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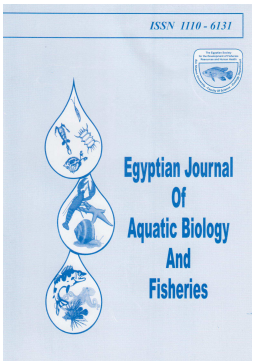

Physiological and haematological responses of the Nile tilapia (*Oreochromis niloticus)* fed on diets supplemented with probiotics

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ABSTRACT

A 12-week feeding trial was conducted to evaluate the effect of dietary probiotic, *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* at a concentration of (10^8cfu/ml).Three isonitrogenous (300 g CP kg^{-1} dry matter, DM) and isocaloric (3500Kcal metabolizable energy kg^{-1} DM) diets were formulated and probiotics was supplemented in the experimental diets. Fingerlings averaging 2.80±0.05g were randomly distributed into 18 glass aquaria (160 liter) and each aquarium holding 15 fish and randomly assigned to one of six replicates of the diets and offered feed at a daily rate of 5% of the total fish biomass. After 12 weeks, fish fed the diets supplemented with the two probiotics showed significantly better final weight, body length, specific growth rate, weight gain, feed intake, feed conversion ratio and protein efficiency ratio than those fed the control diet. The highest red blood cells count (RBCs), Hemoglobin (Hb), hematocrite (Hct), and the lowest mortality rate were recorded for fish fed the diet supplemented with *S. cerevisiae* supplemented compared to the other two groups. Fish fed the diet supplemented with *S. Cerevisiae* followed by *L. acidophilus* recorded the lowest (P<0.001) serum transaminase enzymes (alanine transaminase, ALT and aspartate transaminase, AST). Fish fed *S. cerevisiae* and *L. acidophilus* supplemented diets showed the lowest significant (P<0.001) count of microbial content in surface and muscles of Nile tilapia.

INTRODUCTION

The culture of Nile tilapia, *Oreochromis niloticus*, is one of the most rapidly expanding industries in Egypt (Abdel-Hakim *et al.*, 2001a&b). However, factors including diseases and pollution cause massive mortality in the leading fish countries

(Wang *et al.*, 2005). The diseases that brought the most impact to the industry include viral infections and bacteriosis. Conventional approaches to control diseases with chemicals include use of antimicrobial drugs, pesticides, and disinfectants (Gomez-Gil *et al.*, 2000). Unfortunately, the abuse of such antimicrobials in disease prevention and growth promotion can lead to the evolution of resistant strains of bacteria (Esiobu *et al.*, 2002). Therefore, the research of probiotics for aquatic animals is increasing with the demand for environment-friendly aquaculture (Mehisan *et al.*, 2015, Hassaan and Soltan, 2016).

To our knowledge, the first application of probiotics in aquaculture was relatively recent (Kozasa, 1986), but the interest in such safe and high effective function is increasing rapidly (Gatesoupe, 1999). The microorganisms used as probiotics, including yeasts, Bacilli, lactic acid bacteria, Pseudomonads and so on, have been evaluated in aquatic animals (Ring and Gatesoupe, 1998; Irianto and Austin, 2002). Among lactic acid bacteria, including some *Enterococcus faecium* (*E. faecium*) strains are non-pathogenic, with an ability to produce lactic acid and bacteriocin (Herranz *et al.*, 2001).

The aim of this study was to analyze the effect of a probiotic bacterium, *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* on growth performances and immune responses of tilapia (*O. niloticus*).

MATERIAL AND METHODS

The present study was carried out at the laboratory of fish Nutrition, Faculty of Agriculture, Benha University, Egypt with cooperation of Regional Center for Food & Feed (RCFF), Agriculture Research Center, Egypt. The experimental started at 1st August 2013 and continued until 23th October of the same year (12weeks). It was aimed to assess the role of *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* bacteria as probiotic with special emphasize and its role in cultured *O. niloticus* as a growth promoter and immune stimulant agent.

All-male Nile tilapia, *O. niloticus* fry (2.80±0.03g) were obtained from private farm, Kafr El-Sheikh**,** Egypt. Fish were acclimated to the experimental conditions for two weeks**,** during acclimation period; fish were fed a control diet at a level of 5% of biomass**.** Settled fish wastes with one half of water were siphoned daily and water volume was replaced by aerated tap water from the storage tank. The experiment was conducted in eighteen glass aquaria**.**

Design of the experiment

The present study was carried out to assess the role of *S. cerevisiae* and *L. acidophilus* as a probiotic on growth performance, feed utilization, immune response and the effect of these probiotics on microbial content of muscles and surface of *O. niloticus*. Therefore, three treatments were planned in sex replicates aquaria for each treatment. Each aquarium was stocked with 15 fish and supplied by air pump.

Table1: Experimental design of the present study.

Group	Treatment	Probiotic (ml/kg diet)
T1	*S. cerevisiae*	20ml/kg (10^8cfu/g)
T2	*L. acidophilus*	20ml/kg (10^8cfu/g)
T3	Control	Negative

Preparation of Experimental diets:

Experimental diets were described previously by Hassaan *et al.,* (2014) dry ingredients were homogenized mixture grinder. All dry ingredients were thoroughly mixed with soybean oil, and vitamins and minerals mixture, and then, passing the mixed feed through a laboratory pellet (2-mm die) in National institute of Oceanography and Fisheries, Cairo Governorate, Egypt (CMP California pellet Mill, San Francisco, CA, USA), and stored at-20^{o}C until used. The control diet was formulated using the ingredients as described and the proximate analysis of the basal diet according to the AOAC (1995) in Table 2.

Table 2: Composition of the basal diet (g/kg) and chemical analysis.

Ingredients	Control
Fish meal	160
Soybean meal	340
Yellow corn	350
Gultain	40
Wheat flour	70
Soybean oil	20
Vita & Min [1]	20
Chemical analysis (%Dry matter basis)	
Dry matter	91
Crude protein	30
Crude lipid	8
Crude fiber	5.4
Ash	6
NFE[2]	46.8
ME[3]	3504 (Kcal/Kg)

[1]Vitamin and mineral mix (mg or g / Kg diet): MnSO4, 40 mg; MgO, 10 mg; K2SO4, 40 mg; ZnCO3, 60 mg; KI, 0.4 mg; CuSO4, 12 mg; Ferric citrate, 250 mg; Na2SeO3, 0.24 mg; cholecalciferol, 4000 IU; α-tocopherolacetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg and pyridoxine, 30 mg. [2]NFE (Nitrogen free extract) =100-(crude protein + lipid + ash +fiber content). [3]Metabolizable energy (kJ g-1), calculated based on the physiological fuel values according to (Brett, 1973).

Probiotics strains:

Bacterial strains of *S. cerevisiae* and *L. acidophilus* were obtained from food safety lab, Regional Center for Food and Feed (RCFF), Agriculture Research Center (ARC), and were kept at -20^{o}C until the start of the experimental.

Media and reagents, for *S. cerevisiae* and *L. acidophilus* preparation:

The media used and their ingredients and pH are described in Table 3.

Preparation of S. *cerevisiae* suspension:

S. cerevisiae was propagated into Rose Bengal Agar and incubated at 25^{o}C for 5 days, and the growth was harvested, then washed three times and re-suspended in Brain Heart Infusion Broth (Table 3). The suspension incubated at 37^{o}C for 24 hours. Counting the colony forming unit per ml transfer an aliquot of prepared sample (10^{-1}) to a tested tube contains 9 ml folds of Buffered peptone water (Table 3) from which 1 part is taken to another test tube containing 9 ml folds of the Buffered peptone water to have a final dilution of 10^{-3}. Continue in this manner till reaching to level 10^{-7} microorganisms per ml taking into account good mixing with vortex in each step. One empty and pre-sterilized petri dish is inoculated with a known amount of each dilution before adding about 15ml of molten Rose Bengal Agar (Table 3) previously cooled at 45^{o}C. Mix the inoculums and the medium thoroughly. Incubate the inverted dishes at 25^{O}C for 5 days. Selected average values between 10-100 colonies and report the result multiplied by the dilution factor.

Preparation of *L. acidophilus* suspension:

L. acidophilus was propagated in to MRS Table 3 and incubated at 37^{o}C for 48 hours, and the growth was harvested, then washed three times and re-suspended in Brain Heart Infusion Broth Table 3. The suspension incubated at 37^{o}C for 24 hours to have a final

concentration 10^7 microorganisms per ml. Counting the colony forming unit per ml transfer an aliquot of prepared sample (10^{-1}) to a tested tube contains 9 ml folds of Buffered peptone water Table 3 from which 1 part is taken to another test tube containing 9ml folds of the Buffered peptone water to have a final dilution of 10^{-3}. Continue in this manner till reaching to level 10^{-7} microorganisms per ml taking into account good mixing with vortex in each step. One empty and pre-sterilized petri dish is inoculated with a known amount of each dilution before adding about 15 ml of molten MRS (Table 3) previously cooled at 45°C. Mix the inoculums and the medium thoroughly. Incubate the dishes in inverting position at 44°C for 2 day. Selected average values between 10-100 colonies and report the result multiplied by the dilution factor.

Table 3: Description of media which used in isolation of *S. cerevisiae*, *L. acidophilus* and enumeration of microbial content

Media	Ingridients per (g /l)	pH
Buffered peptone water (Biolife)[1]	Peptomeat 10 g Sodium Chloride 5 g Disodium Phosphate 3.5 g Monopotassium Phosphate 1.5 g	7.0±0.1 at 25°C
Rose bengal agar (LAB M)[2]	Mycological peptone 5.000 Dextrose 10.000 Monopotassium Phosphate 1.000 Magnesium sulphate 0.5 Rose bengal 0.05 Chloramphenicol 0.1 Agar 15.5	7.2 ±0.2 at 25°C
Plate count agar	Tryptone 5.0g Yeast extract 2.5g Glucose 1.0g Agar 9.0g	7.0 ±0.2 at 25°C
MRS Agar (BIOLIFE)[3]	Enzymatic digest of casein 10g Beef extract 10g Yeast extract 4g Glucose 20g Di-potassium Hydrogen Phosphate 2g Sodium Acetate 5g Tri-ammonium Citrate 2g Magnesium Sulphate hepta hydrate 0.2g Manganous Sulpha tetetra hydrate 0.05g Agar 15g Tween 80 1g	7.2± 0.2 at 25°C

[1]The required quantity was prepared as mentioned by the manufacturer; [2to 8] required quantity was prepared as mentioned by the manufacturer then poured in sterile Petri dishes.

Probiotic supplemented diets:

The probiotic test diets T1 and T2 were prepared by gently spraying the required amount of bacteria suspension on the control diet and mixing it part by part to obtain a final probiotic concentration (10^8cfu/g). The probiotic test diets T1 and T2 were packed in sterile poly propylene containers and stored at 4°C for viability studies. Storage period over 14 days period. New diets were prepared bi-weekly to ensure that high probiotic levels remind in the diets for the duration of the trial (Sun *et al.*, 2010).

Feeding system:

Fish were fed the experimental diets at a rate of 5% twice daily at 8.00 am and 4 pm hours. Fish in each aquarium were sampled biweekly and feed amounts were adjusted according to the new fish biomass. Dead fish were daily recorded and removed. The feeding period in the experiment lasted 12 weeks.

Water quality:

Water temperature was recorded daily at 1.00 pm using a mercury thermometer. Dissolved oxygen (DO) was measured at 07.00 am using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectro-photometer (Hash Company, Loveland, CO, USA). A pH was estimated at morning by using a pH meter (Orion pH meter 400, Abilene, Texas, USA). Water temperature ranged from 27.20 to 29.25°C; dissolved oxygen (DO) ranged between 5.32 and 6.81 mg/l; pH values ranged between 8.04 and 8.30 and total ammonia ranged from 0.18 to 0.2 mg/l for the different treatments during the entire experimental period (90 days) of the study. All tested water quality criteria (temperature, pH value, DO and total ammonia) were suitable and within the acceptable limits for rearing *O. niloticus* fingerlings (Boyd, 1990). A photoperiod of 12-h light, 12-h dark (08:00–20:00 h) was used via fluorescent ceiling lights supplied the illumination.

Growth performance and feed utilization parameters:

Records of live body weight (BW/g) and body length (BL/cm) of fish were measured in all fish for each pond and registered every 14 day (two weeks) during the experimental period. Growth performance parameters were measured by using the following equations:

Condition factor (K) = (W/L^3) x 100

Where: W = weight of fish in grams and L = total length of fish in "cm".

Weight gain (WG) = final weight (g) – initial weight (g).

Specific growth rate (SGR) = $\frac{LnW2 - LnW1}{t} x\ 100$

Where: Ln = the natural log; W_1 = first fish weight; W_2 = the following fish weight in grams and t = period in days.

Feed conversion ratio (FCR) = Feed ingested (g)/Weight gain (g)

Protein efficiency ratio (PER) = Weight gain (g)/Protein ingested (g)

Survival rate: (SR) = (Z/X) × 100, Where, Z is the surviving fish number and X is the initial fish number.

Blood sampling:

At the end of the experiment, blood samples collected from the caudal vein in clean tube with 10 % EDTA solution to determine red blood cells (RBCs), hematocrit (Hct), hemoglobin (Hb) and differential leukocytes (WBCs). Blood samples of the other fish were collected also from the caudal vein in clean dry centrifuge tubes, kept for 15 minutes and centrifuged at 3000 rpm for 10 minutes, then kept frozen at -20°C for determination of blood chemistry, asparatate amino transferase (AST) and alanine amino transferase (ALT).

Hematological Parameters:

Hematocrite (Hct), was determined as described by Reitman and Frankel (1957), haemoglobin (Hb) was determined by the haemoglobin kit which is a standardized procedure of the cyanomet haemoglobin method and the total count of white blood cells (WBCs) was carried out by the indirect method (Martins *et al.* 2004). Asparatate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined according to the method described by Reitman and Frankel (1957). Serum creatinine and uric acid were measured by calorimetric method and enzymatic detremenation methods respectively as described by (Henry, 1974).Total count of differential leukocytes (WBCs) was carried out by the indirect method Martins *et al.* (2004) differential counting of leucocytes in the smears stained was carried by Giemsa / May-Grunwald. Total leukocytes number was calculated by the formula:

Leukocytes/µl = (Leucocytes number in the smear × erythrocytes number/ µl) /2,000 erythrocytes counted in the blood smear.

Examination of microbial content of muscles of *O. niloticus*:

Microbiological analysis was performed according to the standard procedure for the enumeration of respective group of microorganisms. All equipment and chemicals were sterilized at 121ºC (15lb pressure) for 15minutes. Total Plate Count (TPC): Tenfold dilution of the homogenate sample were spread plated on plate count agar (oxoid) in duplicate and incubated at 30ºC for 48 hrs and counted using colony counter. Plates between 30 and 300

were taken and recorded. Count for Total Coliform count and Faecal Coliform Count: Tenfold dilution of the homogenate sample were spread plated on Violet Red Bile agar (VRB-agar) (Table3) in duplicate and incubated anaerobically at 37ºC and 44ºC for 24 hr. Staphylococci count: Tenfold dilution of the homogenate sample were spread plated on Baird Parker agar (oxoid) in duplicate and incubated at 37ºC for 2 days. Salmonella Count: Tenfold dilution of the homogenate sample were spread plated on Brilliant Green agar (oxoid) in duplicate and incubated at 37ºc for 24hrs and counted using colony counter. Total Fungal Count of the homogenate sample were spread plated on Rose Bengal agar (oxoid) in duplicate and incubated at 25ºc for 7 days and counted using colony counter.

Statistical analysis:

All data are presented as means± (SE). Growth, hematology and blood chemistry data were analyzed using one way ANOVA, followed by Duncan's multiple range tests which was used to compare differences among individual means, with statistical software ANOVA procedure (SAS, 2004). A probability of 0.05 was utilized to account for the statistical difference between the means. Before the analysis, percentage data were normalized by arcsine-transformation.

RESULTS AND DISCUSSION

Growth performance and feed utilization:

Results in Table 4 showed that *O. niloticus* fed the basal diet supplemented with *S. cerevisiae* (T1) and fish group fed the basal diet supplemented with *L. acidophilus* (T2) showed the highest significant (P<0.05) final body weight (BW), body length (BL), weight gain (WG) and specific growth rate (SGR) compared with fish fed the control diet (T3). The diet supplemented with *S. cerevisiae* (T1) showed the highest significant (P<0.05) BW, WG and SGR when compared with the diet supplemented with *L. acidophilus.* Such increase in the growth in aquatic animals that were fed probiotics supplemented diets may be attributed to the improved digestive activity due to enhancing the synthesis of vitamins and enzymatic activities (Soltan *et al.,* 2016) consequently, improving digestibility and growth performance. Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to increase the growth rate and welfare of farmed aquatic animals (Wang *et al*., 2005; Wang and Xu, 2006; Wang, 2007). Here, for the first time, an enhancement of the growth rate of the tilapia, *O. niloticus*, one of the most important farmed species for the world, was as a result of supplemented the aquaria water with probiotics (Table 4).

Table 4: Effect of probiotics supplemented diets on growth performance and feed utilization of Nile tilapia

Items	Experimental Diets			±SE
	T1	T2	T3	
Body weight (g)	16.57^{a}	15.84^{b}	10.55^{c}	±0.190
Body length (Cm)	9.81^{a}	9.42^{b}	8.48^{c}	±0.050
Condition factor	1.47^{a}	1.45^{a}	1.47^{a}	±0.001
Weight gain (g)	11.93^{a}	10.16^{b}	5.74^{c}	±0.200
Specific growth rate (% day^{-1})	3.60^{a}	3.21^{b}	2.30^{c}	±0.270
Feed intake (g/fish)	21.17^{a}	20.20^{b}	17.50^{c}	±0.100
Feed conversion ratio	1.82^{C}	2.70^{b}	3.31^{a}	±0.010
Protein efficiency ratio	1.52^{a}	1.41^{b}	0.88^{c}	±0.040

Means within the same row sharing the same superscript are not significantly different (P<0.05).

Probiotics have been shown to produce digestive enzymes such as amylase, protease, lipase which may enrich the concentration of intestinal digestive enzymes. In addition, probiotics inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by the competition for nutrients and the alteration of the microbial metabolism (Gatesoupe, 1999). It also improves the nutrition by detoxifying the potentially harmful compounds in

feeds by producing vitamins such as biotin and vitamin B_{12}(Soltan and El-Laithy 2008 and Hassaan *et al.*,2014) who found that supplementation of basal diet with *B. subtilis*, significantly (P<0.001) improved BW, BL, WG and SGR of *O. niloticus*. Similarly, the application of *E. faecium* as a probiotic was found to enhance the growth performance of Nile tilapia, *O. niloticus* (Wang *et al.,* 2008).Al-Dohail *et al.,* (2009) also illustrated that African catfish *Clariasgariepinus* that were fed the *L. acidophilus* showed a better growth performance than the control fish group.

Condition factor of fish is essentially a measure of relative muscle to bone growth and the differing growth responses of these tissues to diet treatment may be reflected by changes in condition factor (Ibrahim *et al.,* 2000, Abou Zead *et al.,* 2008; Soltan *et al.,* 2015).

Results of Table 4 showed that, supplementation of the basal diets with each of *S. cerevisiae* (T1) or *L. acidophilus* (T2) significantly increased feed intake, specific growth rate (SGR), protein efficiency ratio (PER) and improved feed conversion ratio (FCR) compared with *O. niloticus* fed the basal die. 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).

Hematological indices:

Haemoglobin (Hb), hematocrit (Hct) and red blood cells (RBCs) of *O. niloticus* significantly increased when the basal diet supplemented with *S. cerevisiae* (T1) and fish group fed the basal diet supplemented with *L. acidophilus* compared with fish fed the control diet (T3). The diet supplemented with *S. cerevisiae* (T1) showed the highest significant (P<0.05) hematological indices compared with the other experimental diets (T2,T3,T5) (Table 5).

Table 5: Effect of probiotics supplemented diets on hematological indices of Nile tilapia

Treatments	Haemoglobin (g/dl)	Hematocrit (%)	Red blood cells (10^6/mm^3)
S. cerevisiae	8.95^a	30.32^a	2.98^a
L.acidophilus	8.51^a	28.11^b	2.46^b
Control	7.90^b	22.23^c	2.30^b
SE	0.14	0.89	0.09

Means within the same column sharing the same superscript are not significantly different (P<0.05).

Hematology is an important factor that could be considered for the fish diet quality assessment. Ologhobo (1992) reported that one of the most common blood variables consistently influenced by diet is the hematocrit (Ht) and hemoglobin (Hb) levels. On the other hand, *O. niloticus* fed diet supplemented with *B. subtilis*(Soltan and El-Laithy, 2008) or supplemented with *Pediococcus acidilactici* (Ferguson *et al.,* 2010) showed some variation (but not significant) in Hb and Ht content among the control and fish groups fed diet enriched with probiotics. Also, Marzouk *et al.* (2008) reported that both fish groups fed the diet supplemented with dead *S. cerevisiae* and *Bacillus subtilis* showed significant (P<0.05) increase in the Ht level when compared with fish fed the control diet.

White blood cells and differential count:

White blood cells (WBC) count was significantly (P<0.01) increased with each probiotic treatments, and the highest value for WBC count $9.71x10^5$/mm^3 was recorded by fish fed the diet supplemented with the probiotic (*S. cerevisiae*) followed by $9.53x10^5$/mm^3 fish fed the diet supplemented with the probiotic (*L. acidophilus)* while the lowest WBC count $8.41x10^5$/mm^3 was recorded for the control group (fed the basal diet without probiotics (Table 6).

Table 6: Effect of probiotics supplemented diets on hematological indices of Nile tilapia

Treatment	White blood cell 10^5/mm^3	Monocytes (%)	Lymphocytes (%)	Nutrophils (%)	Eosinophils (%)
S. cerevisiae	9.71[a]	27.63[a]	66.53[a]	2.51[a]	3.33[a]
L. acidophilus	9.53[a]	27.66[a]	66.80[a]	2.23[a]	3.31[b]
Control	8.41[b]	29.81[a]	64.10[a]	2.31[b]	3.78[b]
SE	0.23	0.26	0.54	0.99	0.14

Means within the same column sharing the same superscript are not significantly different (P<0.05).

Obtained results are in agreement with those obtained by Zhou *et al.* (2010) who found that the use of *B. coagulans* improved immunity. Also Panigrahi *et al.* (2004) recorded that, in aquaculture the dose of probiotics usually varies from10^6cfu/g feed. The optimum dose of a probiotics can vary with respect to host and also type of immune parameters. Song *et al.*, (2006) recorded high serum lysozyme, phagocytic activity of head kidney leucocytes and complement activities in *O. mykiss* fed for 30 days with *Lactic rhamnosus* strain at 10^{11} cfu /g feed but not at a dose of 10^9 cfu /g feed. Furthermore, there is stimulation of a particular immune response with respect to different tissue/organ. Irianto and Austin (2002) revealed that the feeding of Gram-positive and Gram-negative probiotic bacteria at 10^7 cells/g of feed led to a notably increase in WBC count helps in the nonspecific immunity via neutrophils and macrophages.

Metabolism enzymes

Alanine aminotransferase (ALT) and aspertat aminotranferase (AST) enzymes are important liver enzymes. They indicators for liver health and function through controlling the transferring amino group function of alpha-amino acids to alpha-keto acids. Large amount of ALT and AST are released into animal blood, mostly during liver cell damage.

Results of ALT and AST values as affected by fish fed probiotic (*S. cerevisiae* and *L. acidophilus*) are presented in Table 7 and there were very high significant differences in ALT values between *S. cerevisiae* group and the control group (P<0.001). From the obtained results we noticed that treated diets with probiotic *S. cerevisiae*, or *L. acidophilus* significantly decreased ALT and AST values compared to control group. Soltan and El-Laithy (2008) found that, ALT and AST levels significantly decreased when Nile tilapia fed diets supplemented with probiotics compared to control group. Similarly, Wache¢ *et al.* (2006) observed a decrease in the activity of AST, ALT and lactate dehydrogenase in *O. niloticus* after being fed with diet containing *Pseudomonas spp*. and a mixture of *Micrococcus luteus* and *Pseudomonas spp*.

Table 7: Effect of probiotics supplemented diets on liver enzymes of Nile tilapia

Treatments	ALT(m/L)	AST(m/L)
S. cerevisiae	82.08[a]	15.95[a]
L. acidophilus	81.45[b]	16.81[b]
Control	83.78[b]	19.78[c]
SE	0.34	0.13

Means within the same column sharing the same superscript are not significantly different (P<0.05).

Effect of probiotics (*S. cerevisiae* and *L. acidophilus*) on microbial content in muscles of Nile tilapia

The effect of *S. cerevisiae* and *L. acidophilus* is significant decrease the count of microbial content in muscles of Nile tilapia (Table. 8). The obtained results are in agreement with Kesarcodi-Watson *et al.* (2008). Various ways exist in which probiotics could be beneficial. They can act either singly or in combination**.** Adhesion and colonization of the mucosal surfaces are possible protective mechanisms against pathogens through competition for binding sites and nutrients (Westerdahl *et al.*, 1991). Different lactobacilli have reduced the adhesion of *A. salmonicida, C. piscicola and Yersinia ruckeri* to intestinal mucus from rainbow trout (Balcázar *et al.*, 2006).

Table 8: Effect of probiotics supplemented diets on microbial content in muscles of Nile tilapia

Treatments	Microbial count				
	TPC	TCC	FCC	Sal	TFC
S. cerevisiae	2.26[b]	0.38[b]	0.16[b]	0.11[b]	1.26[b]
L.acidophilus	2.37[b]	0.38[b]	0.15[b]	0.12[b]	1.91[b]
Control	4.21[a]	3.139[a]	1.49[a]	1.21[a]	2.79[a]
SE	0.09	0.12	0.74	0.21	0.18

TPC=Total plate count, TCC=Total coliform count, FCC=Faecal coliform count, Sal=Salmonella count, TFC=Total fungal count. Means within the same column sharing the same superscript are not significantly different (P<0.05).

REFERENCES

Abdel-Hakim, N. F.; Bakeer, M. N. and Soltan, M. A. (2001a). Effect of dietary protein levels on growth performance and pond productivity of Nile tilapia (*Oreochromis niloticus)*, Eel (*Anguilla anguilla*) andGrey mullet *(Mugil cephalus)* reared in polyculture system. Egypt. J. Aqua. Biol. & Fish. 5 (4): 61-85.

Abdel-Hakim, N. F.; Hussein, M. S.; Bakeer, M. N. and Soltan, M. A. (2001b). Effect of protein level and stocking density on growth performance of Nile tilapia (*Oreochromis niloticus)* cultured in tanks. Egypt. J. Nutr. & Feeds, 4:763-780.

AbouZead, M. Y.; Soltan, M. A. and Ibrahim, M. S. (2008). Effect of replacing Soybean meal by sunflower meal in the diets of Nile tilapia, *Oreochromis niloticus*. (L.). Proceeding of the 8th International Symposium on Tilapia in Aqua., 12-14/10/2008, Cairo, Egypt, 787-799.

Al-Dohail, M. A.; Hashim, R. and Aliyu-Paiko, M. (2009). Anaerobic bacteria isolated from Oscars (*Astronotusocellatus*), angel animals: a review. Crit. Rev. Food Sci. Nutr., 43:19-60.

AOAC, (1995). In: Cunni, P.A. (Ed.), Official Methods of Analysis of the Association Official Analytical Chemists, vol. 1, 16thed. AOAC International, Arlington, USA, p. 1298.

Balcázar, J. L., Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. and Muzqui, J. L. (2006). The role of probiotics in aquaculture. Vet. Microbiol., 114:173-186.

Boyd, C. E. (1990). Water quality in ponds for aquaculture.Alabama Agric. Experiment Station, Auburn Univ., Alabama.

Brett, J. R. (1973). Energy expenditure of sockeye salmon, *Oncorhynchusnerka*, during sustained performance. Journal of the Fisheries Board of Canada, 30(12): 1799-1809.

Esiobu, N.; Armenta, L. and Ike, J. (2002). Antibiotic resistance in soil and water environments. Int. J. Environ. Health Res. 12:133–144.

Ferguson, R. M. W.; Merrifield, D. L.; Harper, G. M.; Rawling, M. D., Mustafa, S., Picchietti, S.and Davies, S. J. (2010). The effect of *Pediococcusacidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). J. Applied Microbiology, 109(3): 851-862.

Gatesoupe, F.J. (1999). The use of probiotics in aquaculture. Aqua., 180:147-165.

Gomez-Gil, B.; Roque, A. and Turnbull, J.F. (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aqua., 191: 259-270.

Hassaan, M. S. and Soltan, M. A. (2016). Evaluation of Essential Oil of Fennel and Garlic Separately or Combined with *Bacillus licheniformis* on the Growth,

Feeding Behaviour, Hemato-biochemical Indices of *Oreochromis niloticus* (L.) Fry. J. Aqua., Research & Development.7:3. http://dx. doi. org/ 10.4172/2155-9546. 1000422.

Hassaan, M. S.; Soltan, M. A. and Ghonemy, M. M. R. (2014). Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia *Oreochromis niloticus*. The Egyptian J. Aquatic Research, 40:199-208.

Henry, R.J. (1974). Clinical Chemistry Principles and Techniques, 2nd ed. Harper and Row.Publ, New York, 525.

Herranz, C.; Casaus, P.; Mukhopadhyay, S.; Martínez, J.M.; Rodríguez, J.M.; Nes, I.F.; Hernández, P.E. and Cintas, L.M. (2001). Enterococcus faecium P21: a strain occurring naturally in dry-fermented sausages producing the class II bacteriocinsenterocin A and enterocin B. Food Microbiology, 18:115-131.

Ibrahim, M. K.; Fatma, A. Hafez and Soltan, M. A. (2000). Effect of organic fertilization, supplementary feeding and stocking rate on growth performance of Nile tilapia and silver carp. Egypt. J. Agric. Res. 78 (4):1775-1799.

Irianto, A. and Austin, B. (2002). Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchusmykiss* (Walbaum). J. Fish Dis. 25: 333-342.

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

Kozasa, M., (1986). Toyocerin (*Bacillus toyoi*) as growth promoter for animal feeding. Microbiol. Aliment. Nutr., 4: 121-135.

Martins, M.L.; Nomura, D.T.; Myiazaki, D.; Pilarsky, F.; Ribeiro, K. and Castro, M.P.(2004). Physiological and haematological response of O*reochromis niloticus* (*Osteichthyes: Cichlidae*) exposed to single and consecutive stress of capture. Acta. Sci. Animal. Sci., 26:449–456.

Marzouk, M. S; Moustafa, M. M. and Mohamed, N. M. (2008). The Influence of some probiotics on the growth performance and intestinal microbial flora of *Oreochromis niloticus*. Proceedings of 8th International Symposium on Tilapia in Aqua., Cairo, Egypt, pp. 1059-1071.

Mehisan, M. S.; Gihan M. El-Moghazy.; El-Sayed, A. I.; Iraqi, M. M. and Soltan, M. A. (2015). Role of probiotics in improving growth and immunity and controlling *Aermonashydrophila* in Nile tilapia. Egypt. J. Aqua. Biol. & Fish., 19(4):55-70.

Ologhobo, A. D. (1992). Nutritive values of some tropical (West African) legumes for poultry. J. Applied Animal Research, 2:93-104.

Panigrahi, A.; Kiron, V.; Kobayashi, T.; Puangkaew, J.; Satoh, S. and Sugita, H. (2004). Immune responses in rainbow trout *Oncorhynchusmykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. j. Vet. Immunol. Immunopathol., 102: 379-388.

Reitman, S. and S. Frankel (1957). Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. J. Clinical Pathology, 28: 56-59.

Ring, E. and Gatesoupe, F. (1998). Lactic acid bacteria in fish: a review. Aquaculture 160: 177–203.

SAS (2004).SAS Procedure Guide version 6.12 Ed. SAS Institute Inc., Cary, NC, USA.

Soltan, M. A. and El-Laithy, S. M. M. (2008). Effect of probiotics and some spices as feed additives on the performance and behaviour of Nile tilapia, *Oreochromis niloticus*.Egypt. J. Aqua. Biol. & Fish., 12(2):63-80.

Soltan, M. A.; Fouad, I. and Elfeky, A. (2016). Growth and feed utilization of Nile tilapia, *Oreochromis niloticus* fed diets containing probiotic. Global Veterinaria, 17(5): 442-450.

Soltan, M. A.; Radwan, A. A.; Gomaa, A. H. and Farag, A. M. (2015). Using distillers dried grains as an alternative protein source in Nile tilapia (*Oreochromis niloticus*) feeds. Egypt. J. Aqua. Biol. & Fish., 19(3):23-33.

Song, Z. F.; Wu, T. X.; Cai, L. S.; Zhang, L. J. and Zheng, X. D. (2006). Effects of dietary supplementation with *Clostridium butyricum* on the growth performance and humoral immune response in *Miichthysmiiuy*. J. Zhejiang University Science B, 7(7): 596-602.

Sun, Y. Z.; Yang, H. L.; Ma, R. L. and Lin, W. Y. (2010). Probiotic applications of two dominant gut Bacillus strains with antagonistic activity improved the growth performance and immune responses of grouper Epinepheluscoioides. Fish & Shellfish Immunology, 29(5): 803-809.

Wachec, Y.; Auffray, F.; Gatesoupe, F. J.; Zambonino, J.; Gayet, V.; Labbec, L. and Quentel, C. (2006). Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss*, fry. Aquacult., 258:470-478.

Wang, Y. B. (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeusvannamei*. Aquacult., 269: 259-264.

Wang, Y.B. and Xu, Z. R. (2006). Effect of probiotics for common carp (*Cyprinuscarpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol., 127: 283–292.

Wang, Y. B.; Li, J. R. and Lin, J. (2008). Probiotics in aquaculture: challenges and outlook. Aquacult., 281:1-4.

Wang, Y. B.; Xu, Z. R. and Xia, M. S. (2005). The effectiveness of commercial probiotics in Northern White Shrimp (*Penaeusvannamei* L.) ponds. Fish. Sci., 71: 1034-1039.

Westerdahl, A., Olsson, J.C., Kjelleberg, S. and Conway, P. L. (1991). Isolation and characterization of turbot (*Scophtalmus maximus*) associated bacteria with inhibitory effects against *Vibrio anguillarum*. Applied and Environmental Microbiology, 57 (8): 2223-2228.

Zhou, X.; Tian, Z.; Wang, Y. and Li, W. (2010). Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. Fish Physiol. Biochem., 36(3): 501-509.

ARABIC SUMMARY

الإستجابة الفسيولوجية لأسماك البلطى النيلى المغذاة على علائق تحتوى على البروبيوتيك

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فى تجربة إستمرت لمدة ١٢ أسبوع تم تقييم تأثير نوعين من البروبيوتيك (الخميرة وبكتريا اللاكتوباسلس) حيث تم تكوين ثلاثة علائق متساوية فى محتواها من البروتين (٣٠% بروتين خام) والطاقة (٣٥٠٠ كيلوكالورى/كجم) ثم أضيف إليها الخميرة والبكتريا وتركت العليقة الثالثة بدون إضافات. بعد ذلك تم توزيع أصبعيات أسماك البلطى النيلى (٢,٨ ±٠,٠٥ جرام) على ١٨ حوض زجاجى (١٦٠ لتر) بمعدل ١٥ سمكة للحوض حيث تم إختبار كل عليقة فى ٦ أحواض (مكررات). وكانت الأسماك تتغذى على العلائق المختبرة ٦ ايام أسبوعياً بمعدل ٥% من الكتلة الحية يومياً وحتى إنتهاء فترة التجربة.

فى نهاية التجربة وجد أن الأسماك التى تغذت على العلائق المحتوية علي البروبيوتك (الخميرة أو البكتريا) قد أعطت أفضل مقاييس لوزن وطول الجسم ومعدل النمو والزيادة المكتسبة فى وزن الجسم و كمية الغذاء المأكول ومعدل التحويل الغذائى وكفاءة تحويل البروتين مقارنة بالأسماك التى تغذت على العليقة الضابطة والتى أظهرت أقل مقاييس للصفات سابقة الذكر.

كما أظهرت نتائج التجربة أن الأسماك التى تغذت على العلائق المحتوية البروبيوتك (الخميرة أو البكتريا) قد أعطت أكبر قيم لعدد كرات الدم الحمراء وتركيز الهيموجلوبين والهيماتوكريت وكذلك أقل نسبة نفوق مقارنة بمجموعة الأسماك التى تغذت على العليقة الضابطة. أما بالنسبة لمستوى إنزيمات الكبد فقد اظهرت نتائج التجربة أن الأسماك التى تغذت على العلائق المحتوية البروبيوتك (الخميرة أو البكتريا) قد أعطت أقل قيم لإزيمات الكبد وأقل عدد بكتيرى على السطح وداخل العضلات مقارنة بمجموعة الأسماك الضابطة.