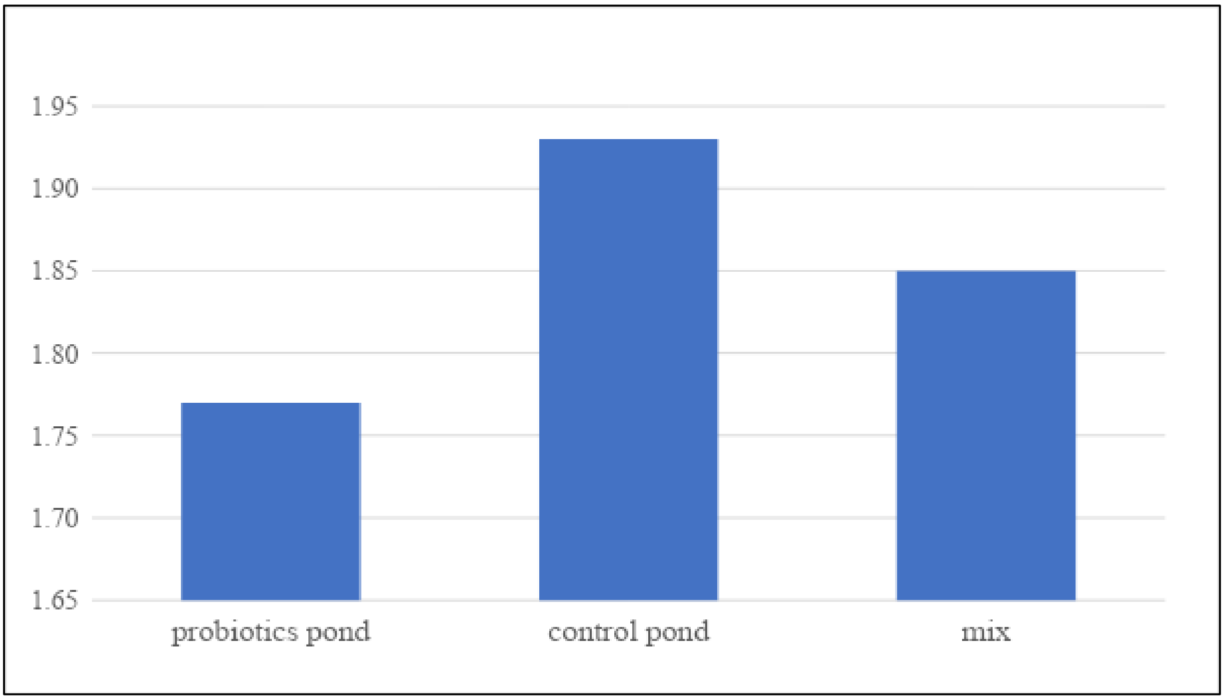

Figure 6.11: FCR in probiotics compared with the mix and control ponds.

The analysis indicates good FCR in probiotics mix and control ponds comparing the feed conversion ratio with the standard FCR for tilapia fish in different life stage and different sex. Probiotics indicate the best FCR (1.77) that means it’s needed 1.77 kg feed to produce 1 kg of tilapia fish, 1.85 kg feed to produce 1 kg of fish in mix pond and 1.93 kg feed to produce 1 kg of fish in control pond.

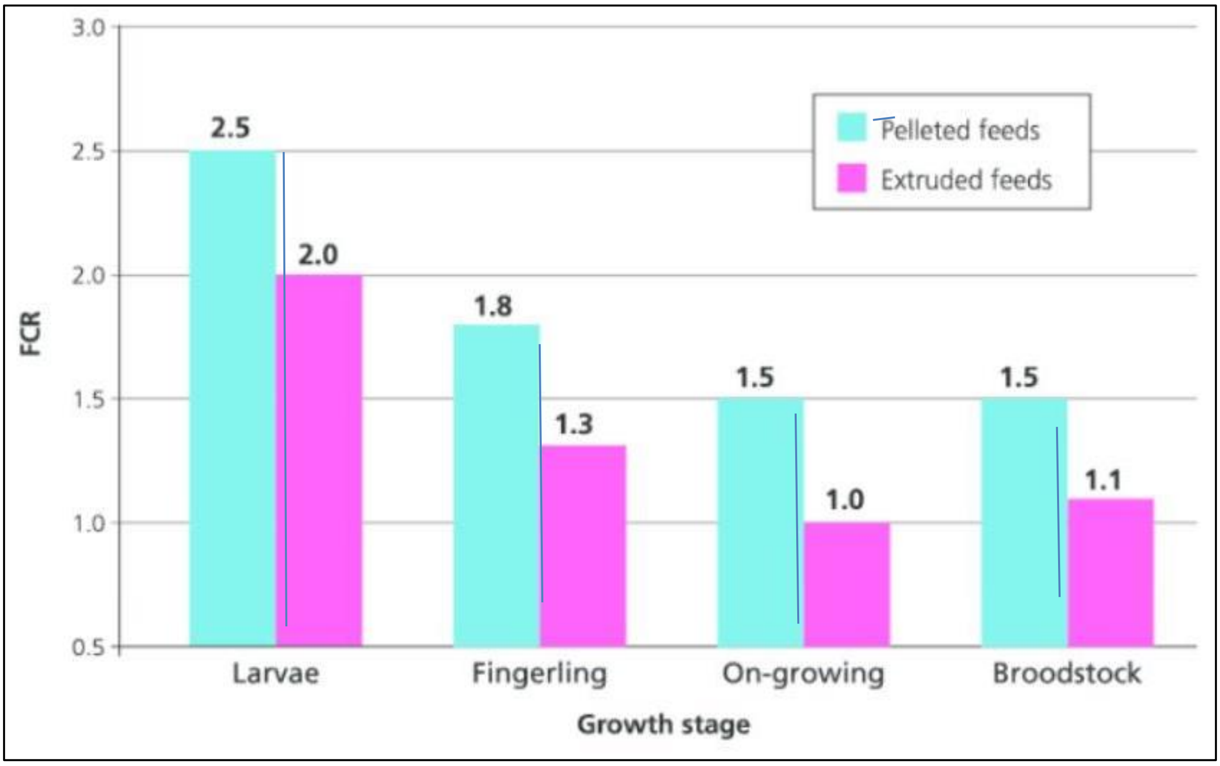

Figure 6.12: FCR field survey, according to EL-Sayed (2004,2006,2007).

The figure (6.13) explains the FCR for male tilapia fish during all life stages that the feed conversion ratio for the small fish is more than the mature fish. The larva fish consume more feed to produce 1 kg of fish and get better in growing and broostock stage.

Chapter Seven

Conclusion and Recommendations

7.1 Conclusion

In intensive and semi-intensive aquaculture practices, high stocking densities of fish along with intense feeding and fertilization often leads to the deterioration of water quality. These are the major constraints in fish culture. In the present study, water quality parameters of the ponds treated with probiotics were observed to be good which might be because of the various roles played by the microbes and bacteria.

In conclusion, the specific functions of probiotics in aquaculture may not be denied. Probiotics confer benefits of increased disease resistance, improved nutrient digestibility, and growth in the host animals, and they also improve culture water quality in general.

Probiotics as an alternative to chemicals and antibiotics have proven to be effective in promoting successful aquaculture, as they have the potential to improve water quality, increase tolerance to stress, and generate high-quality fish.

The probiotics used in the study showed great value in maintaining water quality during the trial period. The probiotics maintained a good level of the chemical and physical properties necessary for raising the fish (ammonium, nitrite, nitrate, pH, temperature, and dissolved oxygen).

The results of the probiotics experiment showed good growth of beneficial bacteria, and the aim of it is to complete the nitrogen cycle in fish water completely with all its components (ammonia, ammonium, nitrite, and nitrates), as any defect in the elements of the nitrogen cycle causes an increase in one of the toxic nitrogenous compounds and thus continuous stress to the fish, it results in poor growth and poor performance of the biological and physiological processes of fish.

The experiment achieved the expected objectives by providing large quantities of water used in raising fish to the maximum extent, as the water change was reduced to 90% of the quantities necessary for raising fish in the natural state. The fish density increased by 25% and this achieved an increase in the productivity of the pond within the same amount. Water in the yeast test pond compared to other ponds. Reducing the amount of feed by 25% and the fish weights were good and close to normal, as the fish feed on plankton, algae, bacteria, and microorganisms in the water.

The study showed a mortality rate of less than 1% in addition to good body score, healthy fish, and normal activity by following the relationship between weight, height, and k factor. The efficiency of food conversion is acceptable for the age stage of fish (1.8-1.9) compared with previous measures and studies (1.5-2) for males only, considering the variety used in the study, the genetic development, and the use of males and females together.

Changing the water once a month to compensate for the loss of evaporation and daily excretion of waste for 10 seconds from the main outlet of the pond is sufficient to maintain the water quality and not to cause any malfunctions in the system for the experiment.

probiotics can be added by mixing them with the feed and presenting it to the fish, which is fine. It can also be added as a solution directly in the water. Add the probiotics at the beginning of the experiment daily to build an adequate bacterial system, and then add it according to the daily checks of the water where the probiotics have proven their efficiency adequately to keep the chemical properties of the water within the safe limit.

7.2 Recommendations

Based on the findings of this research project, which were collected over six-month data gathering and analysis, the following recommendations are proposed within suggested interventions that could enhance fish farming and start using new techniques in aquaculture which are more economically and can uses in any city of Palestine:

1- Probiotics technique can be used to maintain good water quality in the fish farming that can help to grow tilapia fish in different areas in the West Bank of Palestine.
2- The main region needs to pay direct attention to the fisheries sector and to double investment in this area because of its economic importance and facing the challenges in the region.
3- Jericho and the Jordan Valley are mainly suitable for fish breeding due to the abundance of water therein, and their use in the fields of vegetarian agriculture and crop irrigation.
4- Conducting other studies in this field to keep pace with the scientific development in fish farming in neighboring countries to develop the culture of farmers and consumers to contribute to the advancement of an educated society aware of the conditions surrounding the homeland.

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