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# DIETARY PRO-GROW® PROBIOTIC AGAINST HIGH STOCKING DENSITY STRESS:
# 1-EFFECT ON WATER QUALITY, GROWTH PERFORMANCE, FEED UTILIZATION AND CARCASS COMPOSITION OF NILE TILAPIA *Oreochromis niloticus*

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## ABSTRACT

Stocking density (SD) is one of the key factors influencing the perceived level of stress in fish. The present study was designed to evaluate the effects of dietary graded levels of a new commercial probiotic Pro-Grow® and SD on adult Nile tilapia *Oreochromis niloticus*, concerning rearing water quality, growth performance, feed utilization, and fish carcass composition for 15 weeks. Fish with an average initial body weight (55.75 ± 0.6 g) were distributed into six treatments (three replicates per treatment). Fish were stoked at 40 and 80 fish / $m^3$ and fed Pro-Grow® probiotic at three levels (0, 10 and 20 g / kg diet) for each SD. The obtained results revealed that the high SD had negative significant ($P \leq 0.05$) effects on rearing water quality (dissolved oxygen and total nitrogen ammonia, but still within the acceptable ranges for rearing *O. niloticus*), growth performance, feed utilization and fish carcass composition (ash and crude protein) parameters compared to the low SD. However, addition of dietary Pro-Grow® significantly improved these adversely effects of high SD on fish, especially at high level 20 g / kg diet followed by 10 g / kg diet compared to the control group. Hence, the significantly interaction between high SD and high level of Pro-Grow® was detected in most of the above parameters. So, it could be concluded that the high level (20 g / kg diet) of Pro-Grow® probiotic is useful with high SD (80 fish / $m^3$) for enhancing the production performance of *O. niloticus*.

**Keywords:** Nile tilapia – probiotic –stocking density - growth performance – feed utilization.

## INTRODUCTION

Tilapias are one of the most important fish species for freshwater aquaculture and represent a major protein source in many developing countries (Pullin, 1997). Tilapias have become increasingly popular for farming as they are able to reproduce rapidly, easily bred in captivity, tolerate a wide range of environmental conditions, and highly resistant to diseases. Though the fish originated in Africa, and Asian countries have become the leading producers of these fishes (Rana, 1997). Tilapias are second only to carps as the most widely farmed freshwater fish in the world (FAO, 2010). Aquaculture has been increasing in recent decades as a consequence of the increase of fish consumption, since fisheries have possibly reached their maximum due to overexploitation (Ferreira *et al*., 2008). The rapid expansion and intensification of aquaculture production had led to the outbreaks of new pathogens and infectious diseases caused by viruses, bacteria and parasites, inflicting major problems in the fish farming industry (Geng *et al*., 2012).

Attractive feed may be looted and consumed quickly, thus reducing losses by leaching of essential water-soluble components. An addition of chemo-attractants to pelletized feeds may increase ingestion rates and improve growth, survival and food conversion (El- Sayed *et al*., 2005). Nowadays, a number of preparations of probiotics are commercially available and have been introduced to fish, shrimp and molluscan farming as feed additives incorporated in pond water (Wang *et al*., 2005). Probiotics are defined as live microorganisms including many yeast and bacteria, which when administered in adequate amounts could enhance the growth and health of the host (Gatesoupe, 1999 and Irianto and Austin, 2002a). The research into the use of probiotics for aquaculture is increasing with the demand for environment-friendly sustainable aquaculture (Vine *et al*., 2006). Many studies have pointed out that probiotics in fish diet improved growth performance and nutrients utilization (Mehrim, 2009; Ghazalah *et al*., 2010 and Merrifield *et al*., 2010). The benefits of such supplements include improved feeding value and enzymatic contribution to digestion, and inhibition of pathogenic microorganisms, antimutagenic and anti-carcinogenic activity, besides increased immune response. Moreover, probiotic supplementation may provide vitamins, short chain fatty acids and/or digestive enzymes, and therefore may also contribute to host nutrition (Bairagi *et al*., 2002 and John *et al*., 2006).

Particular attention has been drawn to stocking density as one of the key factors influencing the perceived level of stress in fish (North *et al*., 2006). Fish may encounter different types of stress such as, thermal (Akhtar *et al*., 2012); stocking density (Lupatsch *et al*., 2010), anoxia, hypoxia, chemicals and pesticides (DeMicco *et al*., 2010 and Vani *et al*., 2011). To avoid these stressful conditions, intervention with immunostimulants, vaccines and probiotic bacteria, either as a feed supplement or in water, could trigger the defense system and thus ameliorate the harmful effects mediated by different stress factors (Ringo *et al*., 2012). Consequently, the present study was designed to evaluate the effects of graded levels (0, 10 and 20 g / kg diet) of a new commercial probiotic Pro-Grow® and stocking density (40 and 80 fish / $m^3$) on Nile tilapia *Oreochromis niloticus*, concerning rearing water quality, fish growth performance, feed utilization and carcass composition for 15 weeks.

## MATERIALS AND METHODS

**The experimental management:**

Healthy adult Nile tilapia *O. niloticus*, with an average initial body weight 55.75 ± 0.6 g were purchased from the Integrated Fish Farm at Al-Manzala (General Authority for Fish Resources Development – Ministry of Agriculture), Al-Dakahlia governorate, Egypt, and transported to the experimental fish farm at Faculty of Agriculture, Al-Mansoura Univ. via plastic bags with aerated water. Fish were stocked into a rearing fiberglass tanks (1

$m^3$ in volume) for two weeks as an adaptation period, during this period they fed a basal experimental diet. Fish were distributed into six experimental treatments as three replicates per each treatment (Table 1). Each tank (1 $m^3$ in volume) was supplied with an air stone connected with an electric compressor for water aeration. Fresh underground water was used to change half of the water volume in each tank every day. The water quality parameters were measured according to Abdelhamid (1996). Water temperature was measured two times daily (via a thermometer), while water pH (using Jenway Ltd., Model 350-pH-meter), dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter), and water $NH_3$-N (by direct nesslerization method using a CHEMets® test kits (CHEMetrics, Inc, USA) were measured two times day by day according to APHA (1992).

**Table 1: Details of the experimental treatments**

| Treat. | Details |
|---|---|
| $T_1$ | 40 fish / $m^3$ + Basal ration (BR) + 0 g Pro-Grow® / Kg diet |
| $T_2$ | 40 fish / $m^3$ + BR+ 10 g Pro-Grow®/ Kg diet |
| $T_3$ | 40 fish / $m^3$ + BR+ 20 g Pro-Grow®/ Kg diet |
| $T_4$ | 80 fish / $m^3$ + BR+ 0 g Pro-Grow® / Kg diet |
| $T_5$ | 80 fish / $m^3$ + BR+ 10 g Pro-Grow® / Kg diet |
| $T_6$ | 80 fish / $m^3$ + BR+ 20 g Pro-Grow® / Kg diet |

Pro-Grow® was manufactured by Zagro industry company LTD, Korea and distributed by Elyoser Medicine Trading Co., Egypt. One Kg contained *Saccharomyces cervisae*, 4000 × $10^{12}$ colony forming units (CFU), 14 × $10^{12}$ CFU of *Bacillus subtilis*, 150000 IU protease enzyme and 70000 IU amylase enzyme and up to 1 kg lime stone.

The ingredients of the experimental diet were bought from the local market for fish feed, but its proximate chemical analysis was carried out according to AOAC (2000), as shown in Table 2. The ingredients were ground to add the tested probiotic at levels of 0, 10 and 20 g / kg diet, and then referred to treatments No. $T_1$, $T_2$ and $T_3$, respectively, for 40 fish / $m^3$ and $T_4$, $T_5$ and $T_6$, respectively for 80 fish / $m^3$ (Table 1), then all diets were repelleted by manufacturing machine (pellets diameter 1mm). The experimental diets were introduced manually twice daily at 9 a.m. and 15 p.m. at 3% of the fish biomass in each tank for 9 weeks, then 2% for the rest 6 weeks of the experiment. The fish were weighed every two weeks by a digital scale (accurate to ± 0.01 g) to adjust their feed quantity according to the actual body weight changes of the fish present in each tank.

**Fish sampling and fish performance parameters:**

At the start and at the end of the experiment, fish samples were collected and kept frozen (- 20°C) till the proximate analysis of the whole fish body according to AOAC (2000). Their energy content was calculated according to NRC (1993), being 5.64, 9.44 and 4.11 kcal/g for CP, EE and total carbohydrate, respectively.

Fish growth performance parameters such as average total weight gain (AWG, g/fish), average daily gain (ADG, g/fish/day), relative growth rate

(RGR, %), specific growth rate (SGR, %/day) and survival rate (SR, %) were calculated. Feed conversion ratio (FCR), feed efficiency (FE, %), protein efficiency ratio (PER), protein productive value (PPV, %) and energy utilization (EU, %) were calculated acording to Abdelhamid (2009).

**Table 2: Ingredients and proximate chemical analysis (% on dry matter basis) of the experimental basal diet**

| Ingredients | % |
|---|---|
| Fish meal (65%) | 10 |
| Corn gluten (60 %) | 15 |
| Soybean meal (44%) | 30 |
| Yellow corn | 17 |
| Wheat bran | 17 |
| Vegetable oil | 5 |
| Molasses | 5 |
| Vit. & min. premix[1] | 1 |
| Nutrients composition | |
| Dry matter (DM, %) | 92.26 |
| Crude protein (CP, %) | 29.90 |
| Ether extract (EE, %) | 6.56 |
| Ash (%) | 8.27 |
| Total carbohydrate (%) | 55.27 |
| Gross energy (Kcal / 100 g DM) (GE)[2] | 457.7 |
| Protein/energy (P/E) ratio (mg CP / Kcal GE) | 65.32 |

**[1] Vit. & min premix each 1 Kg premix contains; Vit. A, 12000,000 IU; Vit. $D_3$, 3000,000 IU; Vit. E, 10,000 mg; Vit. $K_3$, 3000 mg; Vit. $B_1$ 200 mg; Vit. $B_2$, 5000 mg; Vit. $B_6$, 3000 mg; Vit. $B_{12}$, 15 mg; Biotin, 50 mg; Folic acid 1000 mg; Nicotinic acid 35000 mg; Pantothenic acid 10,000 mg; Mn 80g; Cu 8.8g; Zn 70 g; Fe 35 g; I 1g; Co 0.15g and Se 0.3g).**

**[2] GE (Kcal/100 g DM) = (CP x 5.64) + (EE x 9.44) + (total carbohydrate x 4.11) calculated according to NRC (1993).**

### Statistical analysis:

The obtained data was statistically analyzed using general liner models (GLM) procedure according to SAS (2001) for users' guide (version 9.2), with factorial design (2 × 3 × 3) by using the following model:

$$Yijk = \mu + Li + Mj + LMij + eijk$$

Where, Yijk is the data of water quality, growth performance, feed utilization, and carcass composition of Nile tilapia; μ is the overall mean; Li is the fixed effect of the stocking density (40 and 80 fish / $m^3$); Mj is the fixed effect of the probiotic levels (0, 10 and 20 g / Kg diet); LMij is the interaction effect between the stocking density and probiotic levels and eijk is the random error. Ratio and percent data were arcsine-transformed prior to statistical analyses. The differences between mean were compared for the significance ($P \leq 0.05$) using Duncan's multiple rang test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Quality measurements of rearing water:

Data in Table 3 showed the quality parameters of rearing water for adult *O. niloticus* reared under two stocking densities (SDs, 40 and 80 fish / $m^3$) and fed different levels (0, 10 and 20 g / kg diet) of tested probiotic Pro-

Grow®. The current results indicated that water dissolved oxygen (mg / l) was significantly (P ≤ 0.05) decreased, while water $NH_3$-N was significantly (P ≤ 0.05) increased by increasing the fish SD rate. However, no significant (P ≥ 0.05) differences in both water temperature or pH-value were detected by increasing the SD rate.

On the other hand, no significant (P ≥ 0.05) differences in all tested water quality parameters were observed of fish groups fed different levels of probiotic or diet free probiotic group. Also, the interaction between SD and Pro-Grow® did not show any significant differences in all water quality parameters, except in case of the interaction between the high SD (80 fish / $m^3$) and dietary Pro-Grow®, where fish fed diet supplemented with Pro-Grow® had significantly (P ≤ 0.05) decreased the water $NH_3$-N compared to the control group.

**Table 3: Effect of stoking density (fish / $m^3$), dietary probiotic (Pro-Grow®, g/kg diet) levels and their interaction on water quality parameters of rearing adult *Oreochromis niloticus*.**

| Treat. | Temperature (°C) | pH-value | Dissolved oxygen (mg / l) | $NH_3$-N (mg / l) |
|---|---|---|---|---|
| Stocking density (SD, fish / $m^3$) | | | | |
| 40 | 23.15 | 6.651 | 5.32$^a$ | 0.003$^b$ |
| 80 | 23.50 | 6.549 | 3.61$^b$ | 0.006$^a$ |
| ± SE | 0.097 | 0.039 | 0.171 | 0.007 |
| P- value | 0.013 | 0.067 | 0.001 | 0.006 |
| Probiotic (P, g / kg diet) | | | | |
| 0 | 23.20 | 6.56 | 4.67 | 0.006 |
| 10 | 23.23 | 6.60 | 4.41 | 0.004 |
| 20 | 23.54 | 6.62 | 4.30 | 0.004 |
| ± SE | 0.119 | 0.48 | 0.209 | 0.008 |
| P- value | 0.084 | 0.671 | 0.432 | 0.117 |
| SD * P | | | | |
| 40*0 | 23.01 | 6.60 | 5.44 | 0.004 |
| 40*10 | 23.11 | 6.65 | 5.32 | 0.003 |
| 40*20 | 23.35 | 6.69 | 5.19 | 0.003 |
| 80*0 | 23.39 | 6.53 | 3.91 | 0.009$^a$ |
| 80*10 | 23.36 | 6.55 | 3.50 | 0.005$^b$ |
| 80*20 | 23.74 | 6.60 | 3.41 | 0.005$^b$ |
| ± SE | 0.168 | 0.068 | 0.296 | 0.001 |
| P- value | 0.909 | 0.902 | 0.877 | 0.050 |

**Mean in the same column having different small letters are significantly different (P ≤ 0.05).**

Water quality plays a significant role in the biology and physiology of fish and may impact on the health and productivity of the culture system (Landau, 1992 and Boyd, 1997). Increase in SD may cause deterioration in water quality, resulting in stressful conditions (Pankhurst and Van Der Kraak, 1997). In this topics, negative correlationship between high SD and fish rearing water quality parameters was detected (Mehrim, 2009 and M'balaka *et al*., 2012). However, in the present study, all the water quality parameters were within the acceptable ranges as recommended for rearing *O. niloticus*

(Boyd, 1982 and Ross, 2000), as well as were comparable with those reported by Tharwat (2007). Consequently, it can be noted that the tested probiotic enhanced the water quality parameters under this condition of rearing fish under high SD. This improvement of rearing water quality parameters may be due to the tested probiotic formula, which it contains *S. cervisae*, 4000 × $10^{12}$ CFU, and 14 × $10^{12}$ CFU of *B. subtilis*. From the other hand, this improvement of rearing water quality not only refluxing on fish growth performance or survival rate (Table 4), but also may be on the total fish production, fish health, and final product quality, besides the economic efficiency or the expected friendly environmental effects.

Improved water quality has especially been associated with *Bacillus* sp. The rationale is that Gram-positive bacteria are better converters of organic matter back to $CO_2$ than Gram-negative bacteria. During the production cycle, high levels of Gram-positive bacteria can be minimize 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* (Dalmin *et al*., 2001). These microorganisms (*S. cervisae* and *B. subtilis*) inclusion in the tested probiotic played a potential role for improvement the rearing water quality parameters, which confirmed also by Mehrim (2009) on *O. niloticus* reared under different SD conditions. In this respect, Verschuere *et al.* (2000) suggested a new definition of a probiotic for aquatic environments: 'a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host's response towards disease, or by improving the quality of its ambient environment', or that 'a probiotic is an entire microorganism or its components that are beneficial to the health of the host' (Irianto and Austin, 2002a).

**Growth performance parameters:**

Growth performance parameters of adult *O. niloticus* reared under different SD rates (40 and 80 fish / $m^3$) and fed different levels (0, 10 and 20 g / kg diet) of Pro-Grow® are illustrated in Table 4. The results revealed that high SD (80 fish / $m^3$) caused significant ($P \leq 0.05$) decreases in all growth performance parameters compared to the low SD (40 fish / $m^3$), while survival rate (SR, %) not significantly ($P \geq 0.05$) affected by increasing SD rate. From other hand, fish fed 20 g Pro-Grow® / kg diet reflected significant ($P \leq 0.05$) increases in all growth performance parameters compared to fish fed 10 or 0 g Pro-Grow® / kg diet.

No significant ($P \geq 0.05$) differences were found in all growth performance parameters in the interaction between SD (40 fish / $m^3$) and all dietary probiotic levels. However, fish reared at SD (40 fish / $m^3$) and fed 10 g Pro-Grow®/ kg diet had highest ($P \leq 0.05$) SR among other treatments. Meanwhile, all growth performance parameters significantly increased in case of fish reared at high SD (80 fish / $m^3$) and fed 20 g Pro-Grow®/ kg diet among other treatments. From the other hand, no significant differences were detected in SR of the interaction between high SD (80 fish / $m^3$) and all probiotic levels.

**Table 4: Effect of stoking density (fish / m$^3$), dietary probiotic (Pro-Grow®, g / kg diet) levels and their interaction on growth performance of adult *Oreochromis niloticus***

| Treat. | Final weight (g) | Total weight gain (g) | ADG (g/fish/day) | RGR (%) | SGR (% / d) | SR (%) |
|---|---|---|---|---|---|---|
| Stocking density (SD, fish / m$^3$) | | | | | | |
| 40 | 135.1$^a$ | 79.45$^a$ | 0.756$^a$ | 142.4$^a$ | 0.843$^a$ | 97.88 |
| 80 | 110.5$^b$ | 54.84$^b$ | 0.522$^b$ | 98.34$^b$ | 0.650$^b$ | 96.88 |
| ± SE | 0.920 | 0.920 | 0.008 | 1.663 | 0.007 | 0.777 |
| P- value | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.381 |
| Probiotic (P, g / kg diet) | | | | | | |
| 0 | 119.9$^b$ | 64.20$^b$ | 0.611$^b$ | 115.1$^b$ | 0.722$^b$ | 95.00$^b$ |
| 10 | 121.9$^b$ | 66.20$^b$ | 0.630$^b$ | 118.6$^b$ | 0.738$^b$ | 99.00$^a$ |
| 20 | 126.7$^a$ | 71.05$^a$ | 0.676$^a$ | 127.4$^a$ | 0.780$^a$ | 98.16$^a$ |
| ± SE | 1.127 | 1.133 | 0.010 | 2.025 | 0.008 | 0.952 |
| P- value | 0.003 | 0.003 | 0.003 | 0.003 | 0.001 | 0.02 |
| SD * P | | | | | | |
| 40*0 | 134.1 | 78.40 | 0.746 | 140.6 | 0.835 | 95.00$^c$ |
| 40*10 | 136.1 | 80.40 | 0.765 | 144.1 | 0.849 | 100.0$^a$ |
| 40*20 | 135.3 | 79.56 | 0.757 | 142.6 | 0.844 | 98.66$^b$ |
| 80*0 | 105.7$^b$ | 50.00$^b$ | 0.476$^b$ | 89.63$^b$ | 0.609$^b$ | 95.00 |
| 80*10 | 107.7$^b$ | 52.00$^b$ | 0.495$^b$ | 93.23$^b$ | 0.627$^b$ | 98.00 |
| 80*20 | 118.2$^a$ | 62.53$^a$ | 0.595$^a$ | 112.1$^a$ | 0.716$^a$ | 97.66 |
| ± SE | 1.594 | 1.602 | 0.015 | 2.864 | 0.835 | 1.347 |
| P- value | 0.005 | 0.005 | 0.005 | 0.005 | 0.002 | 0.783 |

**Mean in the same column having different small letters are significantly different ($P \leq 0.05$).**

**ADG: Average daily gain; RGR: Relative growth rate; SGR: Specific growth rate; SR: Survival rate.**

The current results revealed that the high SD had a negative effects on all growth performance parameters. However, addition of Pro-Grow®, especially at high level (20 g / kg diet), enhancing this picture. This improvement may be due to the probiotic itself, its formula with microorganisms (*S. cervisae*, 4000 × 10$^{12}$ CFU and 14 × 10$^{12}$ CFU of *B. subtilis*), enzymes (150000 IU protease and 70000 IU amylase) and up to 1 kg lime stone. Also, the positive effects of probiotic on the rearing water quality parameters (Table 3), may be led to decrease the stress effects of high SD on fish, increase the feed intake (Table 5), and the growth performance of fish (Table 4).

Regarding the depressing effects of high SD on fish growth performance and SR, the results of the present study are in agreement with those obtained by Khattab *et al*. (2004a) and Bakeer *et al*. (2007) for tilapia and EL-Haroun (2007) for catfish. Moreover, Abdelhamid *et al*. (2007b) reported that raising SDs (2, 3 and 4g fish / l) significantly ($P \leq 0.05$) decrease the growth performance of *O. niloticus*. Additionally, Sorphea *et al*. (2010) reported that growth performance and SR are adversely affected by high SDs. But, in some cases this effect is either temporary (Garr *et al*., 2011) or absent (Gokcek and Akyurt, 2007 and Southworth *et al*., 2009). From other hand, some fish species like tilapias can tolerate extreme crowding although competition for food will then limit their growth and lead to poor weight gain (Stickney, 1994). Social interactions through competition for food and/or space can negatively affect fish growth, hence higher SD leads to increased stress and that resulting increase in energy requirements causing a reduction in growth rates and food utilization. Moreover, tilapia is a territorial and aggressive fish so that the density effect on growth might be explainable by their competition for territories, as well as the permanent stress caused by crowding (Yi *et al*.,1996 and Huang and Chiu,1997).

Functional additive, like probiotics, is a new concept on aquaculture (Li and Gatlin III, 2004), where the additions of microorganisms on diets show a positive effect on growth caused by the best use of carbohydrates, protein, and energy (Irianto and Austin, 2002 a, b). In this respect, Marzouk *et al*. (2008) reported that probiotics (*B. subtillis* and *S. cerevisae*) revealed significant improvement in growth parameters of *O. niloticus*. Better results of *O. niloticus* growth performance related with dietary supplemented yeast were reported (Olvera *et al*., 2001 and Lara-Flores *et al*., 2010). In addition, many studies concluded the positive effect of using viable microorganisms in probiotic mixtures into diets of fish (Barnes *et al*., 2006 and Abo-State *et al*., 2009). Moreover, Lara-Flores and Olvera-Novoa (2013) reported that *O. niloticus* fed native bacteria supplemented diets presented significantly higher growth and feeding performance than those fed control diet.

All the probiotic-supplemented diets resulted in fish growth higher than that of the control diets, suggesting that the addition of probiotics mitigated the effects of the stress factors. In this topic, many researchers suggested the significantly positive effects of some commercial probiotics on *O. niloticus* growth performance (Eid and Mohamed, 2008; Mehrim, 2009 and Khalil *et al*., 2012). Recently, Abdelhamid *et al*. (2013 b) suggested the same positive effects on growth performance of *O. niloticus* fed some dietary biological additives. Conversely, Shelby *et al*. (2006) reported that the probiotic used with juvenile channel catfish diet had lack effect on specific growth promoting or immune stimulating aspects. Also, He *et al*. (2009) found that supplementation of dietary DVAQUA® showed no effects on growth performance, feed conversion and survival rate of the hybrid tilapia (*O. niloticus* ♀ × *O. aureus* ♂). The reasons for the differences between fish species have not been elucidated, but might be due to the differences in aquaculture and physiological conditions, besides the type of basal ingredients in diets. Accordingly, to the positive results of the tested probiotic on growth performance in the present study and those obtained by other attempts; probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by breakdown of indigestible components (Irianto and Austin, 2002a).

**Feed and nutrients utilization**:

Results of feed intake and nutrients utilization parameters of adult *O. niloticus* reared at either SDs (40 and 80 fish / $m^3$) and fed different levels (0, 10 and 20 g / kg diet) of Pro-Grow® probiotic are shown in Table 5. Fish reared at high SD (80 fish / $m^3$) had higher FCR and significantly ($P \leq 0.05$) decreased all feed and nutrients utilization parameters compared to fish reared at low SD (40 fish / $m^3$). On the other hand, fish fed dietary 20 g Pro-Grow® / kg diet showed significant ($P \leq 0.05$) increases of FI, PER, PPV, EU and the best FCR compared to other probiotic levels.

No significant ($P \geq 0.05$) differences in all feed and nutrients utilization parameters were detected in case of the interaction between the low SD and all probiotic levels. among all treatments. Meanwhile, fish reared at high SD (80 fish / $m^3$) and fed the high level of tested probiotic (20 g / kg diet) had the highest ($P \leq 0.05$) FI, PER, PPV, EU and the best FCR among other treatments.

**Table 5: Effect of stoking density (fish / $m^3$), dietary probiotic (Pro-Grow®, g / kg diet) levels and their interaction on feed and nutrients utilization of adult *Oreochromis niloticus***

| Treat. | FI (g / fish) | FCR | PER | PPV (%) | EU (%) |
|---|---|---|---|---|---|
| Stocking density (SD, fish / $m^3$) | | | | | |
| 40 | 132.9$^a$ | 1.66$^b$ | 2.01$^a$ | 28.84$^a$ | 18.15$^a$ |
| 80 | 117.3$^b$ | 2.12$^a$ | 1.57$^b$ | 23.08$^b$ | 16.89$^b$ |
| ± SE | 0.609 | 0.026 | 0.010 | 0.231 | 0.140 |
| P- value | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Probiotic (P, g / kg diet) | | | | | |
| 0 | 124.1$^b$ | 1.96$^a$ | 1.73$^b$ | 25.73$^b$ | 16.56$^b$ |
| 10 | 121.7$^c$ | 1.87$^b$ | 1.82$^a$ | 25.77$^b$ | 17.84$^a$ |
| 20 | 129.8$^a$ | 1.83$^c$ | 1.83$^a$ | 26.38$^a$ | 18.15$^a$ |
| ± SE | 1.641 | 0.032 | 0.012 | 0.282 | 0.171 |
| P- value | 0.02 | 0.004 | 0.003 | 0.04 | 0.003 |
| SD * P | | | | | |
| 40*0 | 133.2 | 1.71 | 1.95 | 28.60 | 17.57 |
| 40*10 | 130.3 | 1.59 | 2.10 | 29.07 | 19.17 |
| 40*20 | 135.3 | 1.68 | 1.98 | 27.84 | 17.72 |
| 80*0 | 114.9$^b$ | 2.21$^a$ | 1.51$^b$ | 22.86$^b$ | 15.56$^c$ |
| 80*10 | 112.5$^b$ | 2.16$^a$ | 1.54$^b$ | 21.47$^b$ | 16.51$^b$ |
| 80*20 | 124.4$^a$ | 1.99$^b$ | 1.68$^a$ | 24.92$^a$ | 18.59$^a$ |
| ± SE | 2.322 | 0.045 | 0.017 | 0.400 | 0.242 |
| P- value | 0.007 | 0.004 | 0.003 | 0.009 | 0.001 |

**Mean in the same column having different small letters are significantly different ($P \leq 0.05$).**

**FI: Feed intake; FCR: Feed conversion ratio; PER: Protein efficiency ratio; PPV: Protein productive value; EU: Energy utilization.**

Similarly, with the obtained results in the present study, feed efficiency was adversely affected by high SD. In this respect, Yousif (2002) reported that it is a generally accepted principle, that increasing the number of fish (density) will adversely affect fish feed intake and nutrients utilization. Additionally, Abdelhamid *et al*. (2007b) suggested that increasing SD rate of fish led to significantly ($P \leq 0.05$) increased feed conversion ratio of *O. niloticus*.

As the positive effects of the tested probiotic in the present study, especially at the high level Burr *et al*. (2005) reported that the increased nutrient digestibility associated with prebiotic or probiotic supplementation may be due to the favored microbial community producing enzymes that are either lacking or occurring only at low levels in the host. In addition, probiotics' inclusion of enzymes leads to improvement of growth and feed utilization (Saxena, 2008), since they lead to digestive enzyme activation (Xu *et al.,* 2009). Most probiotics colonize the host and affect the digestive processes through increased numbers and production of microbial enzymes, improving the intestinal microbial balance and consequently the digestibility and absorption of feed and feed utilization (El-Haroun *et al*., 2006 and Mohapatra *et al*., 2012). The supplementation of commercial live yeast, *S. cerevisiae*, improved growth and feed utilization of Nile tilapia (Lara-Flores *et al*., 2003 and Abdel-Tawwab *et al*., 2008). The improved fish growth and feed utilization may possibly be due to improved nutrient digestibility. In this regard, Tovar *et al*. (2002) and Waché *et al*. (2006) found that the addition of live yeast improved diet and protein digestibility, which may explain the better growth and feed efficiency seen with yeast supplements. In addition, Abdel-Tawwab *et al*. (2008) confirmed that the better feed intake with yeast supplemented diets (1.0–5.0 mg / kg diet) may have been due to increased fish appetite resulting in a higher feed intake and therefore improved growth. In this regard, Mehrim (2009) found that Biogen® probiotic had improved growth and feed efficiency of *O. niloticus*. Furthermore, Khalil *et al.* (2012) reported that Hydroyeast Aquaculture® probiotic led to significant improvement of feed and nutrients utilization parameters in both adult *O. niloticus* male and female. Also, recently Abdelhamid *et al*. (2013 c) suggested the same positive effects on feed efficiency of *O. niloticus* fed some dietary biological additives.

**Fish carcass composition:**

Proximate chemical analysis of the whole body of adult *O. niloticus* at the start or at the end of the experiment was summarized in Table 6. Fish reared at high SD (80 fish / $m^3$) had significantly ($P \leq 0.05$) increased DM, EE and EC, while ash and CP were significantly decreased compared to the low SD rate (40 fish / $m^3$). While, fish fed Pro-Grow® at both levels (10 or 20 g / kg diet) showed significant ($P \leq 0.05$) decrease of ash and CP, and significant increase in EE and EC contents of fish carcass compared to the free probiotic diet (control group). However, no significant ($P \geq 0.05$) differences were found in DM among all dietary probiotic levels.

From the other hand, no significant ($P \geq 0.05$) differences in all fish carcass composition parameters in case of fish reared at low SD (40 fish / $m^3$) and fed different levels of Pro-Grow®. However, fish reared at high SD (80 fish / $m^3$) and fed different levels of Pro-Grow® probiotic showed significant ($P \leq 0.05$) increase of EE and EC, while ash was significantly decreased compared to the control group. However, no significant ($P \geq 0.05$) differences in DM or CP were detected among all treatments.

**Table 6: Effect of stoking density (fish / m³), dietary probiotic (Pro-Grow®, g / kg diet) levels and their interaction on carcass composition of adult *Oreochromis niloticus***

| Treat. | DM (%) | On dry matter basis (%) | | | |
|---|---|---|---|---|---|
| | | Ash | CP | EE | EC (Kcal / 100 g) |
| At the start of the experiment | | | | | |
| | 21.69 | 15.23 | 63.90 | 20.80 | 556.70 |
| At the end of the experiment | | | | | |
| Stocking density (SD, fish / m³) | | | | | |
| 40 | 23.57$^b$ | 16.97$^a$ | 59.98$^a$ | 23.04$^b$ | 555.80$^b$ |
| 80 | 24.67$^a$ | 15.70$^b$ | 57.71$^b$ | 26.57$^a$ | 576.40$^a$ |
| ± SE | 0.116 | 0.208 | 0.171 | 0.152 | 1.592 |
| P- value | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Probiotic (P, g / kg diet) | | | | | |
| 0 | 24.20 | 17.10$^a$ | 59.50$^a$ | 23.39$^b$ | 556.40$^b$ |
| 10 | 23.94 | 15.95$^b$ | 58.50$^b$ | 25.54$^a$ | 571.10$^a$ |
| 20 | 24.22 | 15.95$^b$ | 58.53$^b$ | 25.50$^a$ | 570.90$^a$ |
| ± SE | 0.143 | 0.255 | 0.209 | 0.187 | 1.950 |
| P- value | 0.321 | 0.011 | 0.008 | 0.001 | 0.002 |
| SD * P | | | | | |
| 40*0 | 23.65 | 17.06 | 60.55 | 22.38 | 552.80 |
| 40*10 | 23.63 | 16.78 | 59.75 | 23.46 | 558.50 |
| 40*20 | 23.42 | 17.06 | 59.64 | 23.29 | 556.30 |
| 80*0 | 24.75 | 17.14$^a$ | 58.45 | 24.40$^b$ | 560.00$^b$ |
| 80*10 | 24.25 | 15.12$^b$ | 57.25 | 27.62$^a$ | 583.70$^a$ |
| 80*20 | 25.02 | 14.85$^b$ | 57.43 | 27.71$^a$ | 585.50$^a$ |
| ± SE | 0.202 | 0.361 | 0.296 | 0.264 | 2.758 |
| P- value | 0.09 | 0.020 | 0.783 | 0.001 | 0.004 |

**Mean in the same column having different small letters are significantly different ($P \leq 0.05$).**
**DM: Dry matter; CP: Crude protein; EE: Ether extract; EC: Energy content.**

As the current findings in fish carcass composition affected by SD, where stress increases mineral and vitamin mobilization from tissues and their excretion (McDowell 1989), and thus may exacerbate a marginal mineral deficiency or lead to increase mineral requirement. 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 SD rate of tilapia fish. Also, Mehrim (2009) suggested the same negative effects on *O. niloticus* carcass composition by increasing SD rate. On the other hand, yeast supplementation significantly affected the whole-fish body composition (Abdel-Tawwab *et al*., 2008). These results suggest that yeast supplementation plays a role in enhancing feed intake with a subsequent enhancement of fish body composition. The proximate chemical analysis of *O. niloticus* whole body including total lipids and total ash was significantly influenced by dietary protein level only; meanwhile, yeast supplements significantly affected ash content (Abdel-Tawwab, 2012). On the other hand, changes in protein and lipid content in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate (Soivio *et al*., 1989 and Abdel-Tawwab *et al*., 2006).

The results in the present study are in close agreement with those reported by Khattab *et al.* (2004a), 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 to the control treatment. Furthermore, Mehrim (2009) reported positive effects of dietary inclusion of the commercial probiotic Biogen® at a level of 3 g / kg on carcass composition of *O. niloticus* fingerlings reared under different stocking densities. Recently, Abdelhamid *et al*. (2013 a) found the same positive effects on carcass composition of *O. niloticus* fed some dietary biological additives. Additionally, Khalil *et al.* (2012) studied the effect of Hydroyeast Aquaculture® probiotic with both adult males and females of *O. niloticus* and they suggested that fish carcass composition took unclear trends between adult males and females within all treatments, which may be due to the differ in sexes, metabolism, physiological responses and sexual behaviors of fish during this stage of life. Generally, there is a negative relationship between crude proteins and crude fats in the chemical composition of Nile tilapia carcass on one hand (El-Ebiary and Zaki, 2003 and Abdelhamid *et al*., 2007a), and a positive correlation between crude protein and crude ash contents of Nile tilapia, on the other hand (Abdelhamid *et al*., 1998 and 2007b).

Finally, it could be concluded that the high level (20 g / kg diet) of Pro-Grow® probiotic is useful with high SD (80 fish / $m^3$) for enhancing the production performance of *O. niloticus.* Additionally, many studies are required in this level of probiotic, which may be led to increasing the economic efficiency at large scale or high fish intensive system in the fish farms, besides the expected friendly environmental effects.

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**البروبيوتيك الغذائي برو- جرو ضد إجهاد كثافة التخزين العالية:**
**١- التأثير على جودة المياه، أداء النمو، الاستفادة الغذائية ومكونات الجسم لأسماك البلطي النيلي**

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تُعد كثافة التخزين واحدة من العوامل التي يلاحظ مستوي تأثيرها فى الأسماك. صُممت الدراسة الحالية لتقييم تأثيرات إضافة مستويات متدرجة من البروبيوتيك التجاري الجديد ®Pro-Grow وكثافة التخزين على أسماك البلطي النيلي الناضجة فيما يتعلق بجودة المياه، كفاءة النمو، الأداء والاستفادة الغذائية و مكونات الجسم لمدة ١٥ أسبوعاً. وُزعت الأسماك ذات الوزن الإبتدائي (٥٥,٧٥ ± ٠,٦ جم) على ٦ معاملات (٣ مكررات لكل معاملة). خُزنت الأسماك بكثافتي تخزين ٤٠ ، ٨٠ سمكة / م٣ وغذيت على البروبيوتيك ®Pro-Grow بثلاث مستويات (صفر، ١٠ ، ٢٠ جم / كجم علف) لكل كثافة تخزين. أوضحت النتائج المتحصل عليها أن كثافة التخزين العالية كانت ذات تأثيرات سلبية معنوية علي قياسات جودة مياه رعاية الأسماك (الأكسجين الذائب ، نيتروجين الأمونيا الكلية، لكنها مازالت داخل المستويات المقبولة لرعاية أسماك البلطي النيلي)، كفاءة النمو، الاستفادة من الغذاء، مكونات جسم الأسماك (الرماد، البروتين الخام) مقارنة بكثافة التخزين المنخفضة. بينما إضافة البروبيوتيك الغذائي ®Pro-Grow حسنت معنوياً من هذه التأثيرات السيئة لكثافة التخزين العالية على الأسماك خاصة بالمستوي العالي ٢٠ جم / كجم علف يليه المستوى ١٠ جم / كجم علف مقارنة بالمجموعة الضابطة. لذا لوحظ التداخل المعنوي بين كثافة التخزين العالية و المستوى المرتفع من البروبيوتيك فى معظم القياسات السابقة. لذلك يمكن التوصية بأن المستوى العالي (٢٠ جم / كجم علف) من البروبيوتيك ®Pro-Grow مفيد مع كثافة التخزين العالية ٨٠ سمكة / م٣ لتحسين الأداء الإنتاجي لأسماك البلطي النيلي.