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Research Article
Impacts of Pro-Grow® on Growth Performance, Physiological, Immune Responses and Economic Efficiency of Adult *Oreochromis niloticus* (Linnaeus, 1758) under Stocking Density Stress

Ahmed Ismail Mehrim, Fathy Fatoh Khalil, Mohamed Moaaz Refaey and Aram Muhamad Abdalqadir

Department of Animal Production, Faculty of Agriculture, Mansoura University, Al-Mansoura, PC. 35516, Egypt

Abstract

Background and Objective: Fish intensification had led to the outbreaks of stress effects and inflicting major problems in the fish farming industry. Hence, the objective of the present study was to evaluate the effects of dietary probiotic Pro-Grow® contrary to the stocking density stress (SD) on adult *Oreochromis niloticus* (*O. niloticus*) for 15 weeks. **Methods:** The present study was designed to evaluate the effects of graded levels of Pro-Grow® (0, 10 and 20 g kg^{-1} diet) against SD (40 and 80 fish m^{-3}) stress on water quality parameters, growth performance, physiological, immune responses, besides the economic efficiency parameters of adult *O. niloticus*. Data was analyzed using SAS. **Results:** The results revealed that increasing SD (80 fish m^{-3}) significantly ($p \leq 0.05$) decreased the water dissolved oxygen, while water NH_3-N was significantly increased. Also, high SD had drastically effects on all growth and feed efficiency parameters, body composition (ash and crude protein), hematological parameters (hemoglobin, red blood cells and white blood cells) and the economic efficiency compared to *O. niloticus* reared at low SD (40 fish m^{-3}). No significant ($p \geq 0.05$) effects of all tested immune responses parameters of fish reared at both SDs rates. However, addition of 10 and 20 g Pro-Grow® kg^{-1} diet alleviated the severely effects of both SDs stress 40 and 80 fish m^{-3}, respectively on all above tested parameters. **Conclusion:** Thus, it could be concluded that the Pro-Grow® probiotic was successfully using at these levels into *O. niloticus* intensive production systems. Regarding its predicted high fish production, improving physiological responses, besides the high economic efficiency or the environmental friendly effects.

Key words: Aquaculture, intensive systems, Nile tilapia, probiotic, stocking density, immune, economic efficiency

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Corresponding Author: Ahmed Ismail Mehrim, Department of Animal Production, Faculty of Agriculture, Mansoura University, Al-Mansoura, PC. 35516, Egypt Tel: +201002915069

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tilapias are able to easily bred in captivity, tolerate a wide range of environmental conditions, reproduce rapidly and highly resistant to diseases. Thus, they are the most important fish species for freshwater aquaculture and represent a major protein source in many developing countries[1]. 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[2]. The rapid expansion and intensification of aquaculture production had led to the outbreaks of new pathogens and infectious diseases inflicting major problems in the fish farming industry[3].

Attractive food may be looted and consumed quickly, thus reducing losses by leaching of essential water-soluble components. Currently, number of probiotics are commercially available and have been introduced as food additives for fish, shrimp and molluscan[4]. Probiotics are defined as live microorganisms including many bacteria and yeast, which when administered in sufficient amounts could improve the growth and health of the host[5]. Immunomodulatory products, including probiotics are increasingly used in aquaculture production. Thus, the research into the use of probiotics for aquaculture is increasing with the demand for environment-friendly sustainable aquaculture[6]. Many studies have pointed out that probiotics in fish diet improved growth performance and nutrients utilization[7], physiological paramaters[8] and immune responses[9,10]. Moreover, probiotic supplementation may provide vitamins, short chain fatty acids and/or digestive enzymes and therefore may also contribute to host nutrition[11].

Particular attention has been drawn to stocking density (SD) as one of the key factors influencing the perceived level of stress in fish[12]. Fish may encounter different types of stress such as, thermal[13], SD[14], anoxia, hypoxia, chemicals and pesticides[15]. 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[16]. Moreover, the intensification systems of fish are more important subject not only in developed countries but also in developing countries for increasing the total fish production and partially for increasing the fish production per capita. Consequently, the present study was aimed to estimate the effects of graded levels of a new commercial probiotic Pro-Grow® (0, 10 and 20 g kg^{-1} diet) against SD (40 and 80 fish m^{-3}) stress on water quality, growth, feed performance, body composition, haematological, biochemical, immune responses and the economic efficiency parameters of adult *Oreochromis niloticus* for 15 weeks.

MATERIALS AND METHODS

Experimental procedures: This study was conducted during the summer season 2014 in Fish Research Unit, Faculty of Agriculture, Mansoura University, Al-Dakahlia Governorate, Egypt. Adult *O. niloticus*, with an average initial body weight (55.75±0.6 g) were purchased from Integrated fish farm at Al-Manzala (General Authority for Fish Resources Development-Ministry of Agriculture) Al-Manzala, Al-Dakhalia Governorate, Egypt. Fish were stocked into a rearing glass fiber 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 3 replicates per each (Table 1). Each tank was supplied with an air stone connected with electric compressor for aeration the water. Fresh underground water was used to change half of the water volume in each tank every day. Water quality parameters were determined as, water temperature was measured two times daily (via a thermometer). While, water pH, dissolved oxygen and NH_3-N were measured two times day by day. Water pH was measured using a digital Jenway Ltd., Model 350-pH-meter. Dissolved oxygen was measured using a digital Jenway Ltd., Model 970-dissolved oxygen meter. Water NH_3-N was determined by direct Nesslerization method using a CHEMets® test kits (CHEMetrics, Inc, USA) according to APHA[17]. 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.

Experimental diet and probiotic: 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[18], as shown in Table 2. The ingredients were ground to add the tested probiotic at levels of 0, 10, 20 g kg^{-1} 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 treatments for

Table 1: Details of the experimental treatments

Treatment	Details
T_1	40 fish m^{-3}+0 g Pro-Grow® kg^{-1} diet
T_2	40 fish m^{-3}+10 g Pro-Grow® kg^{-1} diet
T_3	40 fish m^{-3}+20 g Pro-Grow® kg^{-1} diet
T_4	80 fish m^{-3}+0 g Pro-Grow® kg^{-1} diet
T_5	80 fish m^{-3}+10 g Pro-Grow® kg^{-1} diet
T_6	80 fish m^{-3}+20 g Pro-Grow® kg^{-1} diet

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

Ingredient	%
Fish meal (65%)	10.00
Corn gluten (60%)	15.00
Soybean meal (44%)	30.00
Yellow corn	17.00
Wheat bran	17.00
Vegetable oil	5.00
Molasses	5.00
Vit. and min. premix*	1.00
Nutrient 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 (kj/100 g DM) (GE)**	1915.01
Protein/energy (P/E) ratio (mg CP/Kj GE)	15.61

*Vitamins and minerals premix each 1 kg 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 80 g: Cu 8.8 g: Zn 70 g: Fe 35 g: I 1 g, Co 0.15 g and Se 0.3 g). **GE (Kj/100 g DM) = (CP×5.64)+(EE×9.44)+(total carbohydrate×4.11) calculated according to NRC[19]

80 fish m^{-3} (Table 1), then all diets were repelleted by manufacturing machine (pellets size 1 mm). The experimental diets were introduced manually twice daily at 9 am and 15 pm at 3% of the fish biomass in each tank for 9 weeks, then 2% for the rest 6 weeks of the experiment. Pro-Grow® was manufactured by Zagro industry company Ltd., South Korea and distributed by Elyoser Medicine Trading Co., Egypt. One kg contained *Saccharomyces cervisae*, 4000×10^{12} CFU (colony forming units), 14×10^{12} CFU of *Bacillus subtilis*, 150000 IU protease enzyme, 70000 IU amylase enzyme and up to 1 kg lime stone.

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 body were done according to AOAC[18]. Their energy content was calculated according to NRC[19]. Fish growth and feed efficiencey parameters such as average weight gain (AWG, g $fish^{-1}$), average daily gain (ADG, g $fish^{-1}$ day^{-1}), specific growth rate (SGR, % day^{-1}), survival rate (SR, %), feed conversion ratio (FCR), protein efficiency ratio (PER) and energy utilization (EU, %) were calculated acording to Halver and Hardy[20].

Blood samples: At the end of the experiment, five fish from each tank were randomly collected, then fish anaesthetized by transferred in a small plastic tank containing 10 L water supplemented with 3 mL pure clove oil (dissolved in 10 mL absolute ethanol). For the hematological parameters analysis, blood samples, (5 mL of whole blood at each collection), were collected from the fish by puncturing caudal venous with a syringe needle and the samples were kept in small plastic vials containing heparin-anticoagulant. Other blood samples were collected in dried plastic tubes and centrifuged for 20 min at 3500 rpm to obtain the blood serum. Serum samples were kept in deep freezer (-20°C) until the biochemical analysis was carried out.

Hematological parameters: Whole blood samples were used for the determination of hemoglobin (Hb) using commercial colorimetric kits (Diamond Diagnostic, Egypt). Total red blood cells ($RBCs\times10^6$ mm^{-3}) and total white blood cells ($WBCs\times10^3$ mm^{-3}) were counted according to Dacie and Lewis[21] on an Ao Bright-Line Hämocytometer model (Neubauer improved, Precicolor HBG, Germany). The packed cell volume (PCV, %) was measured according to Stoskopf[22].

Biochemical and immune responses parameters: Biochemical constituents of serum were determined calorimetrically using commercial kits produced by Diagnostic System Laboratories, INC, USA. Total protein was determined according to the method described by Tietz[23]. Serum albumin was determined according to Doumas *et al.*[24]. The concentration of serum globulin was obtained by subtracting the albumin from the total serum protein concentration according to Doumas and Biggs[25]. Competitive ELISA was used to measure the immunoglobulin M (IgM) concentration in serum, which described by Magnadottir and Guomundsdottir[26].

Statistical analysis: The obtained data was statistically analyzed using SAS (Version 8.2)[27] for users guide, with factorial design (2×3) by the following model:

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

where, Yijk is the data of rearing water quality, growth and feed performance, carcass composition, hematological and biochemical parameters, immune responses and economic efficiency of *O. niloticus*, 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^{-1} diet), LMij is the interaction effect between the stocking density and probiotic levels and eijk is the random error. All ratios and percentages were arcsine transformed prior to statistical analyses. The differences between mean of treatments were

compared using Tukey's *post hoc* significant test and differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Rearing water quality measurements: Data in Table 3 showed water quality parameters of adult *O. niloticus* reared under high stocking densities (SDs, 40 and 80 fish m^{-3}) and fed different levels (0, 10 and 20 g kg^{-1} diet) of tested probiotic Pro-Grow®. The current results indicated that water dissolved oxygen was significantly ($p \leq 0.05$) decreased, while water NH_3-N was significantly ($p \leq 0.05$) increased by increasing the fish stoking density rate. However, no significant ($p \geq 0.05$) differences in both water temperature or pH-value were detected by increasing the stoking density rate. On the other hand, no significant 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 the significant differences in all water quality parameters, except in case of fish reared at high SD (80 fish m^{-3}) and fed different levels of Pro-Grow® had significantly ($p \leq 0.05$) decreased the water NH_3-N compared with the control group (Fig. 1).

Growth and feed efficiency parameters: Growth and feed efficiency parameters of adult *O. niloticus* reared under different SDs rates (40 and 80 fish m^{-3}) and fed different levels (0, 10 and 20 g kg^{-1} diet) of tested probiotic (Pro-Grow®) are illustrated in Table 4. The results revealed that increasing SD (80 fish m^{-3}) caused significantly ($p \leq 0.05$) decreased all growth and feed efficiency parameters (negatively affected on FCR) compared to the low SD (40 fish m^{-3}), while survival rate (SR, %) not significantly affected by increasing SD rate. From other hand, fish fed 20 g Pro-Grow® kg^{-1} diet was significantly ($p \leq 0.05$) increased all growth and feed efficiency parameters (gave the best FCR) compared to fish fed 10 or 0 g Pro-Grow® kg^{-1} diet, respectively.

No significant differences in all growth and feed efficiency parameters in the interaction between low SD (40 fish m^{-3}) and all dietary probiotic levels. However, fish reared in low SD

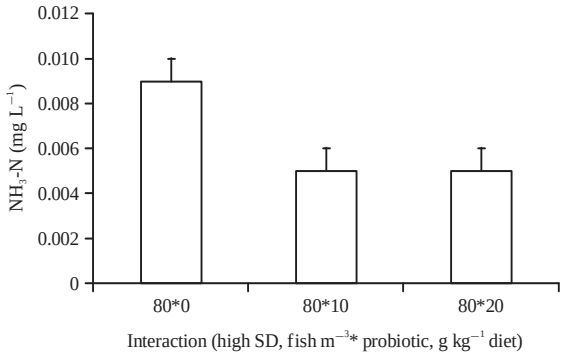

Fig. 1: Effect of the interaction of high stoking density (80 fish m^{-3}) and dietary probiotic Pro-Grow® (0, 10 and 20 g kg^{-1} diet) levels on rearing water NH_3-N of adult *Oreochromis niloticus*

Table 3: Effect of stoking density (fish m^{-3}) and dietary probiotic Pro-Grow® (g kg^{-1} diet) levels on rearing water quality parameters of adult *Oreochromis niloticus*

Treatments	Temperature (°C)	pH-value	Dissolved oxygen (mg L^{-1})	NH_3-N (mg L^{-1})
Stocking density (fish m^{-3})				
40	23.15±0.10	6.651±0.03	5.32±0.13^a	0.003±0.001^b
80	23.50±0.10	6.549±0.05	3.61±0.21^b	0.006±0.001^a
p-value	0.013	0.067	0.001	0.006
Probiotic (g kg^{-1} diet)				
0	23.20±0.12	6.56±0.05	4.67±0.28	0.006±0.002
10	23.23±0.12	6.60±0.05	4.41±0.20	0.004±0.001
20	23.54±0.12	6.62±0.05	4.30±0.19	0.004±0.001
p- value	0.084	0.671	0.432	0.117

Mean in the same column for each category having different small letters are significantly different ($p \leq 0.05$). Mean±SD

Table 4: Effect of stoking density (fish m^{-3}) and dietary probiotic Pro-Grow® (g kg^{-1} diet) levels on growth and feed efficiency parameters of adult *Oreochromis niloticus*

Treatments	Final weight (g)	AWG (g $fish^{-1}$)	ADG (g $fish^{-1}$ day^{-1})	SGR (% day^{-1})	SR (%)	FCR	PER	EU (%)
Stocking density (fish m^{-3})								
40	135.1±0.96^a	79.45±0.97^a	0.76±0.01^a	0.84±0.01^a	97.88±0.77	1.66±0.02^b	2.01±0.01^a	18.15±0.16^a
80	110.5±2.05^b	54.84±2.05^b	0.52±0.02^b	0.65±0.02^b	96.88±1.05	2.12±0.08^a	1.57±0.03^b	16.89±0.43^b
p-value	0.001	0.001	0.001	0.001	0.381	0.001	0.001	0.001
Probiotic (g kg^{-1} diet)								
0	119.9±6.50^b	64.20±6.50^b	0.61±0.06^b	0.72±0.05^b	95.00±1.29^b	1.96±0.21^a	1.73±0.09^b	16.56±0.55^b
10	121.9±6.42^b	66.20±6.42^b	0.63±0.06^b	0.74±0.05^b	99.00±0.45^a	1.87±0.19^b	1.82±0.09^a	17.84±0.48^a
20	126.7±3.84^a	71.05±3.84^a	0.68±0.04^a	0.78±0.03^a	98.16±0.75^a	1.83±0.10^c	1.83±0.05^a	18.15±0.18^a
p-value	0.003	0.003	0.003	0.001	0.02	0.004	0.003	0.003

Mean in the same column for each category having different small letters are significantly different ($p \leq 0.05$). Mean±SD

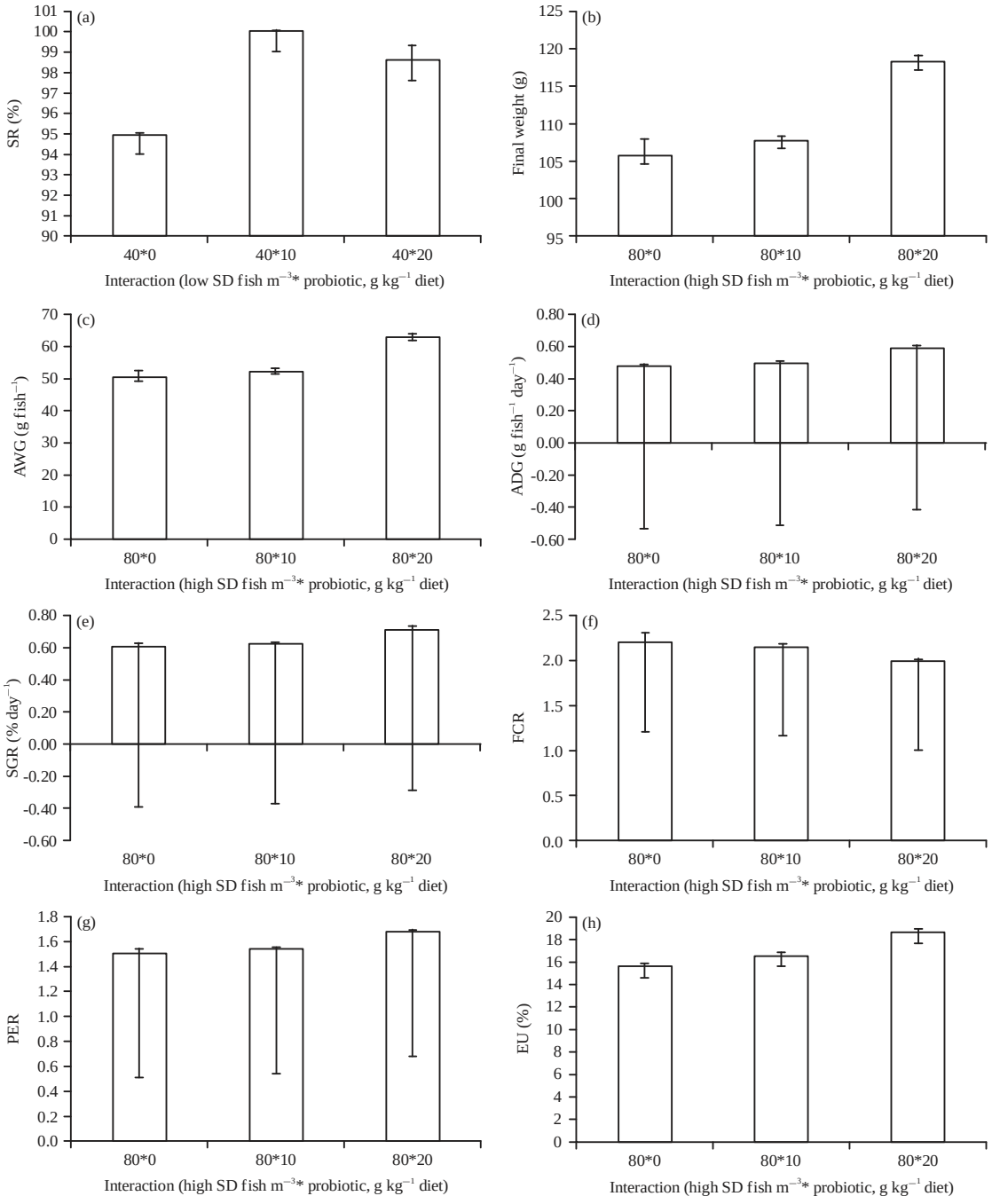

Fig. 2(a-h): Effect of the interaction of stoking density (40 and 80 fish m^{-3}) and dietary probiotic Pro-Grow® (0, 10 and 20 g kg^{-1} diet) levels on (a) Survival rate (%), (b) Final weight (g), (c) Average weight gain (g fish^{-1}), (d) Average daily gain (g fish^{-1} day^{-1}), (e) Specific growth rate (% day^{-1}), (f) Feed conversion ratio, (g) Protein efficiency ratio and (h) Energy utilization (%) of adult *Oreochromis niloticus*

(40 fish m^{-3}) and fed 10 g Pro-Grow® kg^{-1} diet had highest ($p \leq 0.05$) SR among other treatments (Fig. 2a). Meanwhile, all growth and feed efficiency parameters significantly increased in case of fish reared in high SD (80 fish m^{-3}) and fed 20 g Pro-Grow® kg^{-1} diet (Fig. 2b-e, g and h) and gave the best FCR among other treatments (Fig. 2f). 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.

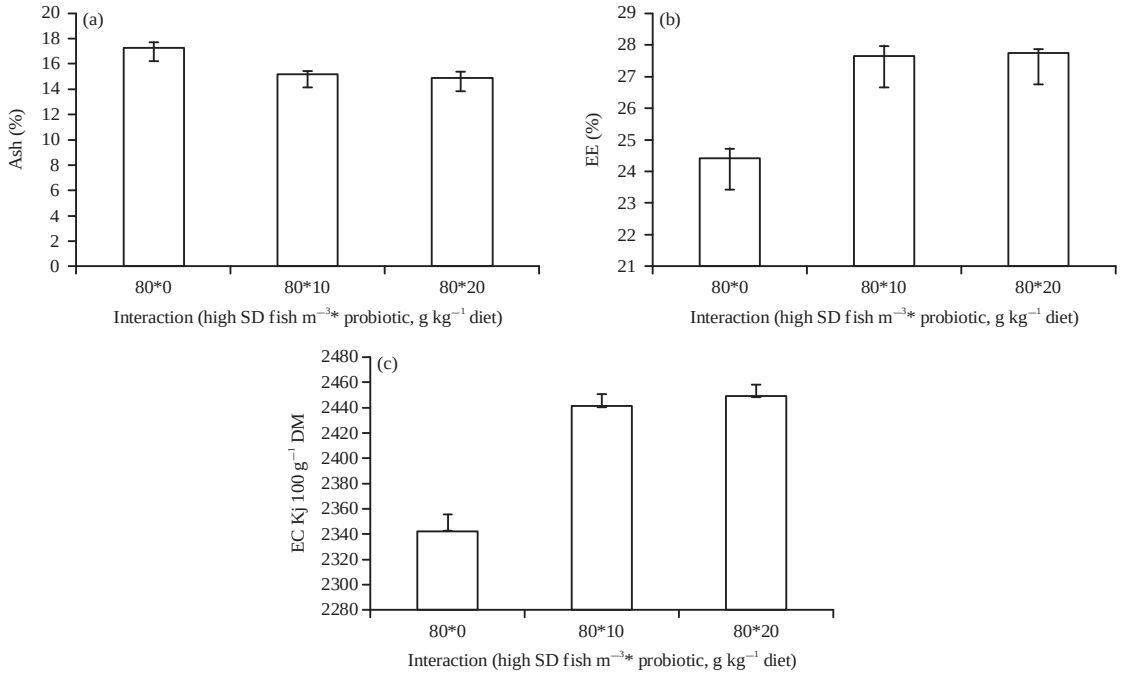

Fig. 3(a-c): Effect of the interaction of high stoking density (80 fish m^{-3}) and dietary probiotic Pro-Grow® (0, 10 and 20 g kg^{-1} diet) levels on some body composition parameters, (a) Ash (%), (b) Ether extract (%) and (c) Energy content Kj 100 g^{-1} DM of adult *Oreochromis niloticus*

Table 5: Effect of stoking density (fish m^{-3}) and dietary probiotic Pro-Grow® (g kg^{-1} diet) levels on body composition parameters of adult *Oreochromis niloticus*

		On dry matter basis (%)			
Treatments	DM (%)	Ash	CP	EE	EC (Kj/100 g)
At the start of the experiment					
	21.69	15.23	63.90	20.80	2329.23
At the end of the experiment					
Stocking density (fish m^{-3})					
40	23.57±0.1[b]	16.97±0.2[a]	59.98±0.4[a]	23.04±0.2[b]	2325.46±5.5[b]
80	24.67±0.2[a]	15.70±0.2[b]	57.71±0.2[b]	26.57±0.6[a]	2411.65±16.4[a]
p-value	0.001	0.001	0.001	0.001	0.001
Probiotic (g kg^{-1} diet)					
0	24.20±0.3	17.10±0.2[a]	59.50±0.5[a]	23.39±0.5[b]	2327.97±8.4[b]
10	23.94±0.2	15.95±0.4[b]	58.50±0.6[b]	25.54±1.0[a]	2389.48±20.9[a]
20	24.22±0.4	15.95±0.6[b]	58.53±0.6[b]	25.50±1.0[a]	2388.64±24.8[a]
p-value	0.321	0.011	0.018	0.001	0.002

Mean in the same column for each category having different small letters are significantly different ($p \leq 0.05$). Mean±SD. DM: Dry matter, CP: Crude protein, EE: Ether extract and EC: Energy content

Fish body 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 5. 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® probiotic in both levels (10 or 20 g kg^{-1} diet) showed significant ($p \leq 0.05$) decrease of ash and CP, while significantly increased EE and EC contents of fish body compared to the free probiotic diet (control group). However, no significant differences in DM among all probiotic levels.

From the other hand, no significant differences in all body composition parameters in case of fish reared at low SD (40 fish m^{-3}) and fed different levels of Pro-Grow® probiotic. 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 (Fig. 3b), EC (Fig. 3c), while ash (Fig. 3a) was significantly decreased compared to the control group (free

Table 6: Effect of stoking density (fish m^{-3}) and dietary probiotic Pro-Grow® (g kg^{-1} diet) levels on hematological parameters of adult *Oreochromis niloticus*

				Blood indices			
Treatments	Hb (g dL^{-1})	RBCs ($\times 10^6$ mm^{-3})	PCV (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	WBCs ($\times 10^3$ mm^{-3})
Stocking density (fish m^{-3})							
40	9.34±0.07[a]	1.83±0.06[a]	43.19±2.90	162.20±2.09	48.55±1.64[b]	30.74±0.79[b]	128.70±2.34[a]
80	8.54±0.05[b]	1.60±0.12[b]	42.62±1.23	159.50±1.56	58.05±2.90[a]	36.43 ±1.29[a]	118.90±3.29[b]
p-value	0.010	0.002	0.792	0.255	0.001	0.001	0.006
Probiotic (g kg^{-1} diet)							
0	8.60±0.06	1.54±0.17[b]	37.59±2.45[b]	158.20±1.82[b]	55.68±4.17[a]	35.21±2.10[a]	116.60±2.22[b]
10	9.28±0.12	1.59±0.04[b]	41.09±1.82[b]	165.20±1.74[a]	58.28±2.28[a]	35.12±1.38[a]	125.60±4.21[a]
20	8.93±0.11	2.01±0.05[a]	50.04±1.32[a]	159.10±2.52[b]	45.93±1.06[b]	30.42±0.62[b]	129.30±3.72[a]
p-value	0.165	0.001	0.005	0.043	0.001	0.002	0.001

Mean in the same column for each category having different small letters are significantly different ($p \leq 0.05$). Mean±SD. MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin and MCHC: Mean corpuscular hemoglobin concentration

Table 7: Effect of stoking density (fish m^{-3}), dietary probiotic Pro-Grow® (g kg^{-1} diet) levels and their interaction on biochemical and immune responses parameters of adult *Oreochromis niloticus*

Treatments	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Albumin/globulin ratio	IgM (mg mL^{-1})
Stocking density (fish m^{-3})					
40	5.80±0.16	3.89±0.15	1.91±0.06	2.05±0.10	7.33±0.50
80	5.75±0.08	3.80±0.07	1.95±0.04	1.94±0.04	6.12±0.50
p-value	0.791	0.600	0.433	0.313	0.110
Probiotic (g kg^{-1} diet)					
0	5.61±0.11	3.80±0.10	1.81±0.04[b]	2.10±0.06	5.93±0.60
10	5.72±0.06	3.83±0.10	1.88±0.04[b]	2.05±0.10	6.75±0.55
20	6.01±0.23	3.90±0.20	2.11±0.05[a]	1.84±0.11	7.50±0.70
p-value	0.223	0.891	0.005	0.142	0.235

Mean in the same column for each category having different small letters are significantly different ($p \leq 0.05$). Mean±SD

diet from the tested probiotic). However, no significant differences in DM or CP were detected among all treatments.

Haematological parameters: In the present study, data of haematological parameters of *O. niloticus* reared in high SD (80 fish m^{-3}) showed significant decrease ($p \leq 0.05$) of Hb, RBCs, WBCs, while MCH and MCHC significantly increased compared to the fish reared in low SD (40 fish m^{-3}). No significant differences of PCV and MCV were detected among fish groups at two SDs. On the other hand, fish fed 20 g Pro-Grow® kg^{-1} diet led to significant increase of RBCs, PCV and WBCs, but significantly ($p \leq 0.05$) decreased all the blood indices (MCV, MCH and MCHC) compared to fish fed other probiotic levels. No significant effects of Hb content were detected among all dietary probiotic levels (Table 6).

The interaction effects between low SD and dietary Pro-Grow® levels did not show any significant differences in all haematological parameters. Also, no significant differences of Hb and PCV were detected among all treatments. However, fish reared in high SD and fed high Pro-Grow® level (20 g kg^{-1} diet) showed significant increase of RBCs (Fig. 4a), WBCs (Fig. 4e) but all blood indices (MCV, MCH and MCHC) were significantly decreased compared to other treatments (Fig. 4b, c and d, respectively).

Biochemical and immune responses measurements: Serum biochemical and immune responses parameters of adult *O. niloticus* reared in high SD and fed dietary Pro-Grow® probiotic are presented in Table 7. Data revealed that both of SDs, Pro-Grow® probiotic, as well as their interaction had no significant effects of all tested immune responses parameters of *O. niloticus*, except the case of fish fed 20 g Pro-Grow® kg^{-1} diet, which significantly ($p \leq 0.05$) increased the globulin concentration compared to other levels of tested probiotic.

Economic efficiency: Fish reared at high SD (80 fish m^{-3}) led to significantly ($p \leq 0.05$) increase the total feed costs (LE kg^{-1} diet) and total outputs (LE kg^{-1} fish), consequently led to significantly decrease of the net return (LE) and economic efficiency (%) compared to the low SD. Fish fed 10 and 20 g kg^{-1} diet of Pro-Grow®, respectively have positively effects on the economic efficiency parameters compared to the control group (Table 8).

In case of the interaction effects between SDs rates and dietary Pro-Grow® levels, fish reared at low SD (40 fish m^{-3}) and fed 10 g Pro-Grow® kg^{-1} diet or fish reared at high SD (80 fish m^{-3}) and fed 20 g Pro-Grow® kg^{-1} diet had gave the highest significant ($p \leq 0.05$) increased of the economic efficiency parameters compared to the control group in each case (Fig. 5 and 6a-d, respectively).

DISCUSSION

All water quality parameters measured herein were within the acceptable ranges as recommended for rearing *O. niloticus*[28]. Where, increasing SD rate of adult *O. niloticus* did not affected on water quality parameters, except significantly decreased water dissolved oxygen and increased water NH_3-N. Increasing of SD may cause deterioration in

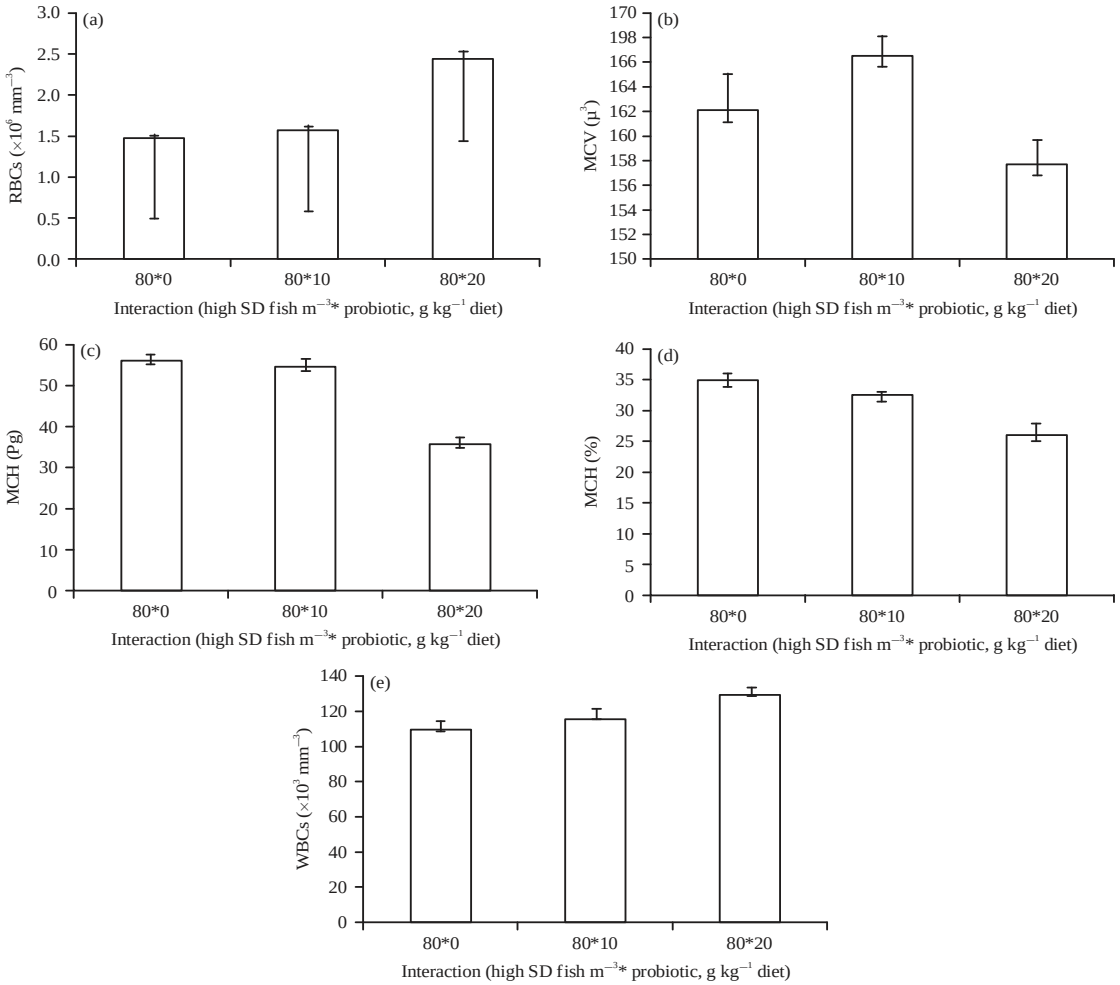

Fig. 4(a-e): Effect of the interaction of high stoking density (80 fish m^{-3}) and dietary probiotic Pro-Grow® (0, 10 and 20 g kg^{-1} diet) levels on some haematological parameters, (a) Red blood cells ($\times 10^6$ mm^{-3}), (b) Mean corpuscular volume (μ^3), (c) Mean corpuscular hemoglobin (Pg), (d) Mean corpuscular hemoglobin concentration (%) and (e) White blood cells ($\times 10^3$ mm^{-3}) of adult *Oreochromis niloticus*

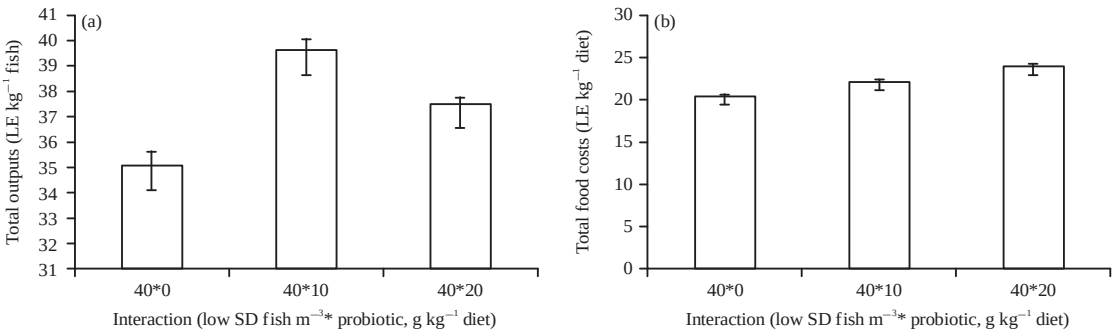

Fig. 5(a-d): Continued

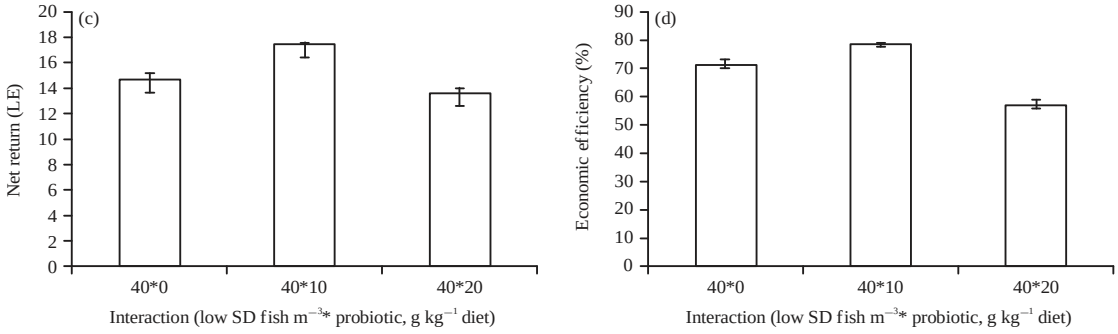

Fig. 5(a-d): Effect of the interaction of low stoking density (40 fish m^{-3}) and dietary probiotic Pro-Grow® (0, 10 and 20 g kg^{-1} diet) levels on the economic efficiency parameters of adult *Oreochromis niloticus*

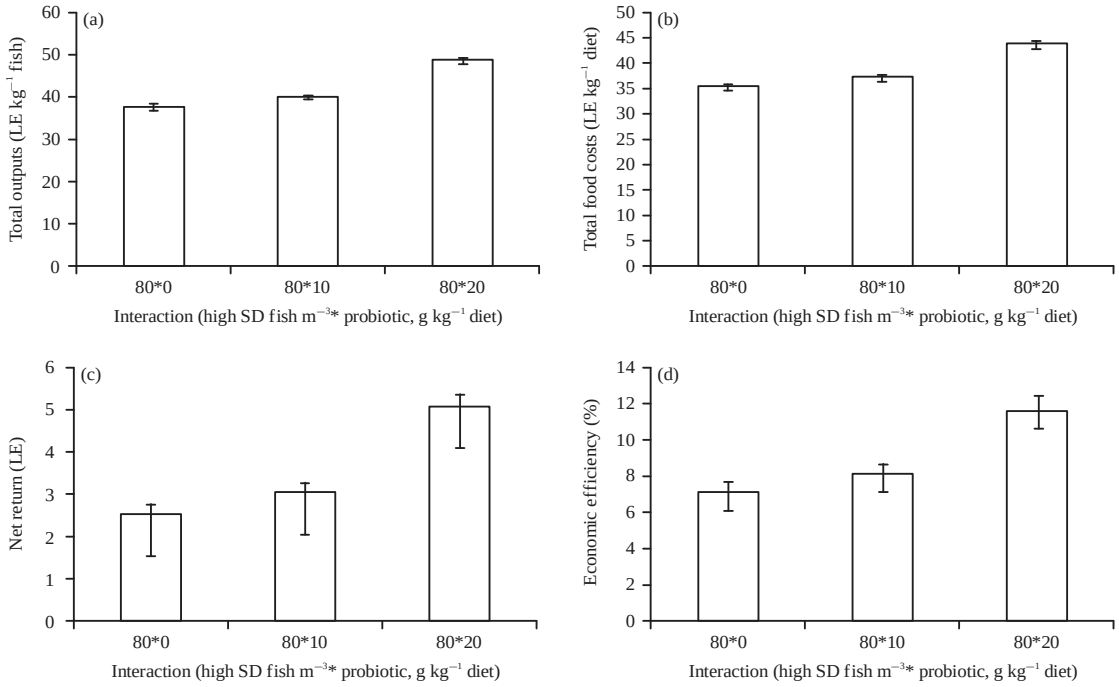

Fig. 6 (a-d): Effect of the interaction of high stoking density (80 fish m^{-3}) and dietary probiotic Pro-Grow® (0, 10 and 20 g kg^{-1} diet) levels on the economic efficiency parameters of adult *Oreochromis niloticus*

Table 8: Effect of stoking density (fish m^{-3}) and dietary probiotic Pro-Grow® (g kg^{-1} diet) levels on the economic efficiency parameters of adult *Oreochromis niloticus*

Treatments	Total outputs*	Total food costs#	Net return@	Economic efficiency$
Stocking density (fish m^{-3})				
40	37.38±0.51^{b}	22.21±0.69^{b}	15.17±0.60^{a}	68.75±3.30^{a}
80	42.34±1.27^{a}	38.77±1.66^{a}	3.54±0.41^{b}	8.96±0.75^{b}
p-value	0.001	0.001	0.001	0.001
Probiotic (g kg^{-1} diet)				
0	36.48±3.33^{c}	27.93±0.74^{c}	8.55±2.70^{c}	39.10±14.32^{b}
10	39.98±3.39^{b}	29.73±0.25^{b}	10.21±3.21^{a}	43.31±15.75^{a}
20	43.13±4.42^{a}	33.86±2.53^{a}	9.31±1.91^{b}	34.16±10.13^{c}
p-value	0.001	0.001	0.006	0.001

Mean in the same column for each category having different small letters are significantly different ($p \leq 0.05$). Mean±SD. *Total outputs/treatment (LE kg^{-1} fish) = fish price×total fish production**. * *Total fish production/treatment = final number of fish×fish weight gain. #Total food costs/treatment (LE kg^{-1} diet) = food costs/1 kg diet×food intake. @Net return/treatment (LE) = total outputs-total feed costs. $Economic efficiency/treatment (%) = (net return/total food costs) ×100. The prices of 1 kg ingredients used were 9.00 LE for fish meal, 5.5 LE for Corn gluten; 4.00 LE for soybean meal, 2.00 LE for yellow corn, 1.80 LE for wheat bran, 6.00 LE for Vegetable oil, 1.20 LE for molasses, 10.00 LE for vit. and min. premix and 25.00 LE for Pro-Grow® according to the local market prices in 2014 of Egypt

water quality, resulting in stressful conditions. 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[29]. Thus, negative correlationship between high SD and fish rearing water quality parameters was previously detected by M'balaka *et al.*[30]. Meanwhile, the tested probiotic enhanced the water quality parameters of *O. niloticus* under high SD rate. 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*. These effective microorgansims played a potential role for improvement the rearing water quality parameters, which confirmed also by Mehrim[8] on *O. niloticus* reared under different SD conditions. Also, Dalmin *et al.*[31], stated that improved water quality parameters, SR, growth rates and increased the health status of juvenile *Penaeus monodon* has especially been associated with dietary *Bacillus* sp. This improvement of rearing water quality herein 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 (Table 8) or the expected friendly environmental effects.

In the present study, high SD rate had negatively effects on all growth and feed efficiency parameters. However, addition of high level of tested probiotic (20 g kg^{-1} diet) enhancement this picture. This improvement may be due to the probiotic itself, its formula components with beneficial microorganisms. 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 rate on fish, enrichment the growth and feed efficiency parameters of fish (Table 4). Regarding the depressing effects of high SD on fish growth and feed efficiency parameters in the present study are in agreement with those obtained by Bakeer *et al.*[32], for *O. niloticus*. Also, Sorphea *et al.*[33], reported that growth performance and SR of tilapia are adversely affected by high SDs. Although, in some cases this effect is either temporary[34] or absent[35]. Some fish species like tilapias can tolerate extreme crowding, nevertheless tilapias are 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[36].

Functional additive, like probiotics, is a new concept on aquaculture[37], where the additions of microorganisms on diets show a positive effect on growth and feed efficiency caused by the best use of carbohydrates, protein and energy[5]. 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[38]. Affirmative results of *O. niloticus* growth and feed efficiency parameters related with dietary supplemented probiotics were reported[39]. Also, the same positive effects on growth performance, feed conversion and protein efficiency ratio were detected for *O. niloticus* fed Biobuds® and yeast[40], some biological additives[41], or native bacteria supplemented diets[42]. Hence, Mehrim[8] suggested that Biogen®, can enhance the metabolism and energy of body cells, raise the efficiency of feed utilization, increase the vitality of cells by supplying oxygen to whole body and improve the immune responses of *O. niloticus*. Conversely, He *et al.*[43], found that supplementation of dietary DVAQUA® showed no effects on growth performance, feed conversion and SR 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 effects of the tested probiotic on growth performance in the present study and those obtained by other attempts, probiotics may stimulate appetite and improve nutrition of fish by the production of vitamins, detoxification of compounds in the diet and by breakdown of indigestible components[5].

As the current findings in fish carcass composition affected by SD or tested probiotic levels, the yeast supplementation significantly affected *O. niloticus* body composition[10], where yeast supplementation plays a role in enhancing feed intake with a subsequent enhancement of fish body composition. Also, results in the present study are in close agreement with those reported by Mehrim[8] and Abdelhamid *et al.*[41]. Reversely, no significant differences were detected on performance and proximal composition of *O. niloticus* fed probiotic *B. amyloliquefaciens*[44]. Additionally, Khalil *et al.*[38], suggested that body composition of adult *O. niloticus* males and females fed Hydroyeast Aquaculture® probiotic were took unclear trends, which may be due to the differ in sexes, metabolism, physiological responses and sexual behaviors of fish during this stage of life.

Haematological parameters of fish are useful tools that aid in studying the immunopotentiators[45]. In the present study, high SD had an adversely effects on haematological parameters of *O. niloticus*. In this respect, Mehrim[8] found the deleterious effects on haematological parameters of *O. niloticus* reared under different SDs. Moreover, Kpundeh *et al.*[46], reported that haematological indices, RBCs, WBCs, Hb, PCV and blood platelets significantly ($p<0.05$) decreased by increasing SD rate of *O. niloticus*. Meanwhile, current positive effects of tested probiotic Pro-Grow® on fish haematological parameters are in agreement with the results

obtained by Mehrim[8]. Furthermore, Abdel-Tawwab *et al.*[10], reported that *O. niloticus* fed 1.0-5.0 g yeast kg^{-1} diet exhibited higher RBCs, Hb and PCV values. In a recent srudy, the clearly positive effects on the homeostatic state were observed of *O. niloticus* fed probiotic *B. amyloliquefaciens*[44]. Inversely, Rawling *et al.*[47], found that PCV, Hb and RBCs levels were not affected of red tilapia (*O. niloticus*) by dietary inclusion of Sangrovit®. Also, Zhu *et al.*[48], reported that *Ictalurus punctatus* fed yeast polysaccharides had no effects on total number of WBCs, granulocytes and lymphocytes.

The effects of stress on the immune system of fish showed how complex these interactions could become[49]. In the present study, the adversely effects of high SD on immune responses of *O. niloticus* were not cleared, but dietary Pro-Grow® probiotic had slightly improved of immune responses parameters of the experimental fish. This improvement may be due to the seriously experimental management and/or functional microorganisms formula of tested probiotic, namely *S. cervisae*, 4000×10^{12} CFU, 14×10^{12} CFU of *B. subtilis*. Hence, probiotics supplementation improves the systemic immune response in fish[50]. Additionally, some factors can regulate their activities such as adhesion properties, attachment site, stress factors, diet, environmental conditions determine the colonization of probiotics in the gut of host, probiotics often exert host specific[51], strain specific differences in their modes of action[52], the source of probiotics[53], achievability[54], dose[55] and duration of supplementation[56].

Modulation of host immunity is one of the most purported benefits of probiotics consumption and fish is no exception[51]. In this context, enhanced the innate immune responses and disease resistance of *O. niloticus* fed 1.0-5.0 g yeast kg^{-1} diet[10], dietary *Lactobacillus* sp.[57], or *L. plantarum*[58] compared to the control group. Recently, Guzman-Villanuev *et al.*[59], confirmed strong synergy in stimulation of immune system between glucan and probiotic strain *Shewanella putrefaciens*. However, not all studies result in enhanced immune function by dietary probiotic supplementation. Thus, Shelby *et al.*[9], did not find any affect on lysozyme activity, alternative complement or total serum immunoglobulin of *O. niloticus* fed probiotic containing *B. subtilis, B. licheniformis*, *P. acidilactici* and *S. cerevisiae*. Generally, there is far less evidence available suggesting that dietary probiotics influence the humoral immune response in tilapia.

SD and SR are important indicators that determine the economic viability of a production system[60]. In the present study, fish reared at high SD led to significantly decrease of economic efficiency compared to the low SD. Fish fed 10 and 20 g kg^{-1} diet of Pro-Grow®, respectively have positively effects on the economic efficiency parameters compared to the control group. From the economic point of view, these positive effects may be due to the role of probiotic to alleviate the stress of SDs and significantly improved the water quality parameters, growth performance, feed utilization, carcass composition, physiological and immune responses parameters of fish reared at high SD, which consequently led to highly economic efficiency. Similarly with our findings regarding the economic efficiency, *O. niloticus* fed Biogen® probiotic[8] or Biobuds® and yeast[40] significantly improved the economic efficiency parameters compared to the fish fed the diet free probiotics. Furthermore, probiotics can reduce the use of antibiotics and synthetic chemicals in the fish food[5], consequently the addition of probiotics to fish diets has become widespread on aquaculture farms. The application of probiotics results in reduced food costs, which plays an important role in determining the practices of aquaculture. Interestingly, previous findings have shown that the beneficial effects of probiotics can manifest as enhanced feed utilization of cultured aquatic animals through the supplementation of digestive enzymes, improved feed efficiency and higher growth, the prevention of intestinal disorders and the pre-digestion of anti-nutritional factors present in mixed feed[61].

CONCLUSION AND FUTURE RECOMMENDATIONS

Finally, the obtained results revealed the adversely effects of high SD (80 fish m^{-3}) stress on the tested parameters of the adult *O. niloticus* compared to the fish reared at SD (40 fish m^{-3}). Also, the potential positive effects of Pro-Grow® at levels 10 and 20 g kg^{-1} diet against the different SDs 40 and 80 fish m^{-3}, respectively were detected too. Consequently, it could be concluded that the using of dietry Pro-Grow® with these levels at the different intensive fish culture systems may be useful and more applicable in the large scale fish farms. Regarding its predicted high fish production, improving physiological responses, besides the high economic efficiency or the environmental friendly effects. Thus, advanced studies are needed not only of this probiotic, but also for other different levels or types of probiotics, prebiotics, synbiotics or other function food additives of different fish species reared under intensive production systems.

SIGNIFICANCE STATEMENTS

This study discover the effects of graded levels of dietary Pro-Grow® probiotic against the stocking density stress on adult *Oreochromis niloticus* that can be beneficial for

avoiding the drastic effects of stocking density stress on fish and increasing fish growth and production, improving the physiological responses. So, this study will help the researcher to uncover the critical areas of intensification of Aquaculture that many researchers were not able to explore. Thus a new theory on this useful probiotic only or possibly with other combinations by prebiotic may be arrived at.

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