JOURNAL OF THE
ARABIAN AQUACULTURE SOCIETY
Vol. 5 No 2 December 2010

Effect of Different Dietary Probiotics on Growth, Feed Utilization and Digestive Enzymes Activities of Nile Tilapia, *Oreochromis niloticus*

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ABSTRACT

A 60 days study was conducted to determine the effects of four probiotic groups, *Bacillus subtilis* NIOFSD017, *Lactobacillus plantarum* NIOFSD018, a mixture containing bacterial isolates (*B. subtilis* NIOFSD017 & *L. plantarum* NIOFSD018) and a yeast, *Saccharomyces cerevisiae* NIOFSD019, isolated from healthy Nile tilapia, *Oreochromis niloticus* on growth performance, feed utilization and digestive enzymes activities of Nile tilapia. Five different experimental diets were formulated. The control diet had no probiotic supplement, diets (1-3) were formulated to contain 10^7 CFU/g diet from *Bacillus subtilis* NIOFSD017 (D1), *Lactobacillus plantarum* NIOFSD018 (D2), a mixture containing bacterial isolates (*B. subtilis* NIOFSD017 & *L. plantarum* NIOFSD018) (D3), while diet (D4) was formulated to contain 10^4 CFU/g diet of a yeast, *Saccharomyces cerevisiae* NIOFSD019. The present results showed that all the diets containing different probiotic groups significantly ($p<0.05$) improved Nile tilapia growth and feed utilization compared to the control diet. These probiotics with the exception of *S. cerevisiae* improved the fish enzyme activities of amylase, protease and lipase in the gastrointestinal tract. *S. cerevisiae* showed a significantly higher amylase activity than the fish fed control diet; however the protease and lipase activities were not affected. The present results recommend the incorporation of probiotics to Nile tilapia feed as supplements to stimulate fish growth and digestion.

Keywords: B. subtilis, L. plantarum, S. cerevisiae , growth, digestive enzymes, O. niloticus

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 (Fuller, 1992; Rinkinen *et al*., 2003), in recent years, the role of probiotics in nutrition and health of certain aquaculture species have also been investigated and subject of reviews (Gatesoupe, 1999; Verschuere *et al*., 2000; Kesarcodi-Watson *et al*., 2008; Ringø *et al*., 2010; 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 gariepinu* (Al-Dohail *et al*., 2009); Senegalese sole, *Solea senegalensis* (Sáenz de Rodrigáñez *et al*., 2009), tilapia, *O. niloticus* (Lara-Flores *et al*., 2003; El-Haroun *et al*., 2006), Japanese flounder, *Paralichthys olivaceus* (Taoka *et al*., 2006), gilthead seabream, *Sparus aurata* and Seabass, *Dicentrarchus labrax* (Carnevali *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 (Verschuere *et al*., 2000; Balcázar *et al*., 2006; Kesarcodi-Watson *et al*., 2008). In addition, the production of vitamins by these gut microbiota could also increase vitamin synthesis and improve fish health (Holzapfel *et al*., 1998).

Endogenous digestive enzymes in fish have been studied by several workers (Kawai and Ikeda, 1972; Das and Tripathi, 1991; Hidalgo *et al*., 1999; Bezerra *et al*., 2005; Jun-sheng *et al*., 2006; 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 different dietary probiotics groups, *B. subtilis* NIOFSD017, *L. plantarum* NIOFSD018, mixture containing bacterial isolates (NIOFSD017 and NIOFSD018) and *S. cerevisiae* NIOFSD019 on growth performance, feed utilization and activity of digestive enzymes of Nile tilapia, *O. niloticus*.

MATERIALS AND METHODS

The present study was carried out in an indoor laboratory includes ten experimental concrete ponds, Fish farm, Inland waters and fish culture branch, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt.

Experimental design and conditions

One hundred ninety two Nile tilapia (24.55 ± 0.03g) were transferred to a laboratory and acclimated to the basal diet for 14 days. Fish was distributed in five experimental treatments in indoor concrete ponds at a density of 15 fish m^{-3} in duplicate 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 sixty days.

Microbiological aspects of microbial flora isolated from O. niloticus gut

Isolation and selection of some microbial isolates as probiotics (in vitro)

Several microbial species were isolated from intestine of ten apparently healthy *O. niloticus* fish, using the method of Trust and Sparrow (1974). One ml of ten fold dilutions seeded on Tryptone Soya Agar (TSA, 1-200, Scharulu Chemie, Barcelona, Spain), De Man, Rogosa and Sharpe (MRS, 1-135, Scharulu Chemie, Barcelona, Spain) and Yeast Peptone Dextrose Agar (YPD, 1-473, Scharulu

Chemie, Barcelona, Spain). The plates were incubated at 35 ± 2°C for 24 to 72 h based on the microorganisms type. The anaerobic counts were determined by incubated anaerobic agar (1-371, Scharulu Chemie, Barcelona, Spain) plates at 35 ± 2°C for 3 days in anaerobic conditions using anaerobic chamber with 5% CO_2 and relative humidity of 50% (Daboor, 2008). The authenticity of the microbial cultures were verified by sub-culturing several times on the same medium and stored at 4 °C for further use. A common way to select probiotic isolates was carried out according to Vine *et al*. (2004) selecting those has the ability to produce secondary metabolites.

Characterization of some microbial isolates

Three selected colonies NIOFSD017, NIOFSD018 (bacterial isolates) and NIOFSD019 (yeast) were characterized and identified following the criteria described in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994) and in parallel to commercial API 50 CH and API 20 CAUX (Bio-Merieux, Marcy I Etoile, France) for bacteria and yeasts, respectively.

Biomass production

B. subtilis NIOFSD017, *L. plantarum* NIOFSD018 and *S. cerevisiae* NIOFSD019 were grown aseptically in 10 ml of nutrient, MRS and YPD broths for 24 h at 35 ±2°C. Five ml was transferred under aseptic conditions into 250 ml of nutrient, MRS and YPD broth and held on a shaker at 150 rpm for 24-48 h at 35 ±2°C. The cells of each isolate were harvested by centrifugation at 10.000 rpm at 4 °C for 15 min. and washed twice with phosphate buffer (PB) having pH 7.0, then dispensed in 5 ml PB.

Experimental diets

Five experimental isocaloric (4382.76 Kcal/kg/gross energy) and isonitrogenous (30.2 % CP) diet were formulated (Table 1). The control diet had no probiotic supplement. Diets 1-4 were formulated to be D1 (*B. subtilis* NIOFSD017, 10^7 CFU/g) D2 (*L. plantarum* NIOFSD018, 10^7 CFU/g), D3 (mixture of NIOFSD017, $0.5x10^7$ CFU/g and NIOFSD018, $0.5x10^7$ CFU/g) and D4 (*S. cerevisiae* NIOFSD019, 10^4 CFU/g). The dry ingredients were mixed with corn oil and the microbial isolates were incorporated into the feed diet components as shown in Table 1 (Salinas *et al*., 2005). 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%. The diets were then stored in a -15ºC freezer until being used.

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

	Experimental diets [3]				
Ingredients (%)	Control	D1	D2	D3	D4
Fish meal	**30.0**	**30.0**	**30.0**	**30.0**	**30.0**
Soybean meal	**22.5**	**22.5**	**22.5**	**22.5**	**22.5**
Wheat bran	**38.0**	**38.0**	**38.0**	**38.0**	**38.0**
Corn oil	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**
Vitamin and Mineral premix [1]	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**
Starch	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**
Chromic oxide	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
B. subtilis $_{NIOFSD017}$ **CFUg^{-1}**		**10^7**			
L. plantarum $_{NIOFSD018}$ **CFUg^{-1}**			**10^7**		
Mix. of *B. subtilis* $_{NIOFSD017}$ **& *L. plantarum*** $_{NIOFSD018}$ **CFUg^{-1}** *				**10^7**	
S. cerevisiae $_{NIOFSD019}$ **CFUg^{-1}**					**10^4**
Chemical composition					
Moisture (%)			**11.20**		
Crude protein (%)			**30.02**		
Ether extract (%)			**10.35**		
Ash (%)			**11.85**		
Crude fiber (%)			**7.10**		
Nitrogen free extract (%)			**40.68**		
Gross energy (kcal/kg) [2]			**4382.76**		

[1]***Vitamin and mineral premix, each kg of premix contained, vitamin A (4,000,000 IU), vitamin D (666,666.7 IU), vitamin H (3,333.3 mg,), vitamin K_3 (333.3 mg), vitamin B_1 (333.3 mg), vitamin B_2 (1,666.7 mg), vitamin B_6 (500 mg), vitamin B_1 (3.33 mg), pantothenic acid (3,333.3 mg), folic acid (333.3 mg), biotin (16.7 mg) niacin (10,000 mg), iron (10,000 mg), manganese (20,000 mg), copper (1,333.3 mg), zinc (166,666.7 mg), iodine (100 mg), cobalt (33.3 mg) and selenium (33.3 mg).***

[2] ***Gross Energy (kcal/kg) was calculated using a caloric values of 5.65 , 9.45 and 4.2 for crude protein, ether extract and nitrogen free extract according to Hepher et al., (1983).***

[3]*D1:* ***B. subtilis*** $_{NIOFSD017}$*, D2:* ***L. plantarum*** $_{NIOFSD018}$*, D3:*****Mixture of B. subtilis*** $_{NIOFSD017}$***; $0.5x10^7$ CFU g^{-1} & L. plantarum*** $_{NIOFSD018}$***; $0.5x10^7$ CFUg,*** *D4:* ***S. cerevisiae*** $_{NIOFSD019}$

Water quality

Temperature, pH, dissolved oxygen (DO) and ammonia were estimated during the experimental period according to APHA (1995).

Analytical methods

The proximate composition for experimental diets and fish carcass were measured according to AOAC (1990). Moisture content was determined by drying samples at 105°C for 24 h. Crude protein (CP) was determined by a micro kjeldahl method using Kjeldahal distillation unit (UDK 127, Velp Scientifica, Milano, Italy), N × 6.25. Crude lipid was extracted by a Soxhlet apparatus using petroleum ether (60-80°C). Ash content was determined using a muffle furnace (M110; Thermo Scientific Heraeus, Hanau, Germany) at 550°C for 12 h.

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

Digestive enzyme activities

Enzymes were extracted by homogenization the gut in cooled phosphate buffer (pH 7.5) with the ratio 1/10 (w/v) using a hand held homogenizer, centrifugation at 4°C and 5000 rpm for 15 minutes (Huang *et al*., 1999; Yanbo and Zirong, 2006). Protein content of the extract was assayed according to Lowry, *et al*. (1951).

Protease activity was assayed according to Anson (1938) and Folin (1928). Amylase activity was determined based on the method of Smith and Roe (1949). Lipase activity was determined by the titrimetric method (Teitz and Fiereck, 1966; Borlongan, 1990) measuring the fatty acids librated.

Statistical Analysis

Results were expressed as means ± standard deviation (SD). Data were statistically analyzed using ANOVA one-way analysis of variance. Duncan's Multiple comparisons among means were made by Duncan (1955) when significant F- values were observed ($P < 0.05$), using SPSS

17 statistical program for Dalhousie University, Halifax, Canada.

RESULTS

Water quality in all ponds were observed to be normal and remained within ranges allowing for high growth rate and production of Nile tilapia (Table 2). Water temperature was maintained at 28.2 °C, pH ranged from 6.90 to 7.45, total ammonia at 0.065 mg/l to 0.082 mg/ l and DO ranged from 6.05 to 6.62 mg/l.

The growth performance including IBW, FBW, SGR, FI, FCR and survival rate of Nile tilapia are shown in Table 3. No significant differences were observed in IBW among treatments. Fish fed the experimental diets D1, D2, D3 and D4 exhibited higher FBW and SGR compared to control diet. Nile tilapia fed diet containing *L. plantarum* NIOFSD018 (D2) showed the highest FBW (56.05 g) and SGR (1.38 % day^{-1}) compared to 40.63g and 0.84 % day^{-1} for fish fed either control diet or D1 respectively. FI for all treatments was statistically higher than the control group. Results showed that fish fed diets containing different probiotic groups had significantly better FCR than those feed the control diet, meanwhile, no significant differences were observed in FCR values among different probiotics tested and the lowest value (1.84) was recorded for fish feed D2.

PER, PPV and ER were significantly increased by supplementation of various probiotics in experimental diets compared to the control diet. The highest PER (1.81), PPV (29.55%) and ER (18.48%) were recorded for fish fed with D2 (Table 4).

Table 2. ***Water qualities of Nile tilapia aquaria measured during the experiment period*** [1]**.**

	Diets [2]				
Items	Control	D1	D2	D3	D4
Temperature (°C)	**28.20 ± 0.30**	**28.20 ±0.30**	**28.20±0.30**	**28.20 ±0.30**	**28.20 ±0.30**
pH	**7.3 ± 0.31**	**7.31 ±0.10**	**6.9 ± 0.14**	**7.12 ± 0.19**	**7.45 ± 0.13**
Ammonia (mg/l)	**0.082 ± 0.01**	**0.078 ± 0.002**	**0.065 ± 0. 002**	**0.079 ± 0.01**	**0.080 ± .005**
Dissolved Oxygen (mg/l)	**6. 40 ± 0.03**	**6.23 ± 0.03**	**6.05 ± 0.14**	**6.62 ± 0.44**	**6.31± 0.14**

[1] ***Values (Mean ± Standard Deviation) of pH, ammonia and dissolved oxygen were measured at morning while temperature was measured at one o'clock after mid-day.***

[2] ***D1: B. subtilis*** $_{NIOFSD017}$***, D2: L.plantarum*** $_{NIOFSD018}$***, D3: mixture of B. subtilis*** $_{NIOFSD017}$ ***and L. plantarum*** $_{NIOFSD018}$***, D4: S. cerevisiae*** $_{NIOFSD019}$

Table 3. ***Growth performance of Nile tilapia fed with different probiotic groups*** [1]

Items	Diets [2]				
	Control	D1	D2	D3	D4
Initial body weight (g)	24.58 ±0.09	24.56 ±0.05	24.50 ±0.02	24.55 ±0.02	24.58 ±0.01
Final body weight (g)	40.63^{c} ±1.51	50.25^{b} ±1.63	56.05^{a} ±2.18	52.92^{ab} ±1.12	54.50^{a} ±0.95
Specific growth rate(%/day)	0.84^{c} ± 0.07	1.19^{b} ±0.05	1.38^{a} ±0.07	1.28^{ab} ±0.04	1.33^{ab} ±0.03
Feed intake (g)	49.24^{b} ±0.03	55.36^{a} ±1.47	58.13^{a} ±1.96	56.12^{a} ±0.34	57.67^{a} ±0.02
Feed conversion ratio	3.08^{a} ±0.30	2.16^{b} ±0.07	1.84^{b} ±0.07	1.98^{b} ±0..07	1.93^{b} ±0.06
Survival rate (%)	100.0	100.0	100.0	100.0	100.0

[1] ***Values (Mean ± Standard Deviation) in the same row sharing the same superscript are not significantly different (P<0.05).***
[2] ***D1: B. subtilis*** $_{NIOFSD017}$***, D2: L. plantarum*** $_{NIOFSD018}$***, D3: mixture of B. subtilis*** $_{NIOFSD017}$ ***and L. plantarum*** $_{NIOFSD018}$***, D4: S. cerevisiae*** $_{NIOFSD019}$

Whole body composition data are presented in Table 5. The Moisture content showed no significant differences among fish fed the experimental diets and it is ranged from 74.05 to 74.47%. Significantly, the uppermost two values (61.65 and 61.36%) of crude protein were achieved for fish fed diets D1 and D2 with no significant difference. Average fat content of fish fed D1, D2, D3, D4 and the control were 22.16±0.01%, 21.82, 21.77, 21.85 and 22.11% respectively. Body ash content ranged from 15.85 to 16.85 %. Nitrogen free extract was not significantly different among the fish fed various experimental diets and they ranged from 0.34 to 0.50 %. The highest significantly gross energy (2340.92 KJ/100g) of carcass was recorded for Nile tilapia fed D1, meanwhile the lowest gross energy (2309.06^{d}±5.36) was observed in D3.

Table 4. ***Feed utilization values of Nile tilapia fed with different probiotic groups*** [1]

Items	Diets [2]				
	Control	D1	D2	D3	D4
Protein efficiency ratio	1.08^{c} ±0.11	1.54^{b} ±0.05	1.81^{a} ±0.06	1.68^{ab} ±0.06	1.73^{ab} ±0.05
Protein productive value (%)	18.28^{c} ±1.93	26.08^{b} ±0.72	29.55^{a} ±1.11	28.06^{ab} ±0.87	28.29^{ab} ±0.36
Energy retention (%)	11.64^{c} ±1.31	16.39^{b} ±0.43	18.48^{a} ±0.78	17.61^{ab} ±0.48	17.77^{ab} ±0.26

[1] ***Values (Mean ± Standard Deviation) in the same row sharing the same superscript are not significantly different (P<0.05)***
[2] ***D1: B. subtilis*** $_{NIOFSD017}$***, D2: L.plantarum*** $_{NIOFSD018}$***, D3: mixture of B. subtilis*** $_{NIOFSD017}$ ***and L. plantarum*** $_{NIOFSD018}$***, D4: S. cerevisiae*** $_{NIOFSD019}$

Table 5. ***Values of carcass chemical composition of Nile tilapia fed with different probiotic groups*** [1].

Items	Initial	Diets [2]				
		Control	D1	D2	D3	D4
Moisture (%)	75.18 ±0.43	74.35^{a} 0.19	74.28^{a} ±0.11	74.47^{a} ±0.15	74.05^{a} ±0.01	74.29^{a} ±0.24
Crude Protein (%)	59.55 ±0.40	60.79^{b} ±0.09	61.65^{a} ±0.15	61.36^{a} ±0.20	60.85^{b} ±0.004	60.90^{b} ±0.04
Fat content (%)	21.25 ±0.21	22.11^{a} ±0.17	22.16^{a} ±0.01	21.82^{ab} ±0.11	21.77^{b} ±0.20	21.85^{ab} ±0.004
Ash content (%)	18.65 ±0.18	16.72^{a} ±0.05	15.85^{c} ±0.18	16.32^{b} ±0.11	16.82^{a} ±0.16	16.85^{a} ±0.08
Nitrogen Free Extract (%)	0.56 ±0.24	0.37^{a} ±0.01	0.34^{a} ±0.04	0.50^{a} ±0.03	0.48^{a} ±0.15	0.41^{a} ±0.11
Gross Energy (KJ /100g) [3]	2258.86 ± 5.47	2319.36^{bc} ± 4.54	2340.92^{a} ± 3.74	2323.42^{b} ± 0.74	2309.06^{d} ± 5.36	2312.03^{cd} ± 0.78

[1] ***Values (Mean ± Standard Deviation) in the same row sharing the same superscript are not significantly different (P<0.05)***
[2] ***D1: B. subtilis*** $_{NIOFSD017}$, ***D2: L.plantarum*** $_{NIOFSD018}$, ***D3: mixture of B. subtilis*** $_{NIOFSD017}$ ***and L. plantarum*** $_{NIOFSD018}$, ***D4: S. cerevisiae*** $_{NIOFSD019}$
[3] ***Gross Energy (kcal/kg) was calculated using a caloric values of 5.65 , 9.45 and 4.2 for crude protein, ether extract and nitrogen free extract according to Hepher et al., (1983).***

The total (Uml^{-1}) and specific (U mg protein^{-1}) amylase activities of Nile tilapia fed different dietary probiotics were significantly higher than the control diet (Table 6). The highest total amylase activities were recorded for

Table 6. ***Amylase enzyme activity of Nile tilapia fed with different probiotic groups*** [1]

Treatments [2]	Protein content (mg ml^{-1})	Enzyme activity [3]	
		Total (Unit ml^{-1})	Specific activity U (mg Protein)$^{-1}$
Control	12.04±0.59	29.20^{d}±1.61	2.43^{d}±0.13
D1	12.13±0.59	34.28^{c}±3.95	2.83^{c}±0.28
D2	12.01±0.55	41.88^{a}±2.92	3.50^{a}±0.31
D3	12.15±0.69	39.58^{b}±3.85	3.27^{b}±0.36
D4	12.28± 0.52	40.87^{ab}±2.97	3.33^{ab}±0.28

[1] ***Values (Mean ± Standard Deviation) in the same column sharing the same superscript are not significantly different (P<0.05)***
[2] ***D1: B. subtilis*** $_{NIOFSD017}$, ***D2: L.plantarum*** $_{NIOFSD018}$, ***D3: mixture of B. subtilis*** $_{NIOFSD017}$ ***and L. plantarum*** $_{NIOFSD018}$, ***D4: S. cerevisiae*** $_{NIOFSD019}$
[3] ***Amylase unit is defined as the amount of enzyme that hydrolyze 1 mg of starch in 30 minutes,***

Table 7. ***Protease enzyme activities of Nile tilapia fed diets supplemented with different probiotic groups*** [1]

Treatment [2]	Protein content (mg ml^{-1}) µ mol tyrosine		Enzyme activity [3]	
			Total (Unit ml $^{-1}$)	Specific activity Unit (mg Protein)$^{-1}$
Control	12.04±0.59	0.057^{c}±0.0091	3.16^{c}±0.58	0.26^{c}±0.04
D1	12.13±0.59	0.098^{a}±0.0107	5.41^{a}±0.59	0.45^{a}±0.06
D2	12.01±0.55	0.099^{a}±0.0125	5.47^{a}±0.69	0.46^{a}±0.06
D3	12.15±0.69	0.081^{b}±0.0091	4.45^{b}±0.50	0.37^{b}±0.04
D4	12.28± 0.52	0.058^{c}±0.0130	3.17^{c}±0.72	0.26^{c}±0.06

[1] Values (Mean ± Standard Deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: B. subtilis NIOFSD017, D2: L.plantarum NIOFSD018, D3: mixture of B. subtilis NIOFSD017 and L. plantarum NIOFSD018, D4: S. cerevisiae NIOFSD019
[3] Protease activity (units ml^{-1}) = µmole tyrosine equivalent released x11/1x2x10
11= the reaction volume, 1 = extract volume, 2 = volume of sample used in the color reaction and 10 = the incubation time for the enzyme action

fish fed either D2 or D4 respectively (41.88 and 40.87 Uml^{-1}) and the lowest significant values were recorded for the control diet. The same trend was observed for specific amylase activities.

Total and specific protease activities in all experimental treatments are shown in Table 7. The highest protease activity was recorded for fish fed the diets either D2 or D1. The highest specific protease activity was observed for fish fed D2 and D1. Lipase total and specific activity are illustrated in Table 8. The supplementation of different probiotic

Table 8. ***Lipase enzyme activities of Nile tilapia fed diets supplemented with different probiotic groups*** [1]

Treatments [2]	Protein content (mg ml^{-1})	Enzyme activity [3]	
		Fatty acid liberated (ml)	Specific activity Unit (mg Protein)$^{-1}$
Control	12.04±0.59	2.26^{c}±0.34	0.189^{c}±0.033
D1	12.13±0.59	3.19^{b}±0.55	0.264^{b}±0.051
D2	12.01±0.55	4.12^{a}±0.34	0.344^{a}±0.034
D3	12.15±0.69	3.12^{b}±0.46	0.258^{b}±0.046
D4	12.28± 0.52	2.47^{c}±0.31	0.202^{c}±0.029

[1] Values (Mean ± Standard Deviation) in the same column sharing the same superscript are not significantly different (P<0.05)
[2] D1: B. subtilis NIOFSD017, D2: L.plantarum NIOFSD018, D3: mixture of B. subtilis NIOFSD017 and L. plantarum NIOFSD018, D4: S. cerevisiae NIOFSD019
[3] Lipase activities was expressed as the volume of 0.05 N NaOH required to neutralize the fatty acids released during the incubation period

isolates to the diet improved the total and specific activity of lipase of Nile tilapia compared to the control diet and D4. The highest total and specific lipase activity were observed for fish fed D2 (4.12 Uml^{-1} and 0.34 unit mg^{-1} protein).

DISCUSSION

The exact mode of action of the probiotic has not been fully elucidated and there is continuous argue about its effect on the water quality. In the present study, there is no obvious effect of the probiotics added to feeds on water quality, this agrees with the finding of Yanbo and Zirong (2006).

The probiotics supplementation of the experimental diets resulted in higher growth and feed utilization as compared with the control diet. The increase in growth of tilapia by inclusion of *B. subtilis* NIOFSD017 may be due to that most of *Bacillus* spp can produce secondary metabolites which have been used industrially for production of antibiotics, bioinsecticides, fine chemicals and enzymes that readily hydrolyze carbohydrates, lipids and proteins into sugars, fatty acids, peptides and amino acids (Sonnenschein *et al*., 1993; Godfrey and West, 1996; Olmos *et al*., 1998). Similar results were found for common carp, *Cyprinus carpio* (Yanbo and Zirong 2006), red drum, *Sciaenops ocellatus*, (Li *et al*., 2005) and Japanese flounder, *Paralichthys. olivaceus* juveniles (Taoka *et al*., 2006). Whereas, *Bacillus* sp. have been successfully used as a probiotic to enhance growth of these fish.

The results indicate that Nile tilapia fed with *L. plantarum* NIOFSD018 showed significantly better growth as compared to diets supplemented with *B. sabitilus* NIOFSD017 and the control diet. It has been reported that *L. bulgaricus*, *L. acidophillus*, *L. sporogenes*, *L. casei*, *L. plantarum*, and *Streptococcus thermophillus* are effective as probiotics in animal nutrition (Ringo and Gatesoupe, 1998; Jacobsen *et al*., 1999; Venkat *et al*., 2004). The beneficial effects of *Lactobacillus* sp on growth response have been observed in Nile tilapia by Lara-Florest *et al*. (2003), sea bream, *Sparus aurata* (Suzer *et al*., 2008) and European sea bass *Dicentrarchus labrax* (Carnevali *et al*., 2006). In the latter study, the better growth and welfare of *D. labrax* was attributed to a decrease in cortisol level in fish, cortisol production may induce proteolysis that may cause a delay in somatic growth in fish and animals (Anderson *et al*., 1991; Vijayan *et al*., 1997).

Yeasts have been used either as live or processed feed ingredients to improve the performance of fish

(Stones and Mills, 2004). In the present study, the inclusion of *S. cerevisiae* NIOFSD019 in Nile tilapia diets significantly improved FBW, SGR and FCR of fish as compared to the fish fed control diet. Such beneficial effects of yeast have been observed in Nile tilapia and other fish species (Lara-Flores *et al*., 2003; Tovar- Ramı´rez *et al*., 2004; Taoka *et al*., 2006). The positive effects of yeast may be due to some unidentified growth factors in the yeast that elicit a response at low concentrations. The ability of yeast, *S. boulardii, S. cerevisiae* and *D. hansenii,* CBS 8339 to secrete polyamines such as putrescine, spermidine and spermine (Tabor and Tabor, 1985; Buts *et al*., 1994; Tovar-Ramı´reza *et al*., 2002) have been linked as essential growth factors (Bardócz, 1993). Those polyamines play a fundamental role in proliferation, rapid growth and regeneration of tissues (Peulen *et al*., 2002). It is possible that polyamine production by yeasts may partly explain its benefic effects on growth of Nile tilapia in this study. It appears that beneficial effects of yeast on growth of fish are associated with the contribution of additional essential nutrients to their diets and better digestibility of macronutrients from feed ingredients by establishment of favorable microbiota in fish gut.

The improvement of feed utilization for fish fed diet supplemented with *L. Plantarum* NIOFSD018 could be due to improvement in intestinal microbial flora balance which in turn will lead to better nutrient digestibility, higher-absorption quality, increased enzyme activities (Tovar-Ramı´reza *et al*., 2002; Lara-Flores *et al*., 2003; Balcázar *et al*., 2006; Waché *et al*., 2006; Al-Dohail *et al*., 2009) and also more degradation of higher molecular weight protein to lower molecular weight peptides and amino acids (De Schrijver and Ollevier, 2000). These contribute towards optimizing use of protein for growth that will result in more efficient protein in fish diets. It appears that, after the passage of probiotic through the stomach into the intestine where sugars (carbohydrates) are utilized for the growth of microorganisms and they also produce several digestive enzymes (El-Haroun *et al*., 2006). That will result in higher growth and feed efficiency, prevention of intestinal disorders and pre-digestion of antinutritional factors present in the feed ingredients (Smoragiewicz *et al*., 1993; Clements, 1997; Thompson *et al*., 1999; Verschuere *et al*., 2000). Moreover, the nutritional benefits of probiotic bacteria have been attributed to synthesis of B vitamins and short chain fatty acids in the intestine and the

higher availability trace elements (Holzapfel *et al*., 1998).

Digestive enzymes are one of the most important factors that influence the efficiency of feed utilization in fish and characterization of these enzymes provides some information regarding the digestive capacity of fish to hydrolyze carbohydrate, protein and lipid of feed ingredients (Lemieux *et al*., 1999). However, information regarding the extracellular enzymes produced by intestinal bacteria and their biochemical significance is limited (Bairagi *et al*., 2002).

In the present study, diets containing different probiotics have appeared to improve the digestion of protein, starch and fat that could be due to higher level of enzymes activities, which may explain the better growth and feed utilization. The addition of probiotic as live supplements in the diet allows probiotic to survive passage through the intestinal tract (Fuller, 1992). Microorganisms and their enzymes have an important role in the digestion process (Munilla-Moran *et al*., 1990) by increasing the total enzyme activity of the gut (Ding *et al*., 2004; Ziaei-Nejad et *al*., 2006) and stimulating the production of endogenous enzymes (Ochoa-Salano and Olmos-Soto, 2006; Wang, 2007) which in turn can increase the food digestibility. In addition, the exogenous enzymes have a broader pH range than endogenous enzymes that prolongs the digestion period and may allow better hydrolysis of substrates. As pointed by several authors the digestive enzymes (amylase, protease and lipase) could be improved by administration of probiotics to the diet (Ziaei-Nejad *et al*., 2006; Taoka *et al*., 2007; Wang, 2007; Gomez *et al*., 2008).

The present study showed that the highest levels of amylase, protease and lipase have been recorded for Nile tilapia fed *L. plantarum* NIOFSD018. This may be attributed to the higher ability of *Lactobacillus* sp to secrete a wide range of exoenzymes or enhance the activities of endogenous digestive enzymes Suzer *et al*. (2008). However, it is difficult to distinguish between endogenous enzymes produced by fish and exogenous enzymes synthesized in the gastrointestinal tract by the probiotics.

CONCLUSION

The present investigation showed a significant improvement of the growth and the digestive enzymes (amylase, protease and lipase) activities by the administration of probiotics to Nile tilapia diet as compared to the control. Unlike *B. subtilis* NIOFSD017, *L. plantarum*

NIOFSD018 or the mixture of NIOFSD017 & NIOFSD018, *S. cerevisea* NIOFSD019 significantly increased the activity of amylase but not protease or lipase enzymes. The present results recommend the incorporation of probiotics to Nile tilapia feeds as supplements to stimulate fish growth and digestion. The beneficial effects of probiotics on fish growth appears to be associated with colonization of favorable microbiota in the gut which produce enzymes that hydrolyze complex molecules, facilitate better digestion and absorption of macronutrients resulting in higher protein and energy retention in the body.

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تاثير بروبيوتكس مختلفة على النمو،الاستفادة من الغذاء وانزيمات الهضم للبلطى النيلى، ايروكروماس نيلوتيكس

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اجريت هذة الدراسة لتحديد اثر محتوى العلائق من انواع مختلفة من البروبيتوكس المعزولة من اسماك البلطى النيلى على النمو و الاستفادة من الغذاء وايضا انزيمات الهضم للبلطى النيلى. تم تغذية البلطى النيلى فى هذه الدراسة على خمسة علائق مختلفة (اربعة علائق مختبرة و عليقة كنترول) حيث اشتملت العلائق المختبرة على اربعة انواع من البوبيوتكس و هي: باسيلس ستلس NIOFSD017، اللاكتوباسليس بلانترم NIOFSD018 وخليط من كلا النوعين، بالاضافة الى نوع خميرة السكروميسيس سيرفيسيا NIOFSD019. و ذلك بمعدل اضافة 10 مليون خليه لكل جرام عليقه فى حاله البكتريا و10 الاف خليه من الخميره لكل جرام عليقة ، اما عليقه الكنترول فلم تحتوى على اى نوع من الكائنات الدقيقه.

تم تسكين اسماك البلطي (24.55 ±0.03جم) فى احواض اسمنتية وتغذيتها لمده 60 يوما ومع نهايه فترة التجربه تم قياس معدل النمو، معدل التحول الغذائى، معدل كفاءة البروتين، القيمه الانتاجية للبروتين، الطاقه المحتجزة بالاضافة الى قياس انشطة انزيمات الهضم ممثلة فى الاميلاز، البروتيز واللايبيز. و قد اشارت النتائج ان التغذية بالعلائق التى تحتوى البروبيوتكس المختلفة عملت على تحسين النمو و معدل الاستفادة من الغذاء بصورة معنوية مقارنة بالاسماك التى تم تغذيتها بالعليقة الخالية من البروبيوتكس (الكنترول). اوضحت النتائج وجود زيادة معنوية فى انشطة انزيمات الاميلاز، البروتيز واللايبز بالتغذية على العلائق المحتوية على البروبيوتك بكتريا بينمنا العليقة المحتوية على الخميرة فلم يكن هناك تاثير معنوى خاصة بالنسبة لانزيمى البروتيز والليبز على الرغم من انها ادت الى تحسين انزيم الاميلاز مقارنة بالنتائج التى تم الحصول عليها من الاسماك المغذاة بعليقة الكنترول. وخلصت الدراسه الى ان تناول اسماك البلطى النيلى لعلائق تحتوى على بروبيوتكس له تأثيرات ايجابيه على تحسين النمو وزيادة انزيمات الهضم مما يؤدى الى زيادة استفادة الاسماك من الغذاء