# CHAPTER ONE

# INTRODUCTION

Tilapias are one of the most important freshwater finfish cultured in the world and they represent approximately 6% of total farmed fish production (**FAO, 2004**). Feed represents a major cost for intensive tilapia production and it is one of the most important factors that influence the ability of fish to attain its genetic potential for growth and maintain proper health. Research on nutrition and feeding of tilapia has been expanded steadily over the past three decades including the use of potential of new functional ingredients, feed additives and probiotics to improve the growth, feed utilization and fish health.

Probiotics are live microorganisms, which have beneficial effects on the host by modifying the host-associated or ambient microbial community of the gastrointestinal tract thus promoting better feed utilization, enhancing the host response towards disease and improving the quality of its ambient environment (**Verschuere *et al,* 2000**).

Although, the importance of probiotics in human and animal nutrition is widely recognized ,in recent years, the role of probiotics in nutrition and health of certain aquaculture species have also been investigated and subject of reviews (**Merrifield *et al.,* 2010**). It appears that probiotics provide benefits by establishing favorable microbial communities such as lactic acid bacteria and *Bacillus* sp. in the gastrointestinal track which may alter gut morphology and produce certain enzymes and inhibitory compounds causing improved digestion and absorption of nutrients as well as enhanced immune response (**Verschuere *et al.,* 2000**). Several studies have demonstrated that the use of probiotics improves health of larval and juvenile fish, disease resistance, growth performance and body composition, however, the mode

of action in fish species may vary between farmed fish species cultured in freshwater and marine environments.

The use of probiotics in feeds to improve growth of different fish species including African catfish, *Clarias gariepinus*; Senegalese sole, *Solea senegalensis*, tilapia, *O. niloticus* (**El-Haroun *et al.*, 2006**), has been investigated. The effects of probiotics have been linked to modulation of gut microbiota and establishment of the beneficial microorganisms, higher specific and total digestive enzyme activities in the brush-border membrane which increases the nutrient digestibility and feed utilization (**Kesarcodi-Watson *et al.*, 2008**). In addition, the production of vitamins by these gut microbiota could also increase vitamin synthesis and improve fish health. Endogenous digestive enzymes in fish have been studied by several workers (**Chan *et al.*, 2008**).

However, information regarding the enzyme producing intestinal bacteria, their source and their effect on fish digestion and metabolism is scarce. So, the present study was designed to evaluate the effect of dietary probiotics groups ***(****B. subtilis, B. licheniformis, Enterococcus faecium, and lactobacillus acidophilus*) on water quality, growth performance, feed utilization and flesh quality of Nile tilapia (*O.niloticus*).

## CHAPTER TWO

# LITERATURE SURVEY

## 2.1. Aquaculture

The nutritional benefits of fish and fish oil consumption on human health, including the prevention of cancer, diabetes and heart diseases, have been well established .As public awareness about the health benefits of fish consumption continues to increase, the global demand for aquatic foods is also expected to continue to rise .Furthermore, the world's population is expected to grow by more than 30 % by 2050, resulting in an estimated 2.3 billion more mouths to feed, with the major growth expected in the developing countries where fish is the primary source of protein. Aquaculture is recognized as the only way to meet these increasing demands for aquatic foods ,half of the sea foods consumed worldwide is from commercial fishing (i.e. fish caught in the wild open waters) (**FAO, 2009**).

The other half is farm-raised fish grown under controlled conditions known as aquaculture,the amount of fish produced globally from aquaculture rose from 6 % in the 1970s to about 50 % of the total fish consumed in the world in 2006. Furthermore, aquaculture is also an important income-generating sector of many economies with considerable prospects for job creation, poverty alleviation, community development, and food security. It provides fish for domestic markets and for international markets. The domestic market improves national food security, and production for international markets creates employment, provides income, and brings in foreign exchange; thereby indirectly contributing to national food security.

The exponential growth of the aquaculture sector during the past two decades is a result of the progressive intensification of production systems. A major contributor to this intensive production system is the use of manufactured feeds formulated to meet the nutritional requirements of the targeted fish species (**FAO, 2009**). Feeds account for up to 70 % of the variable cost of a commercial aquaculture operation (**Webster *et al.*, 1999**) for many fish species. Feed production costs are driven by the cost of fishmeal, an important protein source in fish feeds. The price of fishmeal has increased more than two fold in recent years. It rose from about US$600 per metric ton in 2005 to about US$2000 per metric ton in the first quarter of 2010 .Furthermore; the provisions of fishmeal are not adequate to sustain the current rate of growth of aquaculture in addition to the demand from other animal feed industries.

Flesh quality has gained importance among consumers and in the aquaculture industry because it is directly related to human health and nutrition. Flesh quality comprises several different characteristics. Due to the large number of traits involved and the ensuing complexity, genetic improvement for flesh quality has been almost neglected in breeding programs for aquaculture species.

Quality traits can usually be recorded only on dead fish, and therefore family selection must be practiced in a breeding program (**Gjedrem, 1997**).

In order to meet the increase in human fish demand, aquaculture is increasing along the necessity of supplying fish products of high quality and also diversified product (**Queméner et al., 2002**). Generally, an important success factor is that consumers accept farmed fish to be equivalent or superior to the wild fish (**Olsson et al., 2003**). Quality terms and how they are perceived differ for the fish farmer, processing industry and consumer.

While growth and feed conversion are of great importance to the aquaculturist, these parameters are unlikely to be of indirect interest to the latter. However, producing fish that are positively received by processors and consumers alike is naturally of major concern to the fish farming industry (**Rasmussen, 2001**). The quality of farmed fish has occasionally been reported as being lower than that of wild fish (**Sylvia et al., 1995**).Although, contradictory result have also been obtained (**Jahncke et al., 1988**).

**Hernandez et al. (2001)** reported that wild fish acceptability is greater than that of farmed fish. The term fish quality is all encompassing and its study is difficult owing to the fact that specific parameters that are recognized as being vital in one part of the world are judged to be less important elsewhere. Salmonid aquaculture has focused for many years on enhancing the quantity of fish produced. However, optimization of the quality of salmonids may lead to improvement of consumer acceptance and higher price for the farmed product (**Rasmussen, 2001**). In these connections, **Sahu et al. (2000)** reported that among the commercial characteristics of fish, flesh quality is becoming more important to the aquaculture industry. The consumer dictates the flesh quality and it is a very complex characteristic. An attempt has to be made to define and analyze flesh quality and its relation to carcass characteristics. Carcass quality traits must be defined precisely and should be able to be measured with a high repeatability. Some of the quality traits vary within the carcass. Therefore, a very precise carcass evaluation is necessary to arrive at any useful conclusion. The evaluation of flesh quality of different populations can result in a genotype suitable for aquaculture.

**2.2. Nile Tilapia**

Tilapias are very important in world fisheries, and are the second most important group of food fishes in the world. Nile tilapia, *Oreochromis niloticus* accounted for a harvest of nearly 2.54 million tones in 2009 (**FAO, 2011**), second only to carp as a warm water food fish and exceeding the harvest of Atlantic salmon, *Salmo salar*, although, the value of the Atlantic salmon catch is more than twice that of the tilapia catch (**Maclean et al., 2002**). Although, native to Africa, tilapias are cultured in Asia and the Far East, and occupy two rather separate market niches, being a poor man's food fish in countries such as Israel and the Southern United States (**Maclean et al., 2002**).

Nile tilapia (*Oreochromis niloticus*) is a tropical climate fish of considerable rusticity for cultivation, with a delicate flavor, and a good quality of nutritional aspects with low fat content and free of Yshaped bones (Medri et al., 2009). For these characteristics this is one of the most cultivated species in the world.

## 2.3. Probiotics

Probiotics are a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring an improved use of the feed or enhancing its nutritional value, by increase the host response towards disease, or by improving the quality of its environment (**Verschuere et al. 2000**). Nowadays, probiotics are also becoming an internal part of aquaculture practices to obtain high production. Although considerably low information is available on probiotics application for fish, they offer benefits with regard to improving immune status and fish production (**Cerezuela et al. 2011**). Probiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of specific health-promoting bacteria, which can

improve the host's health (**Gibson et al. 2003**).

Based on the studies of **Mahious and Ollevier (2005)** foodstuff that reaches the colon (e. g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids) is a candidate probiotic (**Yousefian and Amiri, 2009**). However, most of the studies have focused on non-digestiblem carbohydrates, mainly oligosaccharides. Synbiotics are nutritional supplements that combine probiotics and probiotics, enhancing their beneficial effects (**Cerezuela et al. 2011**).

The use of probiotics and probiotics has been regarded during recent years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming as an integral part of aquaculture practices for improving growth and disease resistance (**Rombout et al. 2010**). This strategy offers innumerable advantages to overcome the limitation and side effects of antibiotics and other drugs and also leads to high production (**Sahu et al. 2008**).

In recent years there has been a growing interest in understanding the mechanism of action of probiotics and probiotics, especially in humans and other mammals. Probiotics activity is mediated by a variety of effects that are dependent on the probiotic itself, the dosage employed, treatment duration and route and frequency of delivery.

Some probiotics exert their beneficial effects by elaborating antibacterial molecules such as bacteriocins that directly inhibit other bacteria or viruses and, activity participating in the fight against infections; whereas, others inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules or modulate the inflammatory/immune response (**Cerezuela et al. 2011**).

On the other hand, the potential mechanism of probiotics includes a selective increase/decrease in specific intestinal bacteria that modulate local cytokine and antibody production, an increase in the intestinal short chain fatty acids production, an enhanced binding of these fatty acids to G-coupled protein receptors on leycocytes, an interaction with carbohydrates receptors on intestinal epithelial and immune cells, and partial absorption resulting in a local and systemic contact with the immune system (**Seifert, and Watzl, 2007**).

The alternative methods of disease prevention have been used as a means of reducing the presence of opportunistic pathogens and simultaneously stimulating the host immune responses. However, other effects related have been observed, as improve growth performance, feed utilization, digestive enzyme activity, antioxidant enzyme activity, gene expression, disease resistance, larval survival and gut morphology alter the gut microbiota, mediate stress response, improve nutrition, reduce risk of certain cancers (colon, bladder), produce lactase, alleviate symptoms of lactose intolerance and malabsorption (**Rombout et al. 2010**).

Synbiotic is defined as a combination of probiotic and probiotic. It is presumed to impart the beneficial effects of both ingredients. Few data are available regarding the application of synbiotics in aquaculture (**Zhang et al. 2010**). Synbiotics can help to improve health status, disease resistance, growth performance, feed utilization, carcass composition, gastric morphology, and digestive enzyme activities. As such; many commercial dietary formulations now routinely include probiotics or probiotics.

# CHAPTER THREE

# MATERIAL AND METHODS

## 3.1. Study area

The present study was carried out in fish hatchery at Department of fisheries Science and Wildlife, College of Animal Production Science and Technology, Sudan University of Science and Technology.

## 3.2. Experimental design and conditions

Fish was distributed in four experimental treatments in concrete ponds at a density of 10 fish in groups. Aeration was provided by an air pump for each pond. Water was changed partially every 3 days and entirely every week. Fish was fed at a level of 3% of body weight three times a day (9, 13 and 17 o'clock) for thirty days.

## 3.3. Experimental diets

Four experimental diets were prepared and is nitrogenous (control 16.30 %, D1 19.30%, d2 15.25 and d3 13.32% CP) diet were formulated (Table 1). The control diet had no probiotic supplement. Diets 1-3 were formulated to be (D101%, D2 2.5% and D3 4% *B. subtilis, B. licheniformis*, *Enterococcus faecium*, *lactobacillus acidophilus*) respectively. The dry ingredients were mixed with corn oil and the microbial isolates were incorporated into the feed diet components as shown in Table 1. After a desirable dough quality was obtained, diets were passed through a mincer with a die (2 mm diameter) and the resulting spaghetti-like strings were dried until the moisture levels were at approximately 10%.

**Table (1):** Formulation and composition of the experimental diets (dry matter basis).

| Ingredient (%) | Experimental diets | | | |
|---|---|---|---|---|
| | **Control** | **D1** | **D2** | **D3** |
| Fishmeal | 40 | 40 | 40 | 40 |
| Sorghum meal | 10 | 10 | 10 | 10 |
| Bread flour | 10 | 09 | 09 | 08 |
| Vegetable oil | 06 | 06 | 06 | 06 |
| Vitamin mix | 03 | 03 | 03 | 03 |
| Mineral mix | 1.5 | 1.5 | 1.5 | 1.5 |
| Wheat bran | 10 | 10 | 8.5 | 09 |
| Cornmeal flour | 05 | 05 | 05 | 04 |
| Groundnut cake | 14.5 | 14.5 | 14.5 | 14.5 |
| Probiotic | - | 01 | 2.5 | 04 |
| Total | 100 | 100 | 100 | 100 |

| Chemical composition | Experimental diets | | | |
|---|---|---|---|---|
| | **control** | **D1** | **D2** | **D3** |
| Dry matter (%) | 94.61 | 95.51 | 95.60 | 95.66 |
| Crude protein (%) | 16.30 | 19.30 | 15.25 | 13.32 |
| Crude fat (%) | 5.22 | 4.09 | 7.41 | 7.46 |
| Ether extract (%) | 4.40 | 4.21 | 4.53 | 4.47 |
| Ash (%) | 22.48 | 23.66 | 23.58 | 28.30 |
| Nitrogen free extract (%) | 46.23 | 44.45 | 44.83 | 42.13 |

### 3.4. Water quality

Temperature, pH, dissolved oxygen (DO) and ammonia were estimated by aqua sol kits during the experimental period according to APHA (1995). Physico- water as follows:

### 3.4.1. PH

1. Fill a clean test tube with 5 ml of water to be tested (to the line on the tube).
2. Add 5 drops of High Range pH Test solution, holding dropper bottle upside down in a completely vertical position to assure uniformity of drops.
3. Cap the test tube and invert tube several times to mix solution.
4. Read the test results by comparing the color of the solution to the appropriate High Range pH Color Card (choose either freshwater or Saltwater). The tube should be viewed in a well- lit area against the white area of the card. The closest match indicaters the pH of water sample. Raise the test tube with clean water after use.

### 3.4.2. Dissolved Oxygen

Range: 0.65 – 7.8 ppm

**I.D.O. Fixing:** the dissolved Oxygen requires to be fixed before testing.

1. Rinse the **D.O**. test bottle 2 – 3 times with sample water. Fill it till it overflows with the sample water and then stopper the bottle and ensure that no air bubbles are trapped inside.
2. Now add 10 drops of **D.O.1** followed by 10 drops of **D.O.2**. Mix well. Wait for a minute. A brown precipitate will be formed and start setting. Firmly stopper the bottle and shake the contents thoroughly. Keep the bottle in a safe place for a minimum 20 minutes.
3. Now add 10-12 drops of **D.O.3**. Replace the stopper and shake the bottle till the precipitate dissolves. Add more drops if required to dissolve the precipitate.

   Now this sample is used for testing.

   Proceed for D.O. determination as described in **II**

**II.D.O. determination:**

1. Take 10 ml. of sample (from step 3 of **D.O**. fixing) in the test jar.
2. Add 4 drops of D.O.4. Mix well.
3. Now drop wise* add **D.O.5**, counting the number of drops while mixing, until the blue color disappears.

**Calculation:**

Dissolved Oxygen ppm = 0.65 × [No. of drops of **D.O.5**]

**3.4.3. Total ammonia ($NH_3/NH_4$)**

1. Fill a clean tube with 5 ml of water to be tested (to the line tube).
2. Add 8 drops from Ammonia Test Solution Bottle #1, holding the dropper bottle upside down in a completely vertical position to assure uniform drops.
3. Add 8 drops from Ammonia Test Solution Bottle #2, holding the bottle upside down in a completely vertical position to assure uniform drops.
4. Cap the test tube and shake vigorously for 5 seconds.
5. Wait 5 minutes for the color to develop.
6. Read the test results by comparing the color of the solution to the appropriate Ammonia Color Card (use the fresh water color card). The tube should be viewed in a well – lit area against the white area of card. The closest match indicates the ppm (mg/l) of ammonia in the water sample. Rinse the test tube with clean water after use.

**3.5. Analytical methods**

The proximate composition for experimental diets and fish carcass were measured according to AOAC (1990). As follows:

### 3.5.1 Moisture Content Determination:

The samples were first weight (Initial weight) then dried in an electric oven at 1050C for 24-30 hours to obtain a constant weight. The moisture content was calculated as follows:-

$$\textbf{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Dry weight} \times 100}{\text{Initial weight}}$$

### 3.5.2 Crude Protein Determination:

The Kjeldal method for estimation of nitrogen was applied. Nitrogen content was converted to protein percentage by multiplying by 6.25 as follows:

$$\textbf{Protein \%} = \frac{(Va - Vb) \times N \times 14 \times 6.25}{1000 \times Wt} \times 100$$

Whereas:

Va = volume of HCL used in titration

Vb = volume of sodium hydroxide of known normality used in back titration

14 = conversion factor of ammonium sulfate to nitrogen

6.25= conversion factor of nitrogen to protein

Wt= weight of sample

N= normality of NaoH

### 3.5.3 Crude Fat Determination:

Fat content of each sample was determined according to Soxhlet method by ether extract using 2 gm of fish samples. Extraction continued for 5 hours at 100 0C before finding the weight of the extract fat. Fat percentage was then calculated as follows:

**Fat %** = $\frac{\text{Extracted fat weight x 100}}{\text{Sample weight}}$

### 3.5.4 Ash Content Determination:

Ash was determined by heating 1 gm at 5500C in muffle furnace until a constant weight was obtained. Ash content percentage was given by the following formula: **Ash %** = $\frac{\text{Ash weight x 100}}{\text{Sample weight}}$

### 3.6. Growth and feed utilization

Initial body weight (IBW), final body weight (FBW), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), survival rate, protein efficiency ratio (PER), protein productive value (PPV) and energy retention (ER) were measured using the following equations:

SGR = [In final body weight - In initial
Body weight/time (days)] X 100

FI= fish weight x feeding level /100,

FCR= Feed consumed / Weight gain

WG = FBW (g) - IBW (g)

PER = Weight gain (g) / protein fed (g)

PPV= [Protein gain (g) / protein fed (g)] x 100

ER (Kcal/kg) = [Energy gain (g) /Energy fed (kcal)] x 100

### 3.7. Statistical Analysis

Results were expressed as means ± standard deviation (SD). Data were statistically analyzed using ANOVA one-way analysis of variance. Comparisons among means were made by (LSD) when significant F- values were observed ($P < 0.05$), using SPSS version (21).

# Chapter four

# Results

## Water quality measurements

Weekly temperature, dissolved oxygen (DO), hydrogen ion (pH) and Ammonia ($NH_3$) measurements were taken during the study period. Recorded weekly averages were 32.10– 32.57C° and 4.6– 4.9mg/l for temperature and dissolved oxygen respectively (Figure 4). Lowest DO concentration was recorded in tank one during this study.

## Proximate analysis of feeds

Proximate analysis conducted on all the feeds showed some degree of variation in the protein content from the expected percentages. The expected crude protein percentages for the feeds were 16.30%, 19.30%, 15.25and 13.32% (Table 1).

## Growth and conversion variables

Growth and conversion variables; initial average weight, final average weight, average weight gain, average specific growth rate (SGR), survivor rate (SR %) and feed conversion ratio (FCR) were calculated for studied fish at the end of the study.

**Table (2):** illustrate proximate chemical composition of *Oreochromis niloticus* fed with different protein level feeding.

| **Parameters** **Treatments** | **Moisture** | **C.P** | **Fat** | **Ash** |
|---|---|---|---|---|
| A | 74.81±0.01[c] | 20.77±0.04[a] | 3.32±0.02[a] | 1.59±0.68[a] |
| B | 77.61±0.07[a] | 18.90±0.02 [b] | 2.26±0.02[b] | 1.24±0.03[a] |
| C | 76.52±0.03[b] | 18.64±0.03[b] | 3.12±0.03[a] | 1.72±0.03[a] |
| D | 77.28±0.04[a] | 18.33±0.04[b] | 2.88±0.03[b] | 1.52±0.03[a] |

[a,b,c,] Means in the same column with superscript are significant different at ($p \leq 0.05$).

**Table (3):** growth performance of different tank fed with difference proteins level.

| **Parameters** **No of Tank** | **Initial weight (g/fish)** | **Final weight (g/fish** | **Weight gain (g/fish)** | **SGR** | **Survivor Rate %** |
|---|---|---|---|---|---|
| 1 | 116.7±26.0[c] | 175.0±22.4[a] | 58.28±17.40[a] | 0.30±0.11[a] | 100 [a] |
| 2 | 115.9±18.6[c] | 171.0±20.1[b] | 55.07±7.80[b] | 029±0.06[a] | 100 [a] |
| 3 | 129.8±318[a] | 152.5±29.7[c] | 38.17±10.60[c] | 0.23±0.10[a] | 90[b] |
| 4 | 119.2±27.3[b] | 168.5±23.8[b] | 38.89±13.47[c] | 0.20±0.09[a] | 100[a] |

[a,b,c]Means values in the same column with superscripts are significantly different at level ($P<0.05$)

**Table (4):** physio - chemical parameters of water quality of different ponds.

| **Parameters** | | | | |
|---|---|---|---|---|
| **ponds** | **PH** | **$NH_3$** | **D.O** | **Temperature** |
| 1 | 7.40±0.10[a] | 0.25±0.00[a] | 4.6±3.30[a] | 32.17±1.44[a] |
| 2 | 7.40±o.25[a] | 0.25±0.00[a] | 4.7±3.02[a] | 32.23±1.45[a] |
| 3 | 7.20±0.30[a] | 0.08±0.14[a] | 4.7±2.73[a] | 32.57±1.45[a] |
| 4 | 7.20±0.30[a] | 0.25±0.00[a] | 4.9±2.26[a] | 32.10±0.60[a] |

[a]: Means values in the same column having the same letters are not significantly different (P>0.05)

**Table(5):**ANOVA table for weight gain

| | **Sum of squares** | **df** | **Mean square** | **F** | **Sig** |
|---|---|---|---|---|---|
| **Between groups** | 3381.673 | 3 | 1127.224 | 6.861 | 0.001 |
| **Within group** | 5914.667 | 36 | 164.296 | | |
| **Total** | 9296.340 | 39 | | | |

**Table(6):**ANOVA table for SGR

| | **Sum of squares** | **df** | **Mean square** | **F** | **Sig** |
|---|---|---|---|---|---|
| **Between groups** | 0.071 | 3 | 0.024 | 3.166 | 0.036 |
| **Within group** | 0.270 | 36 | 0.008 | | |
| **Total** | 0.342 | 39 | | | |

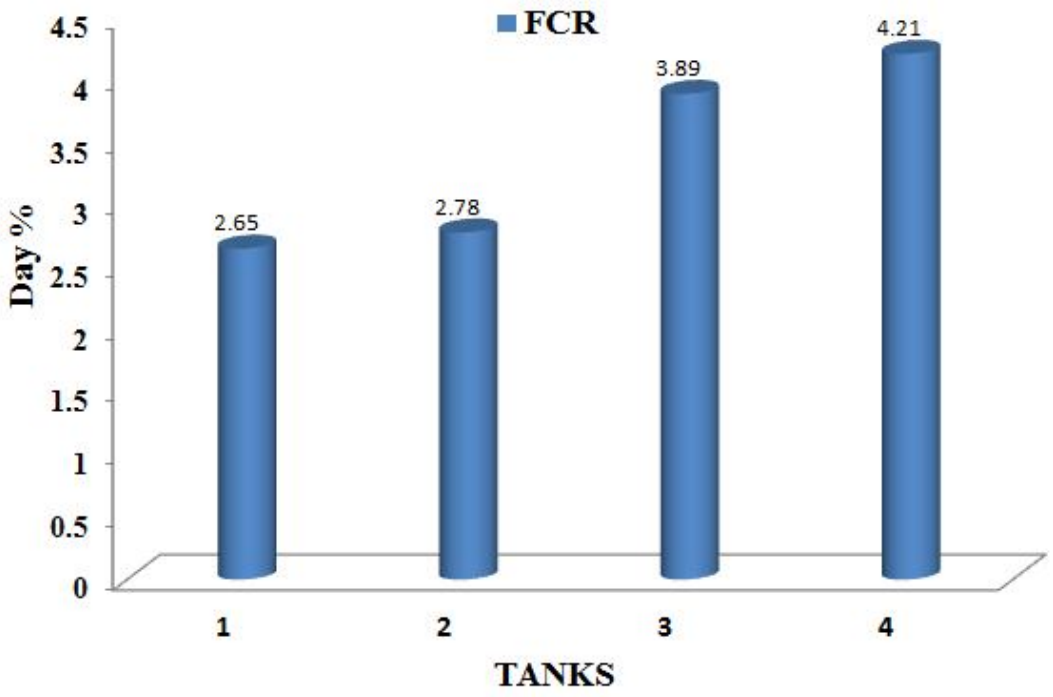


**Figure (1):** Food conversion ratio (FCR) of different tank fed with difference proteins level.

# CHAPTER FIVE

# DISCUSSION

Dissolved oxygen is a very important water quality parameter in aquaculture. Low levels of DO inhibit feed intake, which could subsequently lead to poor growth in fish. This may be due to the reduced oxygen availability to support the energy demands of the fish (**Jobling 1983**). The recorded DO value during the study period was between 4.6 – 4.9 mg/l. This is blow the estimated value of 7.0 mg/l. Therefore, DO levels in the experimental buckets is unlikely to have had adverse effect on the growth of fish in this study.

Temperature is also very vital for optimum growth to be achieved in aquaculture facilities because it affects feeding, growth and maturation of fish. The mean temperatures recorded over the study period were between 32.10 – 32.57˚C. The growth of juvenile in tropical area is highest at temperatures between 28-30˚C (**Jobling 1993**). The temperature in the culture water was within the optimum range for fingerlings tropic char and this is likely to have an effect on the growth of the fish.

**Feed intake and feed utilization**

Results of Figure 1 indicated that, increasing ***(****B. subtilis, B. licheniformis, Enterococcus faecium, and lactobacillus acidophilus*) level in *O. niloticus* diets were followed by a significant increase in feed intake (F4) and a significant improvement of FCR. In practical terms, this means that the use of probiotics can decrease the amount of feed necessary for animal growth which could result in a reduction in the production cost. Several studies on

probiotics have been published in recent years which suggested that, probiotics provide nutritional benefits in diets for tilapia fingerling (**Ferguson et al. 2010)**.

Increasing probiotics levels (from 01, 2.5 and 4%) significantly (P<0.05) increased F4, and significantly improved FCR, PER and PPV (Table 3 and figure 1) which was subsequently followed by an increase in the growth performance. Generally, the high demand for nucleotides occurs during periods of rapid growth (**Carver, 1994**). **Li and Gatlin (2005)** indicated that sub-adult hybrid striped sea bass (*Morone chrysops×M. saxatilis*) fed commercial diet supplemented with probiotic AE, with 10-20 g kg-1 obtained a significantly improved feed efficiency.

Referring to the dietary symbiotic interaction of the experimental diets with, 01%, 2.5% and 4% the mix probiotic (D4) showed the highest FI, the best FCR, SGR and SR compared to other symbiotic treatments and the control group. These results were parallel to that obtained for other growth parameters (BW, BL, WG and SGR) obtained in the present study.

Gastrointestinal bacteria take part in the decomposition of nutrients, provide the microorganisms with physiologically active materials, such as enzymes, amino acids, and vitamins (**Wang, 2007**), and thus facilitate feed utilization and digestion. This may account for the enhanced FCR, PER and PPV by dietary *B. licheniformis* supplementation in the present study and previous studies (**Bagheri et al. 2008**). **Mehrabi et al. (2011)** came to similar results. They found that the addition of symbiotic to the feed of rainbow trout, *Oncorhynchus mykiss* fingerlings produced a better significant (P<0.05) FCR values than the control. Ye et al. (2011) reported that, Japanese flounder fed diet supplemented with (FOS, MOS and *Bacillus clausii*) improved FCR than other diets. **Also, Ai et al. (2011)** showed that juvenile

large yellow croaker, *Larimichthys crocea* fed the diet supplemented with FOS and *Bacillus. Subtilis* 0.96×106 CFU g-1 significantly improved FCR and PER values when compared to fish group fed the control diet.

**Proximate analysis**

Proximate analysis of *O. niloticus* which was affected by ***(****B. subtilis, B. licheniformis, Enterococcus faecium, and lactobacillus acidophilus*) in Table 2. With respect to the effect of ***(****B. subtilis, B. licheniformis, Enterococcus faecium, and lactobacillus acidophilus*) supplemented to the experimental diets, it is shown that all probiotic levels significantly ($P<0.05$) increased dry matter and decreased lipid and protein content when compared to the control group, while ash content was not significantly affected. Soltan and El-laithy, 2008 indicated that, *O. niloticus* fed diet supplemented with *B. subtilis* recorded a high level of dry matter and lipid content than control group with no effect on the ash content. Bagheri et al. (2008) reported that application of 3.8×109 CFU g-1 of *Bacillus spp*. in diet of rainbow trout fry made a significant increase in fish body protein content when compared to the control group.

# Chapter six

# Conclusion and recommendation

## 6.1 Conclusions

The results of the present study clearly indicated that the supplementation of ***(****B. subtilis, B. licheniformis*, *Enterococcus faecium, and lactobacillus acidophilus*) not only enhanced the growth performance and feed utilization of Nile tilapia, Moreover, the supplementation of probiotics ***(****B. subtilis, B. licheniformis, Enterococcus faecium, and lactobacillus acidophilus*) had significant beneficial effects and there were significant interactions between increasing dietary of probiotics level ***(****B. subtilis, B. licheniformis, Enterococcus faecium, and lactobacillus acidophilus*) and proximate composition of the studied fish.

## 6.2 Recommendation

According to the results obtained from this study, we recommended that:

- It is better to add the probiotics to the fish feeding in aquaculture.
- More studies were needed to determine:

  1/the effect of probiotics supplementation on hematological and biochemical blood parameters.

  2/ the effect of probiotics supplementation on fatty acids profiles

  3/ the effect of probiotics supplementation on amino acids

  4/the effect of probiotics supplementation on fish immunology responses.

## References


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