



## Original Research Article

# Effect of *Spirulina platensis* and *Lactobacillus rhamnosus* on growth and biochemical performance of Nile Tilapia (*Oreochromis niloticus*) fingerlings

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## ABSTRACT

### Keywords

Probiotic,  
Spirulina,  
*Oreochromis niloticus*,  
Growth parameters,  
Blood biochemical parameters

The present study was carried out to investigate the effects of probiotics and *Spirulina* on survival, feed conversion ratio (FCR), specific growth rate (SGR), and Blood parameters such as red blood cells (RBCS), white blood cells (WBCS) and hemoglobin (Hb) and Biochemical blood parameters of Nile Tilapia fingerlings (*Oreochromis niloticus*) for period of 45 days. Probiotic organism (the bacteria *Lactobacillus rhamnosus*) and a single cell protein (*Spirulina platensis*) were incorporated into diets at concentrations of 2g/kg, 4g/kg, and 6g/kg. The control diet contained no supplement. *Spirulina platensis* at 6g/kg produced the best and statistically significant weight gain (20.16 g), FCR (3.73) and SGR (0.33). In general, *Spirulina platensis* produced better growth than *Lactobacillus rhamnosus*. The highest FCR (3.73) was obtained in the control. The present investigation shows that incorporation of a probiotic and *Spirulina* in diets for Nile Tilapia results in increased growth rate and biochemical performance. *Spirulina* diets were most effective in stimulating fish growth.

## Introduction

Aquaculture is one of the fast growing systems in the world, which has emerged as an industry possible to supply protein rich food throughout the world (Prasad, 1996). Fish is an important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged throughout the world. According to nutritionists, fish is an excellent substitute of protein for red meat. Fish flesh contains all the essential amino acid and minerals viz., iodine, phosphorus,

potassium, iron, copper and vitamin A and D in desirable concentrations (Sandhu, 2005).

Presently, aquaculture is facing heavy production loss both in hatcheries and grows out systems due to disease outbreak. In many land animals, growth stimulating microorganisms incorporated in the feed are reported to have beneficial effects. Since, the microorganisms or probiotics are found to have the capability of improving the water quality, their application in

aquaculture has gained momentum. The term “probiotic” which literally means “for life” has since been employed to describe these health-promoting bacteria.

The World Health Organization has defined probiotic bacteria as “live microorganisms which when administered in adequate amounts confer a health benefits” (FAO/WHO,2001). Probiotics which are beneficially affect the host by improving its intestinal microbial balance, are quickly gaining interest as functional foods in the current era of self-care and complementary medicine. The use of probiotics in the culture of aquatic organisms is increasing with demand for more environment friendly aquaculture practices (Gatesoupe, 1999). Use of probiotics has been proposed as a measure to maintain healthy environment in aquaculture and to prevent occurrence of disease (Lipton,1998) The microorganisms used as probiotics, including *Lactobacillus*, *Bacillus* and yeasts, have been reported in penaeids and fish (Boonthai *et al.* , 2011).

*Spirulina* is considered as a rich source of protein, vitamins, minerals, essential amino acids, and fatty acids [gamma-linolenic acid (GLA)], antioxidant pigments such as carotenoids and vitamin E and trace elements (Belay *et al.*, 1996). In addition, it is effective as an immunomodulator (Takeuchi *et al.*, 2002). Several studies have been conducted using dried spirulina as a feed supplement (Watanabe *et al.*, 1990; Ungsethaphand *et al.*, 2010; Ahmadzade-Nia *et al.*, 2011; Mukherjee *et al.*, 2011; Roy *et al.*, 2011).

## **Materials and Methods**

### **Isolation and mass cultivation of *Spirulina***

Water samples were collected from El-Khadra Lake in Wadi El-Natroun, Beheira, Egypt at different depths of the lake water.

Enrichment cultures of *Spirulina* were established from El-Khadra Lake samples on liquid Zarrouk’s medium pH 9 (Pandey *et al.*, 2010) supplemented with antibiotics (nystatin (100 mg/ml) and cycloheximide (100 mg/ml) at 35°C for 20 days and incubated in a growth chamber with light flux of 16:8 h illuminated having 500 Klux at the surface of the vessels. Cultures were manually stirred twice for a few minutes every day. The purity of the culture was ensured by repeated inoculation. The algal isolates was routinely checked by streaking on nutrient broth medium and microscopic observations. The identification was accomplished by determining cellular morphology observed by using light and 16S rRNA sequencing

### **Isolation and identification of probiotics bacteria from El- Khadra lake**

#### **Isolation of probiotic bacteria**

After 30 to 40 hours of incubation at 37°C in anaerobic conditions, various numbers of isolated colonies were obtained on de Man, Rogosa and Sharpe (MRS) agar plates (De Man *et al.*, 1960) from water samples of El-Khadra lake.

#### **Identification of probiotic bacteria**

Macroscopic appearance of all the colonies was examined for cultural and morphological characteristics. Bacterial isolates were selected for biochemical test and 16S rRNA sequencing (Mandal *et al.*, 2008; Rouse *et al.*, 2008). Pure cultures were maintained in MRS broth at -20°C with 10% (v/v) glycerol.

#### **Antimicrobial activity assay**

##### **Test organisms**

The test organisms used in this work included (*Escherichia coli* and *Aeromonas*

*hydrophila*) bacteria which were isolated from El- khadra lake . 18 hours incubated peptone broth of clinical isolated cultures ( $10^5$  - $10^6$ cfu/ml) of *Aeromonas hydrophila* and *Escherichia coli* were used in the antibacterial studies.

#### **Preparation of the algal extracts:**

The algal cultures of 10 days old were centrifuged and the pellets were collected weighted and used for extraction of antimicrobial agents. 0.5 g of algal pellets were extracted in 10 ml of the ethanol. The homogenated pellets were then freeze and thawed three times and centrifuged at 10000 rpm for 10 min. The supernatants were harvested and kept at 4 °C till usage.

The inhibitory activity of the *Lactobacillus rhamnosus* was determined by Agar- well diffusion assays (Schillinger and Lucke, 1989). Crude bacteriocin was prepared by inoculating *Lactobacillus rhamnosus* (used to screen for bacteriocinogenic potential) in MRS broth (10mL) and incubated at 30°C. After incubation it was centrifuged at 12000 rpm at 4°C for 30 min and supernatant was collected and filtered through 0.45µm pore size filters in order to eliminate any possibility of remaining cell/contamination.

The cell free supernatant was referred as crude bacteriocin preparation (Ogunbanwo *et al.*, 2003). Crude bacteriocin was neutralized by 1N NaOH and final pH was set at 7.

#### **Agar-well diffusion method (Shanmuga *et al.*, 2002)**

To determine the antibacterial activity of the algal extract of *Spirulina platensis* and crude bacteriocin produced by *Lactobacillus rhamnosus* against the selected fish pathogens. The prepoured nutrient agar

plates were overlaid with 100 µl of overnight culture of tested pathogens (in nutrient broth), then spread well with L-shaped glass rod. After 15 min, wells of 5 mm diameter were made with a sterile cork borer, and then 100 µl of supernatant *Lactobacillus* and the algal extract was added into the well with the help of sterilized micropipette. The plates were kept in an upright position in an incubator until the extracts diffused in the agar at least for 3-4 hr. These plates were then inverted and further incubated at 37°C for 24 hr. The plates were observed for zone of inhibition (mm) around the wells.

#### **Preparation of probiotics for feeding trails**

*Lactobacillus rhamnosus* was grown in an overnight culture at 30 °C in 4.0 L MRS broth under shaking (120 rpm). The cells were harvested by centrifugation at 3000 rpm for 15 min, washed 3 times by 0.1 M phosphate buffer solution (PBS) at pH 7.0. The cells were resuspended in the same buffer to give the final concentration  $10^9$  CFU/ml. One ml of PBS containing the probiotic was added to the commercial sterilized food to give  $10^9$  bacterial cells/ kg fish feed. The probiotic bacteria were added to the surface of the feed via a represented oil suspension (sun flower oil). Diets were dried overnight at room temperature. The moisture content of the feed was eliminated by grinding the feed and drying in an oven at 103 °C until constant weight. The results were expressed in percentage of the dry mass content. Diets were transferred to black plastic bags and freeze stored at -20 °C.

#### **Experimental plan**

Nile Tilapia (*O. niloticus*) fingerlings were brought from a fresh water commercial

farm, Kafr El-Sheikh Governorate, Egypt. Prior to the start of the experiment, fish were kept in a fiberglass tank and randomly distributed into glass aquaria to be adapted to the experimental condition until start of the experiment.

Fish were fed on the control diet for 2 weeks; during this period, healthy fish at the same weight replaced died ones. All the experimental treatments were conducted under an artificial photoperiod equal to natural light/darkness period (12 h light: 12 h darkness).

### **Experimental diets**

Seven diets were formulated containing feed additives for Nile Tilapia fingerlings: one as a control diet without supplements; three diets containing *Lactobacillus rhamnosus* at either a concentration of 1 ml (Lact1), 2 ml (Lact2), or 3 ml (Lact3) /kg diet. The microbe concentrations in the probiotics were  $10^9$  colony forming units (CFU)/g. Spirulina was supplied with three doses (2g (Sp1), 4g (Sp2), 6g (Sp3)/kg diet). The basal and tested diets were formulated from the commercial feed ingredients.

The dry ingredients were grounded through a feed grinder to a very small size (0.15 mm). Experimental diets were formulated (Table 2) to be isocaloric and isonitrogenous (37.58% crude protein and 230.32kcal GE /100 g diet). The ingredients weight mixed by a dough mixer for 20 min to homogenize the ingredients. The estimated amount of oil components (sunflower oil) was gradually added and the mixing operation was continued for 20 min. The diets were pelleted through fodder machine and the pellets were dried under room temperature. The diets were stored in plastic bags in refrigerator at 4°C for further use during the experimental period.

### **Experimental design of rearing fish**

A total of 420 Nile Tilapia fingerlings with an average initial body weight of about 50g  $\pm$  2.3 were randomly divided into seven treatment groups and stocked into 21 glass aquaria (70 L each). Three aquaria were assigned for each treatment. Fresh tap water was stored in fiberglass tanks for 24 h under aeration for dechlorination. One third of all aquaria were replaced daily.

Five air stones were used for aerating the aquaria water. Water temperature ranged between 26 to 28°C. Fish feces and feed residues were removed daily by siphoning. Fish from each replicate were weighted at the start of each experiment and hence was counted and reweight every 2 weeks throughout the experimental period (45days).

Fish in all treatments were daily fed with the experimental diets at a level 3% of the body weight daily. The feed amount was given three times daily in equal proportions for 42 days. Fish were weighed biweekly and feed amounts were adjusted on the basis of the new fish weight.

### **Sample collection and analyses**

At the end of the feeding trial, all fish were bulk-weighed, counted were measured for calculation of weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR).

### **Analytical methods**

#### **Chemical analysis of feed**

Proximate chemical analyses were made of diet ingredients and a sample of fish at the beginning and at the end of the experiment according to standard methods (AOAC 1992) for dry matter, crude protein, ether

extract, crude fiber and ash. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.22 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 1993)

### **Chemical analysis of fish**

At the end of the experiment a total number of 42 fish, 6 fish from each treatment, 2 fish from each replicate were randomly taken, netted, weighted and immediately kept in polyethylene bags in deep freezer (-180<sup>0</sup>C) for chemical analysis. Chemical analysis of repetitive samples of soft tissue was conducted to determine dry matter, crude protein, ether extract (fat) and ash content according to AOAC (1992) methods.

### **Growth parameters**

The growth parameters of the Nile Tilapia fingerlings were assessed by taking their body weight at 45 days. The growth performance was assessed using the following formulas:

Weight Gain (WG) = final fish weight (g) – initial fish weight (g).

Weight Gain % (WG %) = Gain of fish (g) x 100/ initial weight of fish (g).

Average daily gain (ADG) = Gain (g) / time (day).

Specific growth rate (SGR %) = 100 X {(ln W2 – ln W1) / T}

where W2 is the final weight of fish (G), W1 is the initial weight of fish (G), ln is natural log and

T is the period in days.

Feed conversion ratio (FCR) = feed intake (g) / Weight gain (g).

Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g).

Protein productive value (PPV %) = {(Retained protein (g) / (protein intake (g)))} X Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g).

Protein productive value (PPV %) = {(Retained protein (g) / (protein intake (g)))} X 100

### **Blood parameters**

Blood samples were collected at the end of experiment; fish in each aquarium were weighted and 5 fish were taken randomly for blood sampling. The blood was collected using heparinized syringes from the caudal vein.

Blood samples were centrifuged at 4000 rpm for 20 min to allow separation of plasma which was subjected to determine plasma total protein and albumin (Tietz, 1990); the activity of the liver enzymes (Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) were determined by the methods of Young (1990). While, globulin concentrations were determined by subtracting the concentration of total protein from albumin concentration.

Red blood cell (RBCs X 10<sup>6</sup>/mm) and white blood cells count (WBCs x 10<sup>3</sup>/mm): were measured on an a bright –line Haemocytometer, Hemoglobin concentration was estimated according to the method of Zinkl(1986), Packed cell volume (PCV %): was estimated by the microhaematocrite method described by (Dacie and Lewis, 2006).

Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and mean cell volume (MCV) were calculated using the formulae.

$$\text{MCHC (\%)} = \text{Hb} / \text{Ht} \times 100, \quad \text{MCH} \\ (\text{pg}) = \text{Hb} / \text{RBC} \times 10$$

$$\text{MCV} (\mu^3) = \text{Ht} / \text{RBC} \times 10$$

## Result and Discussion

*Arthrospira platensis* and *Arthrospira maxima* were once classified in the genus *Spirulina*. There is now agreement that are in fact *Arthrospira*, nevertheless and somewhat confusing, the older term *Spirulina* remains in use for historical reasons Komarck and Lund (1990). *Spirulina platensis* and *Lactobacillus rhamnosus* were isolated from water of El-Khadra lake and identified as shown in Figs (1&2).

The *Lactobacillus* isolated from El-khadra lake using MRS as the selective medium was Gram positive, catalase negative and non-spore forming long rods. In 16S rRNA sequencing and phylogenetic analysis, 99% similarity was noted with *Lactobacillus rhamnosus*.

*Spirulina* is a microscopic blue-green alga in the shape of a spiral coil, living both in sea and fresh water. *Spirulina* is the common name for human and animal food supplements produced primarily from two species of cyanobacteria: *Arthrospira platensis*, and *Arthrospira maxima* (Vonshak and Tomaselli, 2000). Antimicrobially active lipids and active fatty acids are present in a high concentration in this alga (Lampe *et al.*, 1998). It was hypothesised that lipids kill microorganisms by leading to disruption of the cellular membrane (Bergsson, 2005) as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of

peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. This can probably be explained by the strong fabric of the cell wall of Gram-positive bacteria, which maintain their structure in spite of substantial hydrostatic turgor pressure within the bacteria (Bergsson and Thormar, 2002).

Probiotics has been suggested as an option to improve the health and well being of aquatic animals during culture (Verschuere *et al.* 2000; FAO/WHO Working Group Report 2002), and among the benefits of using them, some authors have reported an improve of growth performance of organisms fed with diets added with probiotic cells (Ghosh *et al.* 2008; Panigrahi and Azad 2007).

It is clear from study that the diameter of the inhibition zone depends mainly on the crude extracts used and the tested bacteria. The extract of *Spirulina platensis* showed maximum antimicrobial activity of 2.7 mm against *Escherichia coli* Table (2).

*S. platensis* is a cyanobacterium that has been largely studied due to its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids in many countries in tropic, subtropical and temperate regions for use in human health food, as an animal feed. *S. platensis* was grown under optimum growth conditions and the produced dried biomass was used in the following investigation in feed additives as growth promoters in Nile Tilapia fingerlings diets.

## Growth performance

Data in Table 3 &4 shows the growth performance and nutrient efficiencies on Nile Tilapia fingerlings fed diets. No significant differences in initial body weight

were found among the different experimental treatments, indicating the accuracy of randomization process between the experimental treatments.

It is clearly shown in (Table 3) that all the tested growth parameters (gain, ADG and SGR) in the diets supplemented with *Spirulina platensis* administered to the fingerlings produced the best growth rate at an optimum concentration of 4 and 6 g/kg. It worth mentioned that the mean value level in the group received 6g/kg is higher in all the tested parameters with significant difference. On the other hand, the group of fish fed with control diet exhibited the lowest final body weight; these results cleared that the optimum dietary level of *S. platensis* for *O. niloticus* is 6 g/kg for 45days to enhance growth performance. Duncan and Klesius (1996) reported that *Spirulina* alga was a good source of protein for animal feed, being containing high amounts of vitamins and minerals, in addition, Nakono *et al.* (2003) recorded that the lack of cellulose from the cellular structure of *Spirulina* render it easily digestible, thus, increase fish appetite, improve feed intake and nutrient digestibility and in turn enhance the health of fish, increasing the ability to fight off infections through the reduction of stress levels. The results in the current study are in accordance with Watanabe *et al.* (1990) and Takeuchi *et al.* (2002) who found that feed supplemented with *S. platensis* powder improved the feed conversion ratio and growth rates in striped jack, *Pseudocaranx dentex*. Lu *et al.* (2002) demonstrated that raw *S. platensis* can be an effective uni-feed for larval tilapia at a feeding rate of 30% (on a dry basis) of body weight. Abdel-Tawwab and Ahmed (2009) recorded that the growth and feed utilization of *O. niloticus* were obtained at 5.0 g fresh culture of *S. platensis* /kg diet.

Results show that, treatments with *Spirulina platensis* and *Lactobacillus rhamnosus* had feed conversion ratios (FCR) significantly lower than those for the control diets. The best conversion ratio (2.33) was recorded for the 6g/kg diet of *Spirulina platensis*) treatments. It is proved that lower the feed conversion ratio higher is the quality of the feed as observed in many fresh water fishes where the feed conversion ratio is known to decrease with increasing dietary protein content (Jha *et al.*, 2009). The best FCR values observed with feed additives - supplemented diets suggest that addition of *spirulina* improved feed utilization. Similar results have been reported for feed additives used in diets for piglets (Gil, 1998). In practical terms, this means that feed additives use can decrease the amount of feed necessary for animal growth which could result in production cost reductions

In general, fish fed with the diets supplemented with feed additives showed better feeding efficiency than those fed with control diet. The protein efficiency ratio (PER) were significantly higher in the treatments containing 6g/kg diet of *Spirulina platensis* than in *Lactobacillus* and control treatments. The lowest PER, were recorded in the control treatments.

The PER and PPV results indicate that supplementing diets with *Spirulina* significantly improves protein utilization in Tilapia. This contributes to optimizing protein use for growth, a significant quality given that protein is the most expensive feed nutrient. The improvement in the biological value of the supplemented diets in these treatments with high population and low dietary protein demonstrated that spirulina supplements performed more efficiently in stress situations (Ringo and Gatesoupe 1998).

Feed additives supplementation significantly affected whole-fish body composition (Table 5). Fish fed with the control diet had the lowest protein content; however, all feed additives supplementation appeared to improved protein content. Carcass lipid content was also affected by dietary protein content, with the highest values in the control treatment, which were statistically different from the supplemented treatments. The lowest overall lipid content (Ether extract) was recorded with *Spirulina platensis* and *Lactobacillus rhamnosus* treatment.

The lowest lipid content content of fish carcass and ash content were recorded in fish groups fed with diets supplemented with *Spirulina platensis* and *Lactobacillus rhamnosus*. From the nutritional stand point of view, increasing the lipid content and ash content of fish tilapia carcass is not acceptable object. In agreement with the results obtained Kalavathy et al. (2003) reported that the relative abdominal fat pad was significantly reduced with adding *Lactobacillus* culture to broiler chick diet, compared to the control group.

These results suggest that *Spirulina platensis* supplementation plays a role in enhancing feed intake with a subsequent enhancement of fish body composition. The better feed intake in spirulina- supplemented diets may have been due to increase fish appetite resulting in a higher feed intake and therefore improved growth. Moreover, due to the high feed intake and nutrient utilization, the deposited nutrients increased. On the other hand, High protein content in group fed 6g/kg diet spirulina algae diet may be due to spirulina improved fish utilization of protein ratio. these results agreed with Soivio et al. (1989) who concluded that the differences in protein content of fish body composition may be related to changes in their synthesis, deposition and difference in

growth rates. Abdel-Tawab and Ahmed (2009) recorded that changes in protein and lipid content in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate.

#### **Blood parameters of *Oreochromis niloticus* fingerlings**

This study was planned to evaluate the effect of *Spirulina* and probiotic on the blood parameters of Nile Tilapia. Concerning the effect of both probiotic and *Spirulina* on the health status of fish. Results in Table(6) showed that, feeding *Spirulina platensis* at levels 6 g/kg diet had significantly higher RBCs, while at level 2 and 4 g/kg diet had significantly higher WBCs. Concerning hemoglobin in fish blood all groups fed on *Spirulina* had higher than control and *lactobacillus* reflecting good nutritional status.

These could be attributed to the fact that, the probiotics used increased the blood parameters values as a result of hemopoietic stimulation (Sarma et al., 2003 ; Manohar, 2005 and Rajesh Kumar et al., 2006). Similar results obtained by Abdel-Tawab et al. (2008a) who stated that 10% Spirulina diet increased RBCs and WBCs counts and their ranges were  $2.07 - 2.54 \times 10^6/\mu\text{L}$  and  $3.29 - 4.02 \times 10^5/\mu\text{L}$ , respectively 0.05;. The low counts of RBCs and WBCs were obtained at the control diet  $1.87 \times 10^6/\mu\text{L}$  and  $3.13 \times 10^5/\mu\text{L}$ , respectively.

#### **Biochemical analysis of *Oreochromis niloticus* fingerlings serum:**

Results in Table 7 showed that, fish fed with diets containing feed additives exhibited higher protein and globulin values. Also, feed additives supplementation significantly decreased AST and ALT values compared with control.

Biochemical analyses often provide vital information for health assessment and management of cultured fish (Pincus, 1996; Cnaani *et al.*, 2004; Řehulka *et al.*, 2004). Serum total protein and globulin in all groups fed on different levels of spirulina have higher concentrations than control whereas albumin showed no significant difference between different treatments indicating that spirulina had enhanced immunity and improved health of *O. niloticus*.

The measurement of AST and ALT in plasma is of considerable diagnostic value in fish, as it relates to general nutritional status as well as the integrity of the vascular system and liver function. This result agrees with Abdel-Tawab *et al.* (2008 b) who investigated the use of commercial baker yeast as a growth and immunity promoter for Nile Tilapia; they found that biochemical parameters were improved in fish fed yeast.

In the current study *Spirulina platensis* and *Lactobacillus rhamnosus* supplementation reduced serum renal injury biomarkers, since it reduced the lipid peroxidation in liver and kidney. In addition there were elevations of hepatic and renal antioxidant enzymes. The antioxidant and protective effects of Spirulina are owed to their content of antioxidant active constituents such as c-phycoerythrin, β-carotene, minerals, vitamins, protein, carbohydrates and lipids (Upasani and Balaraman, 2003).

It can be concluded that the addition of 6g/kg diet spirulina in Tilapia fingerlings diets improves animal growth, and mitigates the effects of stress factors. The two tested organisms used in the present study were effective in stimulating fish performance, though *Spirulina platensis* produced the best results, being the most viable option for optimizing growth and feed utilization in intensive Tilapia culture.

**Table.1** Composition and analysis of the used basal diets

Ingredient	Percentage (%)
Fish meal	15.00
Soybean meal	50.00
Yellow corn	16.50
Wheat bran	5.00
Cellulose	12.00
Vitamin and Mineral mixture*	0.50
Calculated analysis (on dry matter basis) %:	
Dry matter	97.97
Crude protein	37.58
Ether extract	30.49
Crude fiber	8.10
Ash	27.90
Nitrogen free extract (NFE)	3.48
Nutritive value:	
Gross energy k cal/kg**	4909.6
Energy/protein ratio	152.8

Vitamins and mineral mixture: Each 1 kg contains vitamin A, 4.8 ml. U; vitamin D, 0.8 ml.U.; vitamin E, 0.4 g ; vitamin K, 0.8g ; vitamin B1, 0.49 g ; vitamin B2, 1.6 g ; vitamin B6, 0.6 g; vitamin B 12, 0.4 g; Pantothenic acid 4 g; Folic acid 400 mg; Biotin 20 mg; Choline chloride 200 mg; Copper 0.4 g; Iodine 0.4 g; Iron 12 mg; Manganese 22 g; Zinc 22 g and Selenium 0.04g.

Gross energy\*\* the value was calculated from their chemical composition using the factors 5.65, 9.45, 4.22 (K cal/g) for protein, fat, fiber and NFE, respectively (NRC, 1993)

**Table.2** antibacterial activity of *Spirulina platensis* and *Lactobacillus rhamnosus*(mm)

Organism	Diameter of clear zone (mm)	
	pathogens	
	<i>Aeromonas hydrophila</i>	<i>Escherichia coli</i>
<i>Spirulina platensis</i>	2.1	2.70
<i>Lactobacillus rhamnosus</i>	1.6	2.4

**Table.3** Growth performance of Nile tilapia (*Oreochromis niloticus*) fed diet containing *Spirulina platensis* and *Lactobacillus rhamnosus*

Items	Initial body weight (g)	Final body weight (g)	Average daily gain (g/fish/day)	Weight gain (g)	Specific growth rate (%)
Control	51.03	62.37D	0.27D	11.34D	0.22C
Lact 1	50.66	66.34BC	0.37BC	15.67BC	0.27ABC
Lact 2	50.66	63.95CD	0.32CD	13.29CD	0.25BC
Lact3	50.3	64.29CD	0.33CD	14.00CD	0.26ABC
SP1	51.10	66.93BC	0.37BC	15.83BC	0.28ABC
SP2	50.13	68.57AB	0.44AB	18.43AB	0.32AB
SP3	51.50	71.66A	0.48A	20.16A	0.33A
F test	n.s	***	***	***	***
LSD 1%	-	3.31	0.079	2.93	0.079

\* = significant      \*\*\* = Highly significant      n.s= Non significant

**Table.4** Feed intake, feed conversion ratio and nutrient utilization of Nile tilapia (*Oreochromis niloticus*) fed diet containing *Spirulina platensis* and *Lactobacillus rhamnosus*

Items	Feed conversion ratio	Total gain (g)	Feed intake (g)	Protein efficiency ratio	Protein productive value (%)
Control	3.73 A	11.34D	41.74BC	1.83D	27.32E
Lact 1	3.23AB	15.67BC	50.65A	2.00CD	30.95D
Lact 2	2.97BC	13.29CD	39.43BC	2.01BCD	33.82CD
Lact3	2.72BCD	14.00CD	38.02C	2.26ABC	36.80BC
SP1	2.84BC	15.83BC	45.00ABC	2.10BC	36.48BC
SP2	2.16D	18.43AB	46.26AB	2.29AB	38.49B
SP3	2.33CD	20.16A	47.24AB	2.46A	42.90A
F test	***	***	*	***	***
LSD 1%	0.645	2.839	7.951	0.261	3.505

Body composition of *Oreochromis niloticus* fingerlings

**Table.5** Carcass chemical composition of Nile tilapia (*O. niloticus*) fed diet containing *Spirulina platensis* and *Lactobacillus rhamnosus*

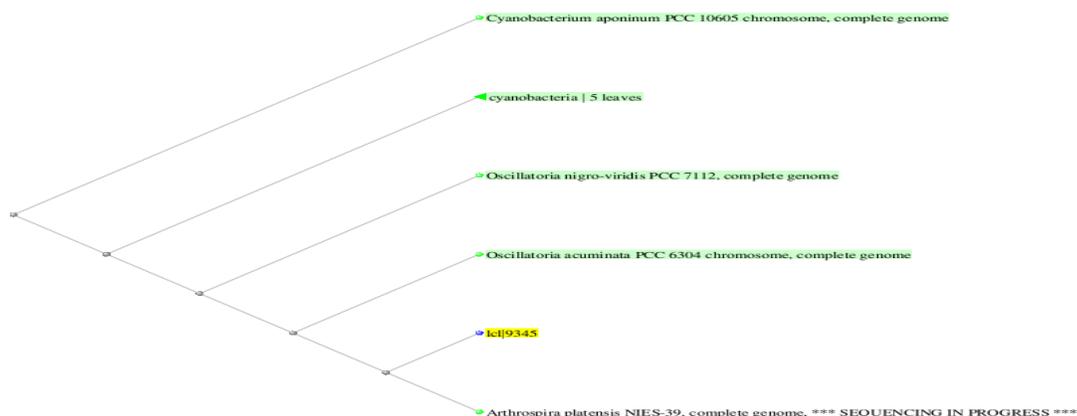
chemical composition	Treatments			
	Dry matter (%)	Crude protein (%)	Lipid content (%)	Ash (%)
Control	24.02D	62.07F	22.13A	13.13BC
Lact 1	24.36CD	63.21E	20.41B	15.38AB
Lact 2	25.27BC	64.12D	18.66C	15.22ABC
Lact3	25.16BC	64.68CD	17.79CD	16.20A
SP1	25.84AB	64.92BC	17.17D	16.53A
SP2	25.65AB	65.52B	17.43D	15.72A
SP3	26.25A	66.40A	18.75C	12.85C
F test	***	***	***	**
LSD 1%	0.939	0.669	0.972	0.0788

\* = significant                      \*\*\* = Highly significant

**Table.6** Hematological parameters of Nile tilapia (*Oreochromis niloticus*) fed diet containing *Spirulina platensis* and *Lactobacillus rhamnosus*

Treatment	RBCS 10 <sup>3</sup> mm	WBCS 10 <sup>6</sup> mm	PCV%	MCV%	MCH %	MCHC %	Hb (g/dl)
Control	1.50F	55.30G	16.66G	113.23C	44.67F	39.40A	6.70F
Lact 1	1.87E	63.23E	25.13F	126.49B	47.37E	28.11D	9.03E
Lact 2	1.92DE	63.46D	26.067E	134.95AB	47.83D	28.38C	9.23D
Lact3	1.98CD	64.20B	28.67D	141.93A	47.50E	30.47B	9.37D
SP1	2.200B	66.70A	33.37B	145.05A	50.30C	27.50F	12.23B
SP2	2.07C	63.77C	29.77C	143.03A	51.87B	27.34E	10.87C
SP3	2.41A	62.80F	34.03A	141.40A	52.33A	27.13F	12.50A
F test	***	***	***	**	**	**	**
LSD 1%	0.111	0.193	0.541	11.52	0.208	0.208	0.193

**Figure.1** Phylogenetic dendrogram showing taxonomic positions of *Spirulina platensis*(*Arthrospira platensis*) type strains based on the 16s rRNA partial sequences



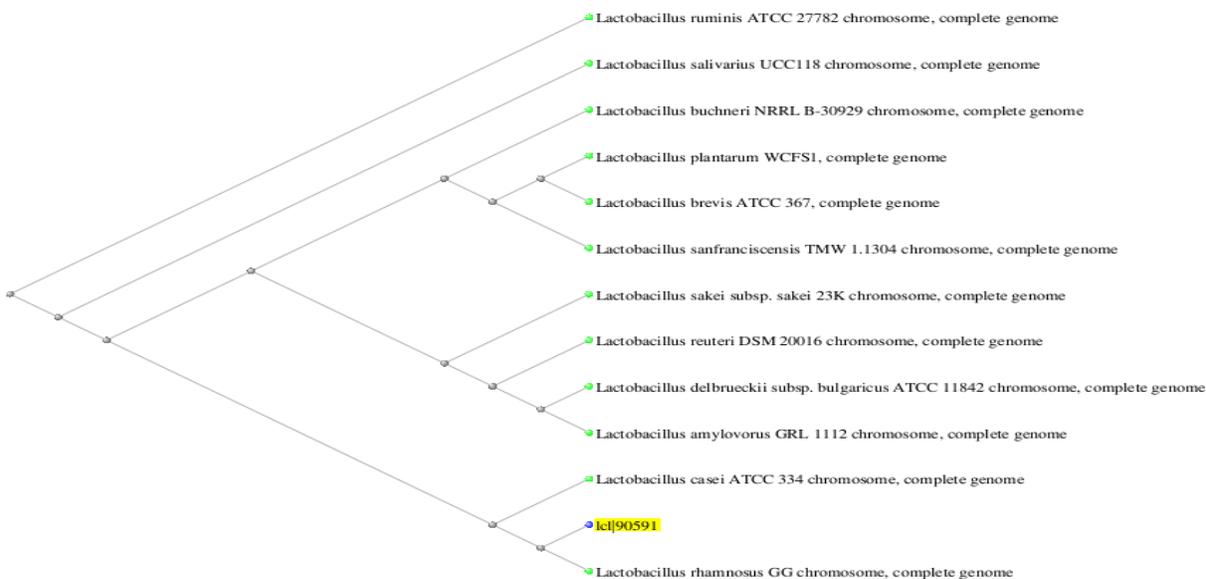
**Table.7** Protein profile and activities of serum enzymes in *Oreochromis niloticus*) fed diet containing *Spirulina platensis* and *Lactobacillus rhamnosus*

Treatment	Kidney enzymes		Immunity index				Liver enzymes	
	Urea	creatinin	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Albumin / Globulin ratio	ALT (Iu/l)	AST (Iu/l)
Control	2.40A	1.37A	4.26E	2.23C	2.03E	1.09A	24.33A	86.67A
Lact 1	2.23AB	0.73BC	4.80D	2.46A	2.33D	1.05B	21.00B	75.00B
Lact 2	2.13BC	0.80BC	4.76D	2.26C	2.50CD	0.91C	18.67C	73.67B
Lact3	2.10BC	0.43C	5.00C	2.40AB	2.60C	0.92C	18.00C	74.00B
SP1	1.96CD	0.76BC	5.14B	2.07D	3.07B	0.67D	23.00A	67.33C
SP2	1.83D	0.90B	5.46B	2.30BC	3.13AB	0.69D	16.33D	65.33C
SP3	1.83D	0.93B	5.63A	2.30D	3.33A	0.63E	14.67E	62.67D
F test	***	**	***	***	***	***	***	***
LSD 1%	0.193	0.402	0.176	0.111	0.223	0.078	1.603	2.320

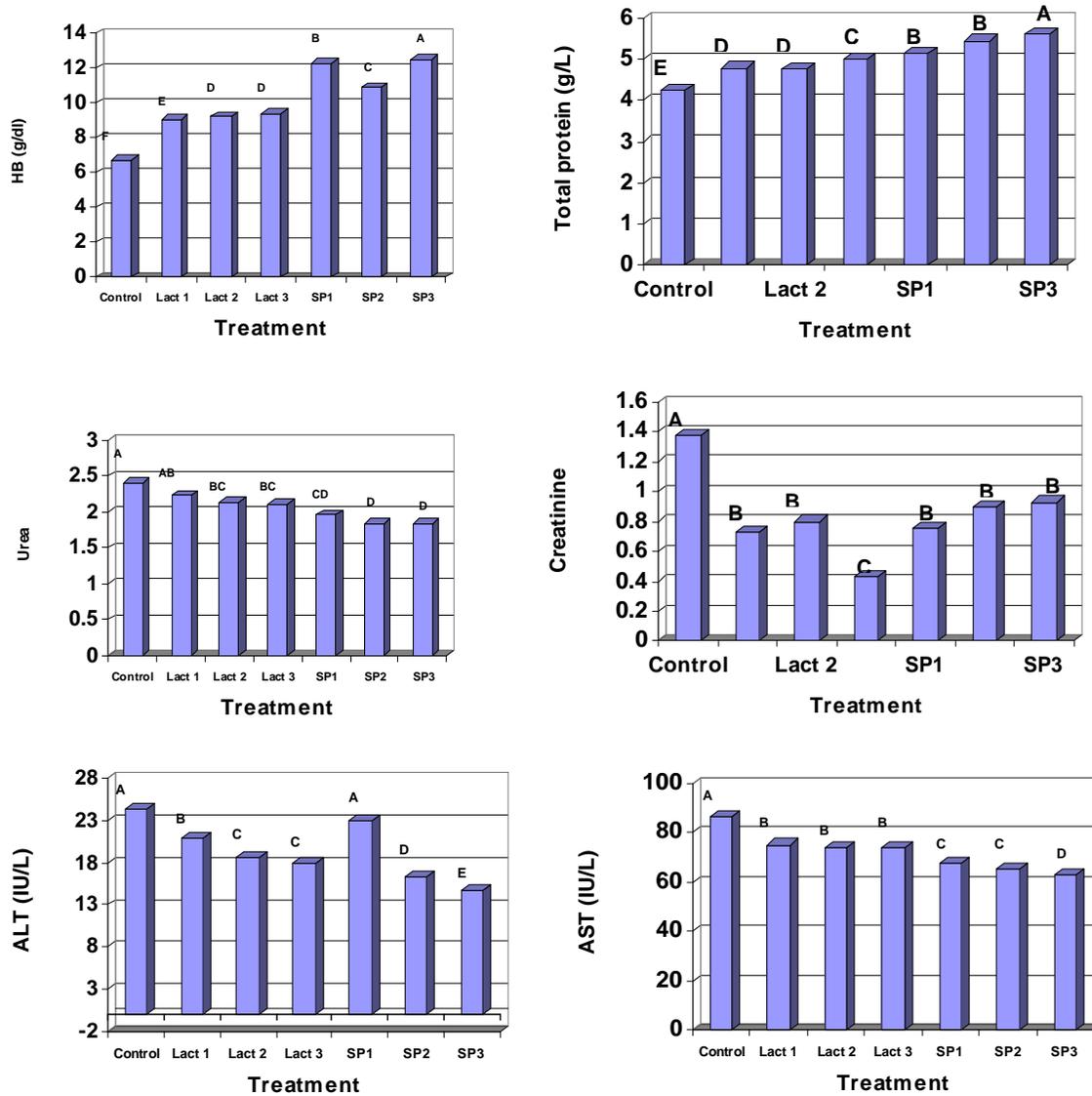
\* = significant

\*\*\* = Highly significant

**Figure.2** Phylogenetic dendrogram showing taxonomic positions of *Lactobacillus rhamnosus*. type strains based on the 16s rRNA partial sequences



**Fig.3** Hemoglobien concentrations, Protein profile and activities of serum enzymes in *Oreochromis niloticus* fed diet containing *Spirulina platensis* and *Lactobacillus rhamnosus*



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