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EVALUATION OF PROBIOTIC STRAIN *SACCHAROMYCES CEREVISIAE* AS A FEED SUPPLEMENT IN NILE TILAPIA (*OREOCHROMIS NILOTICUS* LIN.*)* DIETS

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Abstract

A 70 days feeding trial was carried out to evaluate the effects of *Saccharomyces cerevisiae* as a probiotic (Tonilisat) in Nile tilapia diets (0.00, 0.05and 0.10 %) each with three different sizes of Nile tilapia (0.5, 10 and 20 g/fish) on the growth performance, survival rate, feed efficiency, body chemical composition, blood parameters and economic efficiency of mono-sex Nile tilapia (*Oreochromis niloticus Lin*.) fed on three isonitrogenous, iso-caloric diets (30% Crude protein and 444.58 kcal GE/100g) experimental diets each with three fish sizes each in three replicates, which 15 mono-sex Nile tilapia were stocked in each aquarium (100 l/aquarium). Then, 27 experimental aquaria were used for 9 treatments as a feeding trial. The obtained results showed that the growth performance of fish fed on 0.0 Tonilisat level was the lowest value than fish fed 0.05 or 0.1% Tonilisat. Also the fish weighed 0.5 g /fish fed on diet supplemented with Tonilisat had the best growth performance. The diets containing different probiotic levels significantly ($P<0.05$) improved Nile tilapia growth and feed utilization compared with free supplemented diets. The present results recommend the incorporation of 0. 5g probiotic/ kg in Nile tilapia diet as supplement to stimulate fish growth with no adverse effects on the blood hematological parameters. These results suggested that *Saccharomyces cerevisiae* as a probiotic (Tonilisat) in Nile tilapia fry diets can provide beneficial effects on growth, feed utilization and stimulate health statue. The inclusion of the commercial probiotic Tonilisat at 0.5g Kg^{-1} diet at stocking density rate of 15 fish/m^2 of mono-sex Nile tilapia *(O. niloticus* Lin.) is economically to get the best fish performance with friendly effects on the environment.

KEY WORDS: Nile tilapia, growth performance, probiotic, chemical composition and blood hematological parameters.

INTRODUCTION

Tilapias are the third most important cultured fish group in the world, after carps and salmonids. Tilapia culture is also one of the fastest growing farming activities, with an average annual growth rate of 13.4% during 1970–2002. They are widely cultured in about 100 countries in the tropical and subtropical regions. Nile tilapia is an economically important cultured species in several areas of the world (El-Husseny *et al*., 2007). Nile tilapia is an economically important cultured species in several areas of the world (El-Saidy and Gaber, 2005 and El-Husseny *et al*., 2007). Egypt made an impressive increase in aquaculture tilapia production, from 24916 in 1990 to 504000 ton in 2010 accounting for 80% of Egyptian total fish production (1000000 ton year^{-1}) according to General Authority for Fish Resources Development, (GAFRD, 2010).

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). 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); tilapia, *O. niloticus* (El-Haroun *et al*., 2006), Japanese flounder, *Paralichthys olivaceus* (Taoka *et al*., 2006), gilthead sea bream, *Sparus aurata* and sea bass, *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 increase the nutrient digestibility and feed utilization (Verschuere *et al*., 2000; Balcázar *et al*., 2006 and 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 (Bezerra *et al*., 2005; Jun-Sheng *et al*., 2006 and Chan *et al*., 2008). However, information regarding the enzyme producing intestinal bacteria, their source and their effect on fish digestion and metabolism is scarce. Practical feeds for grow out of tilapia usually contain 25 to 35% CP. However, it has been reported that the dietary CP requirements of fish vary with species, size or age, protein quality, dietary energy level, water quality, feeding and culture management. Meyer and Pera (2001) indicated that tilapia efficiently utilize dietary protein at level between 25 and 35%. The same authors added that, the fish were less efficiently at utilizing 45% crude protein in the diet for growth. The present study was designed to evaluate the effects of different dietary probiotics *(S. cerevisiae) levels* on the growth performance, survival rate, body chemical composition, feed efficiency, blood hematological and economic efficiency parameters of mono-sex Nile tilapia (*Oreochromis niloticus Lin*.) fed on the experimental diets.

MATERIALS AND METHODS

The experiment was conducted for 70 days, using a total number of 405 mono-sex Nile tilapia (*Oreochromis niloticus Lin.) which were*

divided into three average weight groups (0.5, 10 and 20 g/fish) obtained from Abbssa Fish Hatchery, Egypt. The fish were distributed at random into nine experimental dietary treatments, each in three aquaria as replicates in which fish were stocked at a rate of 15 fish/ aquarium. Glass aquaria sized 100l each were used to stock Nile tilapia (*O. niloticus* Lin.) which acclimatized to the lab conditions for 2 weeks. Then, 27 experimental aquaria were used for 9 treatments as a feeding trial. Each aquarium was supplied with an air pump contacted with two air stones for aeration. Tap water has been stored 24 hours in fiberglass tank for dechlorination before filling the aquaria after replacing at 100% of water daily. Water temperature (via a thermometer) was daily measured and the average was between 27.5and 28.5^{o}C during the experimental period. The pH values were measured weekly (using Jenway Ltd., Model 350-pH-meter), ammonia were estimated during the experimental feeding period according to APHA (1995) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter). .

Experimental diets and design:

Commercial probiotic was used to study its effects on the growth performance of Nile tilapia fingerlings fed diets at different levels of 0.00, 0.05 and 0.10%. Probiotic (Tonilisat) is a dried yeast fermentation product as growth promoter containing *Saccharomyces cerevisiaa* as an active live yeast 8000 million cells /g with vitamin B_{12} and healthy for fish. Three basal diets were formulated to contain isonitrogenous, iso-caloric diets (30% Crude protein and 4445.8 kcal GE/100g). Nile tilapia (*Oreochromis niloticus Lin*.) fed on three experimental diets each in three replicates (100l aquaria) in which 15 mono-sex Nile tilapia were stocked in each aquarium during the experimental period (70 days). Feeding level of all experimental diets 5% of the total biomass of the fish per day. The amount of feed was divided into four equal portions and distributed by hand in one side of the aquaria four times daily at 9 am, 11 am, 1 pm. and

3 pm. Every fourteen days, the fish in each aquarium were weighed and the amount of feed was readjusted according to the new fish biomass (El-Banna, 1991).

The ingredients were obtained from local market, finely ground, weighed according to their percentage and mixed together then 30% boiled water was added to each diet to be easily pelleted by pressing through 0.2mm for fry and 0.5 mm diameter for fish weighed 10 and 20g/fish by pelleting unit. The pellets were dried in a drying oven at 60 ^{o}C for 24 hours and stored at – 4 ^{o}C until use during the trial to avoid oxidation and rancidity. After running the feeding experiment, proximate chemical analysis of the dietary ingredients, experimental diets and whole fish at the start and at the end of the feeding trial (10 weeks) were made according to A.O.A.C (1990) methods. The dietary ingredients and their composition are shown in Table 1.

Table 1. Formulation and chemical composition of the experimental diets (% on dry matter basis).

Ingredients (%)	Experimental diets		
	D1	D2	D3
Fish meal	10.0	10.0	10.0
Soybean meal	43.0	43.0	43.0
Corn gluten	18.0	18.0	18.0
Wheat bran	14.0	14.0	14.0
Wheat meddling	8.0	8.0	8.0
Fish oil	1.0	1.0	10
Corn oil	2.0	2.0	2.0
Vit.& Min. premix [1]	2.0	2.0	2.0
Starch	2.0	1.95	1.90
Tonilisat	0.0	0.05	0.10
Total	100	100	100
Chemical composition of the experimental diets			
Experimental diets	**D1**	**D2**	**D3**
DM	91.5 4	91.21	91.53
CP	30.12	30.21	30.24
EE	6.08	6.11	6.14
CF	5.12	5.25	5.33
Ash	5.92	6.08	6.12
NFE[2]	52.76	52.25	52.17
Total	100	100	100
GE(kcal/100g)	446.45	445.67	445.28
P/ E ratio	67.47	67.79	67.91

1 Eco Vit, Egyptian veterinary products and feed additives Co., Damyatta, Egypt. The vitamins and minerals premix provided the following per Kg of experimental diet: 15 000 IU, 0.7 g, 15 000 IU, 2 mg, 2.5 mg,2 mg, 10 mg, 3 mg, 5 mg, 2 mg, 2 mg, 5.5 mg, 200 g, 90 g, 40 g,2.5 g, 48 g, 3.6 g, 23.5 g, 8 g, 450 mg, 200 mg and 20 mg of vitamin A, vitamin C (Stay C_, 35% active), vitamin D_3, vitamin E, vitamin B_2,vitamin K_3, nicotine amide, vitamin B_6, vitamin B_{12}, vitamin B_1, folic acid, Ca-D-pantothenate, calcium, phosphate, sodium, copper, magnesium, manganese, zinc, iron, cobalt, iodine and selenium, respectively.

2-Nitrogen-free extract (calculated by difference) = 100- (protein+ lipid+ ash+ fiber).

Gross energy (GE) was calculated according to NRC (1993) as follow: 5.65, 9.45, and 4.11 kcal/g factors for protein, lipid, and carbohydrates, respectively.

The growth performance and feed utilization parameters:

The growth performance parameters including body weight (B.W.), body weight gain, specific growth rate (SGR), relative growth rate, condition factor (K), survival rate. To determine the growth response of the fish, the following parameters were calculated; Average weight gain (AWG) = W2 – W1, Specific growth rate (SGR) = [ln Wf - ln Wi] x 100/experimental period (d) Where Wf = Final average weight at the end of the experiment, Wi= Final average weight at the beginning of the experiment, Loge =Natural logarithm reading and Time = Number of days for the experiment (91 days). , Relative growth rate (RGR) = [final weight - initial weight/ initial weight] x100, K= Condition factor (W/L^3 x100) and Survival Rate (%) = (number of fish that survived / total number of fish stocked) X 100.

Feed utilization values including feed intake, dry matter intake, protein intake, feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) were measured and calculated as follow: Feed conversion ratio (FCR) = feed intake (g)/live weight gain (g), feed efficiency ratio (FER) = live weight gain (g) / feed intake (g) and protein efficiency ratio (PER) = live weight gain (g)/protein intake (g)

Blood parameters determination:

At the end of the experiment, fish in each aquarium were weighed and 5 fish were taken randomly for blood sampling. Fishes were sampled randomly from each treatments and the blood were taken from the caudal

vein of anaesthetized fishes (MS-222, Ethyl 3-aminobenzoate methane sulfonate, Tricaine; Sigma; 1:2500) by sterile syringe containing anticoagulant solution. Adequate amounts of whole blood in small plastic vials containing heparin (anti-coagulated blood) samples were performed immediately for counting red blood cells. Then, the blood samples were centrifuged at 3500 rpm for 15min to obtain blood plasma for determination of total protein and albumin, (globulin by differences) using commercial kits and spectrophotometer (model 5010, Germany).

Hematological parameters:

At the end of the experiment, blood samples were collected from the fish caudal peduncle of the different groups. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination red blood cells count (RBCs$\times 10^6$/mm), then counted on an A0 Bright –Line Haemocytometer model (Neubauer improved, Precicolor HBG, Germany).

Biochemical parameters:

Total plasma protein (g/l):

It was determined by using a commercial kit (Spain React Company, Spain) according to the method recommended by Gornall *et al.* (1949)

Plasma albumin (g/l):

It was determined by using a commercial kit (Spain React Company, Spain) according to the method recommended by Weichsebum (1976).

Plasma globulin (g/l):

It was calculated by subtracting albumin from total plasma protein concentration according to Doumas and Biggs (1976), and

albumin/globulin (A/G) ratio were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China).

Immunostimulant determination:

Respiratory burst (NBT activity by spectrophotometer assays):

Fifty micron of the blood sample was kept in a micro-titer plate well, and then an equal amount of 0.2% NBT solution was added. Incubation for30 min. at room temperature was carried out 0.05 ml of the NBT-blood cell suspension was taken and added to a glass tube containing 1 ml N, N-diethyl formalin (DMF) (Sigma Chemical Co.). Glass tube was centrifuged for 5 min. at 3000 rpm. Supematant was taken and read in spectrophotometer at 540 nm. Values of the extinction here were transposed according to a standard curve into mg of NBT/l ml of blood. Extinction reading x 4 = mg NBT formazan/ml of blood according to Siwicki (1989).

The economical efficiency:

The economical efficiency of dietary treatments was calculated to estimate the cost of feed needed to produce one kg of fish weight gain. The cost of experimental diets and fish has been calculated in L.E. according to the local market prices at year 2010.

Statistical analysis:

Growth performance, feed efficiency, body chemical compassion and blood hematological and biological parameters were statistically compared using SPSS (1997) for two-way analysis of variance. When F-test was significant, least significant difference was calculated according to Duncan *(*1955).

RESULTS AND DISCUSSION

All the water quality parameters were within the accepted ranges for Nile tilapia (*Oreochromis niloticus Lin.*). During the experimental

period, the water temperature ranged from 27.5 to 28.5°C, dissolved oxygen from 6.7 to 7.2 mg/l, total ammonia was <0.03mg/l and pH ranged from 6.2 to 7.2. The results are similar to those obtained by Abdel-Hakim *et al*. (2002). 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) who found that, there were no adverse effects on water quality criteria among all experimental treatments On the other hand, improved water quality has especially been associated with *Bacillus sp*. Since gram-positive bacteria are better converters of organic matter back to CO_2 .During the production cycle, high levels of gram-positive bacteria can be minimizing the buildup of dissolved and particulate organic carbon. It has been reported that use of *Bacillus sp*. improved water quality, survival and growth rates and increased the health status of juvenile *Penaeus monodon* and reduced the pathogenic vibrios (Dalmin *et al*., 2001).

The proximate composition of the experimental diets (Table 1) showed that the dry matter, ash, lipid and protein contents of the diets were closely related. Several factors including fish size or age, dietary protein source, energy content, water quality and culture conditions have been reported to affect protein requirements of tilapia. For example, many studies indicated that protein requirement for maximum performance of tilapia during larval stages is relatively high (35 - >50%), for tilapia juveniles, the protein requirement ranges from 30-40%, while adult tilapia requires 20-30% dietary protein for optimum performance (El-Sayed and Teshima, 1992). Fish meal and soybean meal were used in the experimental diets, that the protein requirement of a fish could be less than when only soybean meal is used. Shiau and Lan (1996) also reported that different protein sources can affect the protein requirement of a fish. However, it is interesting to note that the growth, feed efficiency and

protein efficiency ratio of the fish fed combined fish meal and soybean meal compare very well with the values obtained from feeding the fish with only soybean meal, an indication that soybean meal alone could meet the protein need of the fish, judging by 30-55% protein requirements for fishes (NRC 1993). This supports the fish as omnivorous herbivore that feeds very low in the food chain, and therefore may require more of plant feedstuffs than animal protein.

The effects of probiotic supplementation on growth performance and feed utilization of Nile tilapia groups are summarized in Table 2 and 3, respectively. The initial live body weight in each group used was almost similar, which confirmed appropriate randomization process.

Meanwhile, it created suitable condition to appraise the effect of dietary treatments on the growth performance of fish. Results showed that the diet contained 0.05 % probiotic as supplementation gave better ($P<0.05$) values for final body weight of fish (10 and 20g/fish) when compared with those containing 0.1% and 0.0%, regarding the tested probiotic levels, results showed that adding either Tonilisat at 1g/kg or 0.5gram per kg Nile tilapia (0.5g/fish) diets recorded significantly ($P<0.05$) higher values compared to the groups fed on diets contained 0.0 % of probiotic. The highest survival rates were recorded with the 0.1 % level of probiotic in fish diets which stocked at lower fish sizes during the feeding experiment (9Table 1). The results indicated relationship between survival rate and Tonilisat levels. But, the differences were not significant ($P>0.05$). The survival rates were not affected significantly with fish sizes suggesting that when fish were small sized, there was no competition for food and space. Moreover, there was a good fish survival at 0.5, 10 and 20 g/fish sizes, respectively. Whenever fish attained larger size indicates the amenability of this fish to the intensive culture practice. The present study concomitant with Huang and Chiu (1997) who

mentioned that survival of Nile tilapia had not been affected significantly by fish density.

Table 2. Effect of dietary supplementation of Tonilisat levels on the growth performance of all male Nile tilapia.

Items	Treatments								
	0.5g/fish			10 g/fish			20g/fish		
Tonilisat levels	T1 0.0%	T2 0.05%	T3 0.1%	T4 0.0%	T5 0.05%	T6 0.1%	T7 0.0%	T8 0.05%	T9 0.1%
Initial weight g/fish	0.55	0.56	0.55	10.23	10.55	10.34	20.73	20.40	20.54
Final weight (g/fish)	10.24^{b}	14.58^{a}	14.94^{a}	35.43^{c}	42.67^{a}	41.46^{ab}	60.46^{c}	70.15^{a}	68.41^{ab}
AWG g/fish	9.69^{b}	14.02^{a}	14.39^{a}	25.20^{b}	32.12^{a}	31.12^{a}	39.73^{c}	49.75^{a}	47.87^{ab}
SGR (%/day)	3.49b^{c}	3.89^{ab}	3.94^{a}	1.48^{ab}	1.66^{a}	1.65^{a}	1.20^{b}	1.45^{a}	1.41^{a}
RWG (%)	1761.81^{c}	2503.57^{b}	2616.0^{a}	246.33^{b}	304.45^{a}	302.0^{4a}	191.65^{c}	243.87^{a}	233.06^{b}
K (%)	1.33^{c}	1.54^{ab}	1.59^{a}	1.40^{b}	1.60^{a}	1.61^{a}	1.46^{b}	1.63^{a}	1.62^{a}
SR (%)	83.30^{b}	93.33^{a}	93.67^{a}	86.89^{b}	91.11^{a}	91.45^{a}	86.67^{b}	89.45^{a}	89.45^{a}

a-c: Means in the same row with different letters are significantly different ($P \leq 0.05$).

Improved growth performance in tilapia fed probiotic diets has been reported by many researchers. Tilapia fed *S. cerevisiae* (Lara-Flores *et al*.,2010), *B. subtilis + S. cerevisiae* (Lara-Flores *et al*., 2003*), Micrococcus luteus* (El-Rhman *et al*., 2009*), Bacillus subtilus, Lactobacillus plantarum, B. subtilis + L. plantarum,* (Essa *et al*., 2010*), Lactobacillus acidophilus, Streptococcus faecium* (Lara-Flores *et al*., 2010), the commercial probiotic mixtures Organic Green® (Aly *et al*., 2008), Biogen® (El-Haroun *et al*., 2006 and Mehrim, 2009), and Premalac® (Ghazalah *et al*., 2010) have all shown to increase growth performance in tilapia. However, other researchers reported no effect of some dietary probiotics on growth. Non-viable *S. cerevisiae* (Marzouk *et al., 2008), Pseudomonas spp.* (El-Rhman *et al.,* 2009) and *P. acidilactici* (Bactocell PA10 MD®), viable *S. cerevisiae* (Levucell SB 20®) (Shelby

et al., 2006) have shown no affect on growth of tilapia. Although improved growth has been linked to the production of digestive enzymes stimulated by probionts as reported earlier, metabolite production and improved nutrient utilization may also be responsible for improved feed efficiency and growth performance in tilapia fed probiotics. In most cases, the mechanism for improved growth performance is not known or reported. It is difficult to draw concrete conclusions and provide specific recommendations on the effects of dietary probiotics on growth performance of tilapia given that the studies vary widely with regard to fish age and size, stocking density, diet composition, dietary probiont concentration, feed allowances, feeding duration, and of course, type and source of probiont (Welker and Lim., 2011).

The results (Table 2) indicated a positive acceptable effect of the used probiotic mainly *Saccharomyces cerevisiaa* (yeast) in Nile tilapia diets. Abd El-halim *et al*. (1989) found that the addition of living yeast in diet improved the performance of *O. niloticus*. Also, Scholz *et al*. (1999) reported that *S. cerevisiae* improved the growth and survival of sea bass fry. They attributed this action to adherence of *S. cerevisiae* cells to the gut and the secretion of amylase enzymes which shared in the increased digestibility of the diet. On the other hand, the increased growth performance of *O. niloticus* treated with commercial products Megalo and Diamond-V yeast containing living *S. cerevisiae* with *B. subtilis* and dead *S. cerevisiae* respectively could be also attributed to the inhibition of some intestinal bacterial flora and increasing the non-specific immunity of the treated *O. niloticus*.

Feed utilization results (Table 3) indicated that, diets supplemented with 0.1 %Tonilisat (tested probiotic) had recorded significantly better values than the corresponding diet without supplementation. These results may be explained that probiotic (Tonilisat) optimizing protein utilization for Nile tilapia growth. The best

values of live body weight, FCR and PER were obtained with 0.1% diets followed by fish fed on diets contained 0.05% probiotic. The lowest values of final body weight, body weight gain, SGR, survival rate and feed efficiency were recorded by fish fed on diets without supplementation of probiotic. The best FCR values were observed with probiotic-supplemented diets suggested that, the addition of probiotics improved feed utilization, in practical terms this means that probiotic used can decrease the amount of feed necessary for fish growth which could result in production cost reduction (Table, 3). Similar results have been reported by Lara-Flores *et al*. (2003). Also, Laxmi Prasad *et al*. (2012) reported that, the best growth performance and feed efficiency was obtained in *Macrobrachium rosenbergii* postlarvae fed on diet supplemented with 0.5% *Lactobacillus sporogenes* containing 1.67 x 10^5 colony forming units/100 g feed.

The PER results (Table 3) indicated that supplementing diets with probiotic significantly improved protein utilization in tilapia. This contributes to optimizing protein use for growth which is the most expensive feed nutrient. The improvement in the biological value of the supplemented diets in these treatments with high population demonstrated that the probiotic supplements performed more efficiently in stress situations. This agrees with the results obtained by Ringo and Gatesoupe. (1998).

The improvement of feed utilization for fish fed diet supplemented with Tonilisat 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 and 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, 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 predigesting of anti- nutritional factors present in the feed ingredients (Smoragiewicz *et al*., 1993; Clements, 1997; Thompson *et al*., 1999 and Verschuere *et al*., 2000). These positive effects in fish growth performance may be related with supplementation of commercial and natural probiotic Biogen®, which can enhance the metabolism and energy of fish body cells, raise the efficiency of feed utilization, increase the palatability of feed, promote the secretion of digestive fluids and stimulate the appetite (Mehrim, 2001).

Table 3. Effect of different levels of dietary protein and supplementation of Tonilisat on the feed utilization of all male Nile tilapia.

Items	**Treatments**								
	0.5g/fish			**10 g/fish**			**20g/fish**		
Feed efficiency	**T1 0.0%**	**T2 0.05%**	**T3 0.1%**	**T4 0.0%**	**T5 0.05%**	**T6 0.1%**	**T7 0.0%**	**T8 0.05%**	**T9 0.1%**
Feed intake g/fish	16.47^{b}	20.84^{a}	21.54^{a}	57.37^{c}	64.15^{a}	61.08^{ab}	95.62^{b}	97.52^{a}	91.79^{c}
DM intake g/fish	15.12^{c}	19.00^{b}	19.67^{a}	52.52^{c}	58.51^{a}	55.91^{ab}	88.11^{b}	89.84^{ab}	84.60^{c}
Protein intake g/fish	6.04^{b}	7.59^{a}	7.87^{a}	15.81^{c}	17.78^{a}	16.91^{ab}	22.92^{a}	22.77^{ab}	21.40^{bc}
FCR	1.70^{a}	1.487^{b}	1.497^{c}	2.277^{a}	2.000^{b}	1.963^{bc}	2.407^{a}	1.973^{b}	1.930^{b}
FER	0.588^{b}	0.673^{a}	0.669^{a}	0.439^{b}	0.501^{a}	0.509^{a}	0.416^{b}	0.507^{a}	0.519^{a}
PER	1.604^{b}	1.847^{a}	1.828^{a}	1.340^{b}	1.807^{a}	1.840^{a}	1.733^{b}	2.185^{a}	2.237^{a}

a-e: Means in the same row with different letters are significantly different ($P \leq 0.05$).

The obtained results (Tables 2 and 3) could be attributed to the ability to adhere to the intestinal mucosa of *O. niloticus* producing a wide

range of relevant digestive enzymes (amylase, lipase and protease) which have the ability to denaturate the indigestible components in the diets, the ability to detoxify the potentially harmful components of feed and the ability to produce a lot of essential vitamin B. complex members particularly biotin and vitamin B_{12}, the matter of which resulted in high food utilization and an increase in digestibility of different diet components. These results supported those of Kennedy *et al*. (1998) who used *B. subtilis* in the food of common snook (*Centropomus undecimalis*) and found that these probiotic bacteria increased the food absorption by enhancing the protease level and consequently gave a better growth. Also, El-Haroun *et al.* (2006) in his study with Biogen® as food additive containing *B. subtilis* came to the conclusion that, this organism germinates in the intestine of fish, using a large numbers of sugar (carbohydrates) and produces a wide range of digestive enzymes (amylase, lipase and protease) which have a beneficial effects including higher growth rate and higher feed efficiency. Also the incorporation of *S. cerevisiae* as a probiotic in fish diet was investigated by a lot of researchers in which similar results were obtained. Olvera *et al*. (2001) concluded that yeast have a positive effect on fish performance when cultured under stress condition of lowering dietary protein, leading to improving growth and feed efficiency. The adherence capacity of S cerevisiae and *B. subtilis* to the intestinal mucosa inhibits the attachment of the other intestinal bacteria to these binding sites and so preventing the disease occurrence with its negative impact on fish growth. These results supported the results of Esteban *et al*. (2001) who reported that the cell wall constituents of *S. cerevisiae* play a significant role in stimulation of innate immune response and protect the fish against infection. Raa (1996) reported also that mannose rich proteins from yeast are belonging to the category of compounds which adhere to receptors used by pathogenic microbes and so prevent their colonization in the fish gut. He reported also that the yeast cells could also produce group of substances namely

glutamine, glutamic acid and keto-glutric acid which are known to be energy substrates for intestinal cells and which contribute to healthy gut. Finally, Raa (1996) reported also about the peptides content of yeast cells which regulates the digestive enzymes secretion in fishes. The results indicate that Nile tilapia fed with 0.1 % Tonilisat showed significantly better growth as compared to diets supplemented with 0.05% Tonilisat and the control diets (T_1, T_4 and T_7). Yeasts *(S. cerevisiae*) have been used either as live or processed feed ingredients to improve the growth performance of fish (Stones and Mills, 2004). In the present study, the inclusion of *S. cerevisiae* in Nile tilapia diets significantly improved FBW, SGR and FCR of fish as compared to the fish fed T_1, T_4 and T_7 diets (Tables 2 and 3). 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 and 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 are able to secrete polyamines such as putrescine, spermidine and spermine (Tabor and Tabor, 1985; Buts *et al*., 1994 and Tovar-Ramı´reza *et al*., 2002). 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.

Few studies have examined the impact of probiotics on nutrient uptake and utilization in fish, including tilapia. However, there are reports of improved nutrient utilization through probiotic use in tilapia and other species of fish. Rainbow trout exhibited relief from vertebral column compression syndrome due to improved bone formation/mineral

utilization from improved mineral uptake in fish fed diets containing the probiotic *P. acidilactici* (Merrifield *et al*., 2011). The authors hypothesized that improved mineral uptake may have been caused by acidification of the intestinal environment through short-chain fatty acid and lactic acid production by the supplemented probionts. No evidence has been reported supporting improved mineral uptake in tilapia through probiotic use, but probiont-assisted production and utilization of other nutrients has been observed. Premalac® or Biogen®, commercially available probiotic mixtures, supplemented in diets containing varying levels of protein, produced better growth performance in tilapia, suggesting improved protein utilization (Ghazalah *et al*., 2010). Tilapia fed the probiotic diet had better growth than fish fed diet without probiotics. No explanation on the mechanism responsible for the improved protein utilization was provided, but bacteria, including Aeromonads commonly found in the gut of tilapia are known to produce proteases (Nayak, 2010). Gut microbes produce amino acids that are utilized by tilapia. Newsome *et al.*, 2011 found that tilapia appeared to be able to acquire their essential amino acid requirements directly from the GI microbiota when dietary sources were insufficient. Volatile, short-chain fatty acids can be produced from anaerobic microbes in the gut of tilapia by fermentation of dietary carbohydrates (Smith *et al*., 2005).

In the present study (Table 4) the dry matter, lipid and ash contents of the initial sample were significantly lower than the values after feeding the fish with experimental diets, while the protein was significantly higher. Carcass lipid and protein contents of Nile tilapia fed graded Tonilisat levels were positively correlated with growth rate and feed efficiency, while the ash contents of the fish had inverse relationship with dietary Tonilisat levels. Similar results on carcass body protein of other species of fish have been reported (Gunasekera *et al*., 2000). The observed inverse relationship between the carcass protein and lipid values has also been noted with other fish species (Khan *et al.*, 1993; Arzel *et*

al., 1995 and Yang *et al.* 2002).Increasing dietary protein levels in fry groups increased carcass protein values at the end of the experiment. Also, this observation is in contrast with that of Shiau and Lan (1996) who reported positive correlation between carcass lipid of grouper (*Epinephelus malabaricus*) and dietary protein levels.

Table 4. Effect of dietary supplementation of Tonilisat levels on whole body carcass composition (% on DM basis) of all male Nile tilapia.

Items	**Treatments**								
	0.5g/fish			**10 g/fish**			**20g/fish**		
At the start of trial	**T1 0.0%**	**T2 0.05%**	**T3 0.1%**	**T4 0.0%**	**T5 0.05%**	**T6 0.1%**	**T7 0.0%**	**T8 0.05%**	**T9 0.1%**
Moisture	80.08			74.35			72.45		
Crude protein (CP)	53.63			56.62			57.13		
Ether extract (EE)	17.44			23.19			24.15		
Ash	28.93			20.19			18.32		
At the end of the trial									
Moisture	75.32^{a}	74.00^{b}	73.83^{b}	72.13^{c}	70.35^{d}	70.81^{d}	68.13^{e}	66.32^{f}	65.14^{g}
Crude protein (CP)	57.43^{e}	59.12^{d}	59.88^{cd}	60.35^{c}	63.14^{a}	64.08^{a}	58.14^{e}	60.43^{bc}	61.15^{b}
Ether extract (EE)	24.00^{a}	23.13^{b}	22.34^{bc}	21.75^{c}	18.43^{d}	18.13^{d}	18.85^{d}	18.63^{d}	18.32^{d}
Ash	18.37^{c}	17.05^{d}	17.18^{d}	17.38^{cd}	18.13^{cd}	17.58^{cd}	22.35^{a}	20.43^{b}	20.12^{b}

a-e: Means in the same row with different letters are significantly different ($P \leq 0.05$).

Results in Table 5 showed that RBCs count, total protein, albumin, globulin, and erythrocytes count in the experimental fish of T_3 were increased significantly ($P \leq 0.05$) compared with the probiotic free diets. While, no significant ($P \geq 0.05$) differences were recorded in blood indices between T_1, T_4 and T_7. On the other side, increasing the dietary probiotic levels of fish diets resulted in significant recorded the highest

blood parameters compared with the $T_{1,}$ T_4 and T_7. However, T_4 (diet free with Tonilisat) recorded the lowest values in the blood parameters values among all experimental treatments. The promising positive results obtained in hematological blood parameters led to increase the immune status of fish and decreased mortality among all the treated fish by using Tonilisat at 0.1 and 0.05%. These findings related to Tonilisat, improved the physiological function of fish and immune response. These results are in agreement with those obtained by Mohamed (2007) who revealed the increase in plasma total protein of *O. niloticus* fingerlings fed on probiotic and yeast. Yet, Diab *et al.* (2002) reported that there were no significant differences in serum total protein in fish fed diets containing 0.5%, 1.0% and 1.5% of Biogen®. Moreover, Eid and Mohamed (2008) found no significant differences ($P > 0.05$) in plasma total protein, albumin and total globulins of fish fed the experimental diets containing different levels of probiotics (Biogen® and Pronifer®) in comparison with the control diet. On the other hand, Wang *et al.* (2008) found that there were no remarkable differences ($P > 0.05$) in the total serum protein, albumin and globulin concentrations and albumin/ globulin ratio between the *O. niloticus* supplemented with the probiotic bacterium, *Enterococcus faecium* ZJ4 and the control fed the basal diet.

Table 5. Effect of supplementation of Tonilisat levels on blood parameters of all male Nile tilapia.

Items	Treatments								
	0.5g/fish			10 g/fish			20g/fish		
	T1 0.0%	T2 0.05%	T3 0.1%	T4 0.0%	T5 0.05%	T6 0.1%	T7 0.0%	T8 0.05%	T9 0.1%
RBCs ($x10^6/mm^3$)	1.82^{e}	2.58^{ab}	2.61^{a}	1.74^{e}	2.41^{cd}	2.45^{bc}	1.79^{e}	2.27^{d}	2.33^{cd}
Total protein (g/l)	35.64^{i}	54.36^{b}	55.63^{a}	37.12^{g}	51.32^{c}	50.49^{d}	36.31^{e}	47.12^{f}	48.65^{h}
Albumin (g/l)	1.317^{f}	2.235^{a}	2.289^{a}	1.419^{e}	1.931^{c}	1.998^{d}	1.633^{d}	1.635^{d}	1.677^{d}
Globulin (g/l)	22.46^{d}	32.01^{b}	32.74^{a}	22.93^{d}	32.01^{b}	30.51^{c}	19.96^{e}	30.77^{c}	31.82^{b}
A/G ratio	0.059^{bc}	0.069^{ab}	0.069^{ab}	0.061^{bc}	0.060^{b}	0.065^{ab}	0.081^{a}	0.053^{cd}	0.053^{cd}
NBT(mg/l)	0.234^{f}	0.632^{a}	0.641^{a}	0.237^{f}	0.603^{b}	0.610^{b}	0.257^{e}	0.572^{c}	0.552^{d}

a-f: Means in the same row with different letters are significantly different ($P \leq 0.05$).
RBCs= red blood cells ($x10^6/mm^3$)

A number of specific modes of action by probiotic microorganisms have been attributed to physiological benefits in fish. Although gut colonization is often identified as the most important characteristic of effective probionts, the reality is that the benefits incurred by the host from probiotic supplementation are likely a synergistic product of multiple biological effects (some of which have nothing to do with gut colonization), including production of inhibitory compounds, competition for chemicals or available energy, competition for adhesion sites, inhibition of virulence gene expression or disruption of quorum sensing, enhancement of the immune response, source of macro and/or micronutrients, enzymatic contribution to digestion, and stimulation of local and systemic immune responses (Merrifield *et al*., 2010). Also, the goal of probiotic supplementation (immune stimulation, disease resistance, growth performance, etc.) must also be taken into account (Merrifield *et al*., 2010).

The NBT values was better in group fed on diets supplemented with Tonilisat compared with free Tonilisat diet groups. The lowest

values were recorded by T_4, T_1 and T_7, respectively. Live yeast (*S. cerevisiae*) in diets improved survival and increased immune values of NBT activity in Nile tilapia serum (Table 5). These results were agreement with Tovar-Ramirez *et al*. (2004) who demonstrated that live yeast reduced malformation in seabass larvae. Also, *S. castelli increased immune parameters by increasing NBT activity and lysozyme level in common carp serum.*

Economical efficiency:

Data in Table 6 show that the best values of economical efficiency expressed as feed cost /kg gain in weight and relative economic efficiency were for diets containing 30% CP and supplemented with Tonilisat. Meanwhile, the worst values were recorded by fish weighed. Consequently, from the obtained results, this result would be effective from the economical point of view; Tonilisat at a level of $0.05\%Kg^{-1}$ diet with fry of mono-sex Nile tilapia *O. niloticus* is useful to get the best body weight gain. Also, Tonilisat at a level of $0.05\%Kg^{-1}$ diet had the best economic efficiency in groups sized 10 and 20 g/fish, respectively.

CONCLUSIONS

In conclusion, all results obtained indicated that 0.5 or 1g Tonilisat /Kg feed produced a positive effect on growth and feed utilization of tilapia (fry and fingerlings). In addition, the immune responses were substantial in both treatment groups However, the probiotic Tonilisat when added to fish diet at 0.05%/Kg; produce a steady improvement of tilapia growth compared 0.00 Tonilisat diets. It could be concluded that the inclusion of the commercial Tonilisat at a level of $0.05\%Kg^{-1}$ diet with fry of mono-sex Nile tilapia *O. niloticus* is useful to get the best fish performance and there were no adverse effects on water quality criteria among all experimental treatments. Also, the probiotic, Tonilisat was beneficial for tilapia in terms of increasing growth

performance and the concentrations of serum protein and globulin and enhancing immune responses.

Table (6). Effect of probiotic supplementation levels on the economic efficiency of the experimental diets.

Items / Treatments	Feed-cost/ton LE	Feed cost/ kg gain LE	Relative feed cost / kg gain*%
T1	3250	5.525	70.63
T2	3280	4.877	61.89
T3	3310	4.955	63.35
T4	3250	7.400	94.60
T5	3280	6.56	83.87
T6	3310	6.50	83.10
T7	3250	7.822	100
T8	3280	6.471	82.73
T9	3310	6.369	81.42

* Relative to T7.
Feed cost/ kg gain LE =FCR x Feed-cost/ton LE

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تقييم أداء البروبيوتك كأضافة غذائية فى علائق أسماك البلطى النيلى

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قسم تغذية الأسماك ، المعمل المركزى لبحوث الثروة السمكية ، مركز البحوث الزراعية ، وزارة الزراعة ، مصر.

الملخص العربى

أجريت تجربة التغذية لمدة ٧٠ يوما لتقييم أداء ثلاثة مستويات من البروبيوتك كإضافة غذائية بمستويات صفر ، نصف و واحد جرام لكل كيلو جرام عليقة كلا مع ثلاثة أحجام مختلفة من البلطى النيلى على معدلات النمو ، معدل الإعاشة ، الكفاءة الغذائية ، تحليل جسم الأسماك و تحليل الدم و الكفاءة الأقتصادية للعلائق المستخدمة لأسماك البلطى النيلى وحيد الجنس . تم تغذية أسماك البلطى على ثلاثة علائق تجريبية متماثلة فى محتواها البروتينى (٣٠% بروتين خام) ومحتواها من الطاقة الكلية ٤٤٤.٥٨ كيلو كالورى/١٠٠جم من العليقة كلا مع ٣ مجموعات وزنية من الأسماك و بكل معاملة ثلاثة مكررات و بكثافة ١٥ سمكة للمتر وقد استخدم ٢٧ حوض زجاجى للتسعة معاملات لتجربة التغذية. أوضحت نتائج التجربة أن معدلات النموكانت أقل مع عدم اضافة البروبيوتك عن تلك المعاملات المضاف لها ٠.٠٥ جرام أو ١ جرام من البروبيوتك لكل كيلو جرام عليقة كانت أفضل للنمو بينما معدلات النمو كانت أقل مع عدم إضافة البروبيوتك عن تلك المعاملات المضاف إليها. أيضا أوضحت النتائج المتحصل عليها أن أدء النمو أفضل مع إضافة ٠.٥ جرام من البروبيوتك لكل كيلوجرام لعلائق يرقات البلطى النيلى. إن إضافة البروبيوتك ذو تأثير معنوى على معدلات النمو و الكفاءة الغذائية كما تشير النتائج أيضا إن إستخدام ٠.٥ جرام من البروبيوتك لكل كيلو جرام من العليقة يحسن من أداء النمو مع عدم وجود تأثير عكسى على نتائج تحليل الدم ونستخلص من الدراسة الحالية أن أستخدام الخميرة الحية كبروبيوتك فى علائق يرقات البلطى النيلى وحيد الجنس ذو تأثير إيجابى على معدلات النمو و الكفاءة الغذائية المرتفعة مع زيادة الحالة الصحية و لكن من الناحية الأقتصادية فأن أستخدام نصف جرام من البروبيوتك لكل كيلو جرام فى علائق أسماك البلطى النيلى وحيد الجنس بكثافة ١٥ سمكة للمتر المربع ذوكفاءة أقتصادية و بدون التأثيرالسلبى على بيئة الاسماك.

الكلمات الدالة: البلطى النيلى، أداء النمو،البروبيوتك، تحليل الجسم ونتائج تحليل الدم.