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EFFECTS OF STOCKING DENSITY AND PROBIOTIC ON THE GROWTH PERFORMANCE AND NUTRIENTS EFFICIENCY OF NILE TILAPIA (*OREOCHROMIS NILOTICUS* L.)

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Abstract

The present study was carried out to evaluate the effects of stocking densities and probiotic (Tonilisat) as supplementary diet (0.00, 0.025, 0.05, 0.10 and 0.20%) on the growth, survival rate, feed efficiency, body chemical composition and hematological study of all male Nile tilapia (*Oreochromis niloticus* Lin.) fingerlings. Fish were reared at two different densities each with different levels of probiotic during the feeding trial (10weeks).Fish with initial body weight (mean weight, 3.3 g/ fish) were stocked at 10 and 20 fish/m-2 each with five experimental diets (three replicates), fish fed on diets at 5% as a feeding rate, four times daily, all experimental diets contained 30% crude protein and 446 kcal gross energy/100 g diet. The final mean weights of the fish stocked at low density had the highest record. Growth rate was fast up to 10 and 20 fish m^2 with 0.1% probiotic The corresponding SGR values were higher in T5 and T9 groups (2.317 and 2.307% day–1, respectively). The obtained results revealed that stocking density had significant effects on growth, and survival rates chemical composition of whale fish of Nile tilapia fingerlings at lower stocking density. Fish reared at the highest density exhibited lowest growth rate and survival rates compared with lower stocking density. But the differences were not significant ($P<0.05$) of survival rates. Hematological parameters were recorded the highest value with low stocking densities and higher level of probiotic in diets. It could be concluded that the inclusion of the Tonilisat at 0.1 %/kg diet at lower or higher stocking densities of all male Nile tilapia fingerlings is useful to get the best growth performance with no adverse effects on the environmental and enhance the blood hematological parameters. Hence, an optimum density level in terms of economic viability of tilapia culture must be established. Thus, this study enables us to postulate an optimum stocking density level of tilapia for maximum utilization of food and space with minimum stress and energy expenditure resulting in higher growth potential of the fish.

Key Words: Nile tilapia, growth performance, probiotic, chemical composition and blood hematological parameters.

INTRODUCTION

Aquaculture has become an important economic activity in many countries (Subasinghe, 1997). However, this aquaculture develop show many problems as widespread epizootics, feed efficiency and growth performance (Fegan, 2001; Gaiotto, 2005 and Balcazar *et al.*, 2004). This is principal caused by the large-scale production facilities, where aquatic animals are exposed to stressful conditions, problems related to disease, inadequate balance of nutrient in the artificial diets an deterioration of environmental conditions, since the physiological stress is one of the primary contributing factors of aquatic organisms disease, poor growth and mortality in aquaculture (El-Haroun *et al.*, 2006 and Rollo, 2008).

Nile tilapia is an economically important cultured species in several areas of the world (El-Husseiny *et al*., 2007 and El-Saidy & Gaber, 2005). 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 t year-1). (General Authority for Fish Resources Development, GAFRD, 2010).

Nutrition is the most important factor of the culture process; it is often represent the major operating cost of aquaculture. Under intensive culture system, fish totally depend on complete balanced diets during their life stages. Aqua-culture should know the optimum quality and quantity of feeds introduced to fish to avoid poor growth, health and productive performance. Fish cannot grow well without feeds and they should not be underfed. From the economical point of view, fish producers mostly use the cheaper and more balanced fish diets to cover the nutrient requirements of fish during the growing and production periods (Magouz *et al*., 2002).

The improvement in the efficiency of protein utilization at lower stocking densities was found in the work of many researchers (Sharms and Chakrabarti 1998, El-Sagheer, 2001, Baumgarner *et al*., 2005, Ridha, 2006 and Piccolo *et al*., 2008). There have been several reports indicating that rate of survival become reduced when fish are held at high stocking densities. This reverse relationship could be attributed to social stresses, stress-related disease and depuration the productive performance (Sharma and Chakrabarti, 1998 and Bolasina *et al*., 2006), Increased mortality related with higher stocking density was frequently reported by (El-Sagheer, 2001 and Piccolo *et al*., 2008). The principal aims of the aquaculture industry are increase the growth or survival performance, feed efficiency, and resistance of aquatic organisms which show a positive effect on production costs (Gatlin, 2002).

The word “probiotic” was introduced by Parker (1974). According to his original definition, probiotics are ‘Organisms and substances which contribute to intestinal microbial balance’. Fuller (1989) revised the definition as ‘A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’. This revised definition has put forward the importance of live cells as the essential component of a potential probiotic and it clears the confusion created by the use of the term ‘*substances*’. However, “an effect on intestinal microbial balance” has been defined and demonstrated only in a few cases. This was noted by Tannock (1997), and he proposed the definition “living microbial cells administered as dietary supplements with the aim of improving health”. The concept of microfloral manipulation was first appreciated by Metchnikoff (1907) who examined the consumption of yoghurt and found an effect on the longevity of Bulgarian peasants, stocking density is a key factor in determining the productivity and profitability of commercial fish farms. Knowledge of fish stocking density practices is vital to judge the impact of the importance of any density limits on economic sustainability; Commercial farmers are thought to use a combination of intuition and experience to decide upon the most appropriate stocking density (Ellis *et al*., 2002). The use of probiotics in aquaculture has been accompanied by an increase in nutrient utilization through providing enzymes capable of converting certain components of the diet into more digestible nutrients for the host. In this connection, Geovany *et al*., (2007) showed that feeding probiotics may improve appetite and growth performance of the farmed fish or what?? Species. However, the specific function of probiotics may differ depending on the host animal and more on the characteristics of the probiotics. Guillian *et al*. (2004). Eid and Mohamed (2008) found that *Bacillus* spp. May modulate the mucosal immune system enhancing their resistance to enteric pathogens and so improved survival and growth of tilapia. Also, Venkat *et al*., (2004) found that *Lactobacillus* spp. have an inhibitory effect on the harmless gram negative bacteria present in gut microflora of *Macrobrachium rosenbergii* post-larvae, and so reduced the mortality significantly. Laxmi Prasad *et al*. (2012) reported that, the best growth performance and feed efficiency was obtained in *Macrobrachium rosenbergii* postlarvae fed on diet supplement**ed** with 0.5% *Lactobacillus sporogenes* containing 1.67 x 105 colony forming units/100 g feed.

The present experiment was carried out to study the effects of stocking density and probiotic on the growth performance, efficiency of feed, protein utilization, and body chemical composition and blood hematological parameters of all male Nile Tilapia (*Oreochromis niloticus L.*) fingerlings with average initial body weight of 3.3 grams.

MATERIALS AND METHODS

Experimental fish:

All male Nile tilapia (*Oreochromis niloticus* Lin.) fingerlings were obtained from Abbassa Hatchery belong to GAFRD, Abou Hammad, El-Sharkia Governorate They seemed healthy and had an average initial weight (3.3g/fish). Fish distributed randomly into 30 glass aquaria. Each treatment including 3 replicates (aquaria) in which 10 or 20 fish were stocked in fifteen glass aquaria.

Fish feeding:

The feeding trial lasted 10 weeks (1st of the June2010 to 15 of the August. 2010). The experimental diets included 10 diets which fed at level of 5% of the total biomass of fish daily. The fish were fed 4 times a day at 9.00, 12.00, 15.00 and 18.00 hours. The amount of feed was adjusted biweekly according to the change in fish weight, using a total number of 450 Nile tilapia (*Oreochromis niloticus* Lin.). The fingerlings were distributed at random into ten experimental dietary treatments, each in three aquaria (60x50x40 cm) as replicates in which fingerlings were stocked at rates of 10 and 20 fish/ m^2 square. Water temperature in the experiment of aquaria ranged between 27.4-27.6ºC with photo period of 12h light and 12h darkness, Water samples were weekly taken for analysis of total ammonia nitrite, nitrate and pH levels using standard methods (APHA, 1992) to adjust the appropriate water quality parameters for tilapia cultivation.

Glass aquaria measuring (60 x 50 x 40 cm) of 120 litters each were used to stock Nile tilapia (*O. niloticus* Lin.) fingerlings, which acclimatized to the lab conditions for 2 weeks. Then, thirty experimental glass aquaria were used for 10 treatments to execute 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 and filling the aquaria after replacing at 100% of water daily. Water temperature (via a thermometer) was daily measured and the average was between 27.5-28.5^{o}C during the experimental period were measured weekly. The pH (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 being was used to study its effects on growth performance of Nile tilapia fingerlings fed diets with different levels of 0.00, 0.025, 0.05, 0.10 and 0.20%. 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. The basal diets were formulated to contain the recommended 30% crude protein levels (Table, 1). The feeding experiment was conducted in 5x2 factorial designs and included ten treatments. Feeding level of all experimental diets was 5% of the total biomass of the fish daily and the amount of feed was divided into four equal portions and distributed by hand in one side of the aquaria four times daily. 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). This trial consisted of ten treatments to investigate the effects different levels of probiotic on the growth performance, feeding efficiency and physiological measurements of Nile tilapia (*O. niloticus* Lin.) fingerlings. The probiotic (P1, P2, P3, P4 and P5) were added to Nile tilapia fingerlings diets 0.0, 0.25 0.5, 1.0 and 2.0 g/kg diet each with 10 and 20 fish per square meter. The All experimental diets were formulated to cover all nutrients requirements by Nile tilapia as recommended by National research council (NRC, 1993).

Table (1): The composition of the experimental diets.

Ingredients %	$Diet_1$ 0.00 probiotic	$Diet_2$ 0.025 probiotic	$Diet_3$ 0.05 probiotic	$Diet_4$ 0.10 probiotic	$Diet_5$ 0.20 probiotic
Fish meal	10	10	10	10	10
Soybean meal	43	43	43	43	43
Yellow corn	18	18	18	18	18
Wheat bran	15	15	15	15	15
Corn starch	5	5	5	5	5
Sunflower oil	2	2	2	2	2
Fish oil	3	3	3	3	3
Carboxy methyl cellouse	2	1.975	1.95	1-90	1.80
Probiotic	0.0	0.025	0.05	0.10	0.20
Vit. & min.premix1	2	2	2	2	2

1 Eco Vit, Egyptian Veterinary products and feed additives Co., Demyatta, Egypt. The vitamin and 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 D3, vitamin E, vitamin B2,vitamin K3, nicotine amide, vitamin B6, vitamin B12, vitamin B1, folic acid, Ca-D-pantothenate, calcium, phosphate, sodium, copper, magnesium, manganese, zinc, iron, cobalt, iodine and selenium, respectively.

Experimental diets:

The dietary ingredients and their composition are show in Table (1) were 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 0.5 mm diameter by pelleting unit. The pellets were dried in a drying oven at 60 ^oC for 24 hours and stored at – 4 ^oC until use during the trial to avoid oxidation and rancidity. The dietary ingredients and the experimental diets were analyzed according to standard methods of Association of Official Analytical Chemists (AOAC, 1990) for moisture, protein, total lipids, and ash. Moisture constant weight at 60 ^{o}C in drying oven (GCA, model 18 EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxllet apparatus (Lab. Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours and ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Lowa, USA) at 550 ^{o}C for 6 hours .The chemical composition of the experimental diets is show in Table (2).

Table (2): The chemical composition of the experimental diets (% on DM basis).

ITEMS	$Diet_1$ 0.00	$Diet_2$ 0.025	$Diet_3$ 0.05	$Diet_4$ ٠.10	$Diet_5$ 0.20
DM	91.51	91.31	91.21	91.53	91.34
CP	30.12	30.15	30.29	30.24	30.28
EE	6.68	6.89	6.66	6.77	6.95
Ash	7.92	7.98	8.08	8.12	6.85
CF	5.12	5.17	5.25	5.33	5.34
NFE*	50.16	49.81	49.82	50.54	50.58
GE(kcal/100g)**	446.45	446.21	445.67	445.28	445.33
P/E ratio	67.47	67.60	67.79	67.91	67.99

* Nitrogen free extract (NFE) = 100 - (CP% + EE% + ash% + crude fiber)

** Gross Energy (kcal/kg) was calculated using caloric values of 5.65, 9.45 and 4.١ for crude Protein, ether extract and nitrogen free extract, respectively (NRC, 1993).

The growth performance parameters:

The performance parameters included, the growth performance parameters and feed utilization values including body weight (B.W.), body weight gain, specific growth rate (SGR), condition factor (K) and survival rate, feed intake, protein intake, feed conversion ratio (FCR), and feed efficiency ratio (FER), protein efficiency ratio (PER) were measured. After running the feeding experiment, proximate chemical analysis were made according to A.O.A.C (1990) methods for experimental diets and whole fish at the start and at the end of the feeding trial (10 weeks).

Blood 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 of hemoglobin (Hb) by using commercial kits (Diamond Diagnostic, Egypt). Also, total erythrocytes count (RBCs) and total leukocytes count (WBCs) were measured on an A_o Bright -Line Haemocytometer model (Neubauer improved, Precicolor HBG, Germany). Other blood samples were collected and transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for determination of total protein according to Gornall *et al.*, (1949), albumin according to Weichsebum (1976), globulin by difference according to Doumas and Biggs (1972). As well as, at the end of the experiment some fishes from all treatments were sacrificed and fish dorsal muscles were sampled. Samples were fixed in 10% neutralized formalin solution to histometric examination according to Pearse (1968).

Statistical analysis:

Growth performance, feed efficiency and blood hematological 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 acceptance range for Nile tilapia (*Oreochromis niloticus Lin.*).The water temperature ranged from 27.5 to 28.5 ^{o}C, dissolved oxygen from 5.8 to 6.5 mg/l, pH from 6.3 to 8.1 and ammonia (NH_3) from 0.03 to 0.04 mg/l. the results are similar to those Abdel-Hakim *et al*. (2002).

Table (3) shows the summary of the initial and final average weights (g), survival rate condition facto (K) of fish. The highest survival rate of 92.00% was recorded with the 0.1 % level of probiotic in fish diet which stocked at lower stocking density during the experiment. The results indicated an inverse relationship between survival rate and stocking density. But, the differences were not significant (P>0.05). The survival rates were not affected significantly with fish density suggesting that when fish were small sized, there was no competition for food and space. Moreover, the good fish survival at high density when 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.

Treatment 4 with 10 fish /m^2 had the highest final mean weight of 31.66 g, while T6 group had the lowest final weight of 21.12 g. Values of final weight were not significantly different for T9, T8, T4, T5, T3 and T10 groups. The body weight gain values were increased by increasing probiotic levels in Nile tilapia diet.

Results also showed that fish fed diets containing different probiotic levels groups had significantly better condition factors than those fed on the control diets (T1 and T2), meanwhile, significant differences were observed in K values among different probiotics tested and the lowest value (1.42) was obtained by T6 group while the highest value was obtained by T4 followed by T5 group. The differences between T4, T5, T3, T9 and T10 were not significant (Table, 3).

Table (3): Effect of different stocking densities and dietary supplementation of Tonilisat on growth performance of all male Nile tilapia**.**

Treatments	Initial weight g	Final weight g	Weight gain g	SGR %/day	Condition factor (K) %	Survival rate (SR) %
T1 (D_1S_1)	3.36	21.12^d	17.76^d	2.01^c	1.48^c	89.33
T2 (D_2 S_1)	3.33	25.89^b	22.56^b	2.11^b	1.47^c	92.00
T3 (D_3S_1)	3.34	31.61^a	28.38^a	2.29^a	1.70^a	92.00
T4 (D_4S_1)	3.31	31.58^a	28.27^a	2.30^a	1.71^a	91.67
T5 (D_5S_1)	3.39	31.33^a	27.98^a	2.32^a	1.71^a	91.33
T6 (D_1S_2)	3.36	21.12^d	17.76^d	1.88^d	1.42^d	98.33
T7(D_2S_2)	3.33	25.89^b	22.56^b	2.09^b	1.48^c	90.00
T8 (D_3S_2)	3.34	31.62^a	28.38^a	2.30^a	1.64^b	91.67
T9 (D_4S_2)	3.31	31.78^a	28.27^a	2.31^a	1.69$_a$	91.00
T10 (D_5S_2)	3.39	31.33^a	27.98^a	2.28^a	1.65^b	91.33

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

D1=diet1	D2=diet2	D3=diet3	D4=diet4	D5=diet5

S1=lower stocking density and S2=higher stocking density

AWG = Average weight gain (g/fish), Specific growth rate (SGR, %/day) = [ln final weight - ln initial weight] x 100/experimental period (d), K= Condition factor (W/L^3 x100) and Survival rate (SR %) = end number of the live fish/the beginning number of the fish x 100

The ability of the fish to convert feed given to flesh decrease as stocking density increased, except for treatment 1 which had a higher feed conversion ratio than treatment 2. Fish production increased as the stocking density increased but the final weight decreased. On the other hand, the production was generally high in this experiment. High final body weight gain obtained could be attributed to effect of supplementary probiotic; the favorable physico-chemical conditions of the water quality. The results indicated that the probiotic significantly promoted final body weight, body weight gain and survival rate (%) in experimental treatments in comparison with T1 and T6 treatments (P < 0.05). Also, the condition factor (K) had significantly increased (P > 0.05) by increasing probiotic values with lower stocking density. The lowest K value was recorded by T6 (control group with higher stocking density).The higher body weight obtained in treatment of T4 and T5 (lower stocking density with supplemented by probiotic) while this parameter in T6 (control treatment with higher stocking density with no supplemented by probiotic) was lower. The differences between T9, T8, T4, T5, T10 and T3 in final body weight values were not significant. The lower growth performance of tilapia at higher stocking density could have been caused by voluntary appetite suppression, more expenditure of energy because of intense antagonistic behavioral interaction, competition for food and living space (Diana *et al*., 2004) and increased stress(Ouattara *et al*.,2003). It was also reported that increasing stocking density of Nile tilapia fry might have lead to diminishing social dominance, resulting in lower individual growth rates Dambo and Rana, 1992). In addition, the resting plasma cortisol concentrations of Nile tilapia fingerlings were found to rise with increased stocking density (Barcellos *et al*., 1999).

In finfish the use of probiotics demonstrated beneficial effects on the growth performance, feed efficiency and digestibility of organic matter and protein, when used yeast (Lara-Flores *et al*, 2009) In some case this beneficial effect had attribute to the capacity of the probiotic to stimulate and/or produce some enzymes on the intestinal tract. For example, Lara-Flores *et al*. (2000) observed a high activity of alkaline phosphatase in Nile tilapia (*Oreochromis niloticus*) when administered probiotics in the diet, the result may be show a high activity reflected a possible development of brush border membranes of enterocytes that can be stimulated by the Tonilisat and this it can be a indicator of carbohydrate and lipid absorption and explain the higher weight gain and the best feed efficiency. 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). In this trend, Abdelhamid *et al*. (2007) reported that raising the stocking density (2, 3 and 4g fish liter $^{-1}$) of the experimental Nile tilapia *O. niloticus* resulted in a significantly ($P \leq 0.05$) decrease of the growth performance parameters (final weight, weight gain, average daily gain, relative growth rate and specific growth rate) of fish. However, they added that increasing the stocking density rate of fish led to significantly ($P \leq 0.05$) increased feed conversion ratio of the experimental fish, but survival rate of fish was not influenced by raising the stocking density. Yet, they added that increasing dietary Betafin® level caused a significant improve in this picture. As well as, Bakeer *et al*. (2007) found that body weight and length were negatively correlated to the stocking density of tilapia fish. Moreover, results of the present study are in agreement with those of Khattab *et al.* (2004a) for tilapia and EL-Haroun (2007) for catfish. Also, EL-Haroun *et al.* (2006) reported that the growth performance and nutrient utilization of Nile tilapia were significantly ($P \leq 0.01$) higher in the treatment receiving probiotic (Biogen®) than the control diet. Yet, Mohamed *et al.* (2007) reported that *O. niloticus* fingerlings fed on diets supplemented with probiotics exhibited greater growth than those fed the control diet. Also, they added that the diet containing 30% protein and supplemented with Biogen® at level of 0.1% produced the best growth performance. Recently, Eid and Mohamed (2008) revealed that using Biogen® at level of 0.1% was the best in terms of growth performance of mono-sex *O. niloticus* fingerlings. Moreover, Wang *et al.* (2008b) mentioned that tilapia (*O. niloticus*) supplemented with the probiotic bacterium, *Enterococcus faecium* ZJ4 showed significantly ($P < 0.05$) better final weight and daily weight gain (DWG) than those fed the basal diet (control). Recently, Marzouk *et al*. (2008b) reported that both fish groups fed on diet supplemented with probiotics (dead *Saccharomyces cerevisae* yeast and both of live *Bacillus subtilis* and *Saccharomyces cerevisae*) revealed significant (P < 0.05) increases in the body weight gain, specific growth rate and condition factor (K). While, they added that a significant (P < 0.05) decreased in feed conversion ratio was recorded comparison with the control group fed on probiotic-free diet. On the other hand, Abdelhamid *et al.* (2002) found that Biogen® supplementation (2 and 4 g kg^{-1} diet) did not significantly improve fish growth performance. Also, Diab *et al.* (2002) reported that Biogen® addition to fish diet at 0.5, 1.0 and 1.5% gave insignificant increase in fish growth performance.

The PER results (Table, 4) indicated that supplementing diets with probiotics 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 30 %CP dietary protein demonstrated that the probiotics supplements performed more efficiently in stress situations. This agreed with the results obtained by Ringo and Gatesoupe. (1998).This study highlights the effects of probiotic (*Saccharomyces cerevisiaa*) on the enhancement of growth parameters (Table, 3) and feeding efficiency (Table, 4) of Nile tilapia fingerlings. In experimental treatments the probiotic (Tonilisat) had positive and significant effects on the feed conversion efficiency (FCR),), feed efficiency ratio (FER) and protein efficiency ratio (PER) (P<0.05). A significant (P < 0.05) decreased in feed conversion ratio was recorded comparison with the control group fed on probiotic-free diet. The higher FER obtained in T3 followed by T4, T8, T9 and T10groups (Table, 4). The lowest value of PER was obtained by T6 group (higher stocking density) fed on probiotic-free diet. In this trend, Abdelhamid *et al*. (2007) reported that raising the stocking density (2, 3 and 4g fish $liter^{-1}$) of the experimental Nile tilapia *O. niloticus* resulted in a significantly (P ≤ 0.05) decrease of the growth performance parameters (final weight, weight gain, average daily gain, relative growth rate and specific growth rate) of fish. However, they added that increasing the stocking density rate of fish led to significantly ($P \leq 0.05$) increased feed conversion ratio of the experimental fish.

Table (4): Effect of different stocking densities and dietary supplementation of Tonilisat on feed efficiency of all male Nile tilapia fingerlings.

Treatments	Feed intake g/fish	Protein intake g/fish	FCR	FER%	PER%
T1 (D_1S_1)	35.32^{d}	10.64^{c}	1.73^{b}	0.58^{b}	1.93^{c}
T2 (D_2 S_1)	39.42^{bc}	11.89^{b}	1.72^{b}	0.58^{b}	1.93^{c}
T3 (D_3S_1)	41.21^{abc}	12.45^{ab}	1.48^{c}	0.68^{a}	2.23^{ab}
T4 (D_4S_1)	42.03^{abc}	12.71^{ab}	1.48^{c}	0.67^{a}	2.23^{ba}
T5 (D_5S_1)	43.75^{a}	11.93^{c}	1.55^{c}	0.64^{a}	2.37^{a}
T6 (D_1S_2)	33.98^{d}	11.84^{b}	1.91^{a}	$0.0.52^{c}$	1.74^{d}
T7 (D_2S_2)	39.25^{c}	11.85^{ab}	1.74^{b}	0.58^{b}	1.91^{c}
T8 (D_3S_2)	42.54^{abc}	12.85^{ab}	1.50^{c}	0.67^{a}	2.21^{b}
T9 (D_4S_2)	42.86^{ab}	12.96^{a}	1.50^{c}	0.67^{a}	2.20^{b}
T10 (D_5S_2)	41.84^{abc}	12.67^{ab}	1.49^{c}	0.67^{a}	2.21^{b}

a-d: Means in the same column different letters are significantly different ($P \leq 0.05$).

D1=diet1	D2=diet2	D3=diet3	D4=diet4	D5=diet5

S1=lower stocking density and S2=higher stocking density

Feed conversion ratio (FCR) = feed intake (g)/live weight gain (g) , Feed efficiency ratio (FER)= live weight gain (g) / feed intake (g) x 100 and Protein efficiency ratio (PER) = Live weight gain (g)/Protein intake (g),

In fish farming practices, stocking density is considered to be one of the important factors that affect fish growth, feed utilization and fish yield (Liu and Chang, 1992). On the other hand, Abdelhamid *et al.* (2002) found that Biogen® supplementation (2 and 4 g kg^{-1} diet) did not significantly improve fish growth performance. Also, Diab *et al.* (2002) reported that Biogen® addition to fish diet at 0.5, 1.0 and 1.5% gave insignificant increase in fish growth performance. It was found that the highest weight, length, daily weight gain, growth rate and protein content were observed for the 20000 fish/ha density class. Thus, culture of monosex tilapia at a density of 20000 fish/ha can be considered ideal for augmented production of the fish under Indian context (Suman and Samir, 2010). It could be concluded that the inclusion of the commercial probiotic Tonilisat at a level of 1g Kg^{-1} diet at stocking density rate of 10 fish/m^2 of all male Nile tilapia *O. niloticus* Lin. is useful to get the best fish performance and nutrients efficiency.

Comparison of the chemical composition of Nile tilapia fingerlings body at the beginning and at the end of experiment (Table, 5) indicates significant changes which took place in the raw fat content, where the values in treatments T_3 and T_2 significantly increased. Changes took place also in the content of total protein in treatment T_6, the protein content significantly decreased. For the remaining body components, like dry matter and raw ash, no significant changes were recorded.

There were significant ($P \geq 0.05$) differences at the end of the feeding trial in moisture, crude protein and ash of *O. niloticus* between T_1 and T_3 While, there were significant ($P \leq 0.05$) decreased in ether extract in fish fed free diet supplementation by Tonilisat compared with the others group). However, by increasing the stocking densities of fish in treatments no significant ($P \geq 0.05$) differences were recorded in crude protein and ether extract comparing with T_6 group. While, increasing the stocking densities of fish led to significant ($P \leq 0.05$) decreased in both of ether extract compared with the control group (Table, 5). These positive effects in carcass composition of experimental fish may be due to the dietary supplementation with Tonilisat which caused the good growth performance compared with the control group (Table, 5). Since, Tonilisat may be enhancing the metabolism and energy of fish body cells and raise the efficiency of feeds. These results are in agreement with that obtained by Mehrim (2001). In this topic, Khattab *et al*. (2004b) reported that crude protein, total lipids and ash were significantly (P < 0.01) affected by protein level and increasing stocking density rate of tilapia fish. Yet, Abdelhamid *et al*. (2007) added that increasing the stocking density rate of fish was responsible for increased % of DM, leading to increases in CP and ash, but EE percentages of the whole fish body decreased. Yet, they added that increasing dietary Betafin® (betaine) level caused a significant improve in this picture. On the other side, results in the present study are in close agreement with those of Khattab *et al.* (2004a), Srour (2004), EL-Haroun *et al.* (2006) and Mohamed *et al.* (2007) for tilapia and EL-Haroun, (2007) for catfish. Moreover, Eid and Mohamed (2008) found that no statistical differences were observed in whole body moisture, crude protein, ether extract and ash of mono-sex *O. niloticus* fingerlings fed diets containing different levels of commercial feed additives (Biogen® and Pronifer®), compared with the control treatment.

Table (5): Effect of different stocking densities and dietary supplementation of Tonilisat on carcass composition (%) of all male Nile tilapia fingerlings.

Treatments	Moisture	Crude protein (CP)	Ether extract (EE)	Ash
At the start **At the end**	77.77	56.34	19.94	23.82
T1 (D_1S_1)	72.59^{a}	62.77^{d}	20.88^{b}	16.56^{b}
T2 (D_2 S_1)	72.05^{ab}	65.87^{ab}	19.15^{d}	14.69^{c}
T3 (D_3S_1)	71.12^{bc}	66.33^{a}	19.71^{cd}	13.58^{a}
T4 (D_4S_1)	70.93^{bc}	65.76^{ab}	19.81^{d}	15.00^{c}
T5 (D_5S_1)	70.63^{c}	65.12^{abc}	19.66^{cd}	14.96^{c}
T6 (D_1S_2)	72.41^{a}	60.35^{e}	22.65^{a}	17.73^{a}
T7 (D_2S_2)	71.99^{abc}	64.15^{e}	20.37^{bc}	14.12^{cd}
T8 (D_3S_2)	71.35^{abc}	65.89^{ab}	19.59^{cd}	13.38a
T9 (D_4S_2)	71.12^{bc}	65.53^{ab}	19.73^{cd}	15.12^{c}
T10 (D_5S_2)	71.76^{abc}	64.73^{bc}	20.12^{bcd}	14.76^{c}

a-d: Means in the same column different letters are significantly different (P ≤ 0.05).

D1=diet1	D2=diet2	D3=diet3	D4=diet4	D5=diet5

S1=lower stocking density and S2=higher stocking density

Results in Tables (6) showed that RBCs count, total protein, albumin, globuline, NPT and Erthrothine count in the experimental fish of T_5 were increased significantly ($P \leq 0.05$) compared with the control. While, no significant ($P \geq 0.05$) differences were recorded in blood indices between T4, T5 and T3 at lower stocking density. On the other side, by increasing the stocking densities of fish, no significant ($P \geq 0.05$) differences were recorded in all above mentioned blood parameters compared with the control (T_1 and T_6). However, T_6 (stocking density of 20 fish/m^2 plus feeding diet free supplemented with Tonilisat) recorded the worst 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 prevent mortality among all the treated fish by increasing the stoking densities of fish fed on Tonilisat at 0.1% (Table, 6). These findings were related to Tonilisat, improved the physiological function of fish and immune response which in turn increased the ability of exposed fish to resist the stress effect. These results are in agreement with those obtained by Mohamed (2007) revealed the increase in plasma total protein of *O. niloticus* fingerlings fed on probiotic and yeast. On the other hand, 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. Also, Wang *et al.* (2008) found that there was no remarkable difference ($P > 0.05$) 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. There were no adverse effects on water quality criteria among all experimental treatments. Consequently, from the obtained results, it could be concluded that the inclusion of the commercial probiotic Tonilisat at a level of 1g Kg^{-1} diet at stocking density rate of 10 fish/m^3 of mono-sex Nile tilapia *O. niloticus* is useful to get the best fish performance with friendly effects on the environment.

Table (6): Effect of different stocking densities and dietary supplementation of Tonilisat on the blood parameter and plasma proteins of all male Nile tilapia.

Blood parameters / Treatments	RBC	Total Protein	Alb.	Gl.	NPT	Erythrocytes
T1 (D_1S_1)	1.80^b	34.12^a	14.23^f	19.96^f	0.35^d	1.55^d
T2 (D_2 S_1)	1.88^b	48.92^d	16.52^e	32.40^d	0.38^d	1.65^c
T3 (D_3S_1)	2.55^a	64.37^b	18.93^c	45.44^a	0.69^b	1.98^b
T4 (D_4S_1)	2.59^a	62.53^c	20.31^b	42.22^c	0.71^b	2.04^{ab}
T5 (D_5S_1)	2.46^a	65.77^a	19.51^c	44.26^b	0.71^b	1.99^{ab}
T6 (D_1S_2)	1.83^b	36.37^e	17.35^d	19.02^f	0.39^{cd}	1.56^{cd}
T7 (D_2S_2)	1.89^b	49.37^d	18.05^d	31.12^e	0.42^c	1.65^c
T8 (D_3S_2)	2.59^a	66.12^a	20.63^b	45.49^a	0.75^a	2.08^{ab}
T9 (D_4S_2)	2.52^a	65.73^a	21.07^{ab}	44.66^a	0.77^a	2.07^{ab}
T10 (D_5S_2)	2.56^a	65.42^{ab}	21.54^a	43.88^b	0.76^a	2.09^a

a-e: Means in the same column different letters are significantly different ($P \leq 0.05$).
RBCs= red blood cells (Leukocytes).

D1=diet1	D2=diet2	D3=diet3	D4=diet4	D5=diet5

S1=lower stocking density and S2=higher stocking density

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تأثير كثافات الاستزرا ع والبروبيوتك على أداء النمو و الكفاءة الغذائية لاسماك البلطى النيلى

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المعمل المركزي لبحوث الثروة السمكيه، مركز البحوث الزراعية، القاهرة، مصر.

الملخــص العربــى

اجريت هذه الدراسة لتقييم أداء البروبيوتك (تونليسات) كأضافة غذائية بمستويات صفر ، ربع ، نصف ، واحد ، اثنين جرام لكل كيلو جرام عليقة على النمو، معدل الاعاشة، تحليل جسم الاسماك، الكفاءة الغذائية و تحليل الدم لاصبعيات البلطى النيلى وحيد الجنس. تم رعاية الاسماك فى كثافتين كلا مع خمسة مستويات من البروبيوتك خلال فترة التجربة الغذائية (عشرة أسابيع). كان وزن البداية للاصبعيات ٣.٣ جم للسمكة وخزنت بكثافتين عشرة او عشرين فى المتر المربع كلا مع خمسة علائق تجريبية (ثلاث مكررات) وغذيت الاسماك بمعدل خمسة بالمائة من الوزن على اربعة فترات يوميا وكان محتوى البروتين الخام ثلاثين وطاقة كلية مقدارها اربعمائة ستة واربعون كيلو كالورى لكل مائة جرام عليقة. ومن نتائج التجربة كان أفضل وزن نهائى للاسماك المخزنة بكثافة أقل او أعلى ومحتوى العليقة لهما ١ جرام من البروبيوتك لكل كيلو جرام. ان معدل الوزن النوعى كان الافضل فى المعاملتين الخامسة والتاسعة وأوضحت النتائج المتحصل عليها ان كثافة التخزين اثرت على معدل النمو، تحليل جسم الاسماك ومعدل الاعاشة لاصبعيات البلطى النيلى خاصة مع الكثافات الاقل. كانت الاسماك المخزنة بكثافات أعلى ذات معدل نمو واعاشة أقل مقارنة بالاسماك الاخرى ذات الكثافة الاقل ولكن الاختلافات لم تكن معنوية فى قيم معدلات الاعاشة. ومن نتائج تحليل الدم تم تسجيل أعلى قيم مع كلا من الكثافتين وخاصة مع زيادة مستوى البروبيوتك فى العلائق التجريبية. ويمكن القول ان واحد جم من البروبيوتك فى العلائق فى كلتا الكثافتين لم يكن لهماتأثيرا عكسيا على البيئة ولكن حسن من نتائج تحليل الدم. ويجب الأخذ فى الحسبان ان افضل كثافة للبلطى هى التى تعطى أفضل استفادة من الغذاء فى نفس المساحة مع أقل جهد او فقد للطاقة بما ينعكس على زيادة النموللاسماك.

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