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IMPACTS OF COMMERCIAL PROBIOTICS ON GROWTH PERFORMANCE, DISEASE RESISTANCE AND PROFITABILITY OF NILE TILAPIA (*Oreochromis niloticus*) UNDER STOCKING DENSITY STRESS

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ABSTRACT: The current research aimed to reduce the effects of increasing Nile tilapia (*Oreochromis niloticus*) fish stocking density (SD) as stress factor by using dietary commercial probiotic supplementation (biogen® or Bactocell®). The experimental Nile tilapia (mean weight of 4.00 g) were distributed by random into 18 hapa (1.5 X 2 X 2 m), representing to 6 treatments (3 replicates per treatment). In a 2 × 3 factorial arrangement, fish were divided into two main groups. The first group was stocked at 100 fish/m^3 and the second was stocked at 150 fish/m^3. Each group was divided into 3 sub-groups; the 1st sub-group was fed on a diet without supplementation with commercial probiotics, the 2nd and 3rd sub-groups were fed on diet supplemented with 2 g Biogen and 0.5 g Bactocell/kg diet, respectively. The obtained results showed that all tested water quality measurements were suitable for rearing Nile tilapia fingerlings. With increasing fish stocking density, final body weight (FBW), daily weight gain (DWG), specific growth rate (SGR) and daily feed intake (DFI) of fish significantly ($P<0.001$) decreased and feed conversion ratio (FCR) significantly ($P<0.001$) impaired while, survival rate significantly ($P<0.001$) increased. The highest value for each of ($P<0.001$) FBW, DWG, SGR and DFI were achieved in fish groups fed diets supplemented with probiotic, while FCR significantly ($P<0.001$) improved. The interaction between fish SD and dietary probiotic supplementation significantly ($P<0.01$) affected FBW, DWG and DFI, while SGR and FCR were insignificantly affected. All blood components studied were insignificantly affected with fish SD. Hemoglobin, RBCs, WBCs, total protein, albumin and globulin of Nile tilapia fish significantly ($P<0.001$) affected with probiotic treatments. The interaction between fish SD and dietary probiotic supplementation significantly ($P<0.01$) affected WBCs only. Approximate analysis of body composition of Nile tilapia fish showed that, ash percentage only significantly ($P<0.05$) affected with SD, while crude protein, ether extract and ash percentages were significantly ($P<0.001$) affected with probiotic treatments. Within low SD the percents relative level of protection (RLP) after the challenge infection test using *A. hydrophila* in fish groups fed diet supplemented with biogen or bactocell were 100 and 60%, respectively. While, with high SD the RLP was 100 and 75% in fish groups fed diet supplemented with biogen and bactocell, respectively as compared with control fish group in the two levels of SD which recorded 0% of RLP. Final profit margin increased by 30.37 and 10.37%, in fish group stocked at high density and fed diet supplemented with biogen or bactocell, respectively when compared with fish group stocked at low density and fed diet without probiotic. The obtained results recommended that dietary probiotic (biogen or bactocell) supplementation to Nile tilapia (*Oreochromis niloticus*) diets reduce the effects of increasing stocking density as stress factor and achieve better growth performance, high disease resistance and high margin.

Key words: Nile tilapia, stocking density, biogen, bactocell, growth performance, disease resistance.

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INTRODUCTION

The industry of aquaculture is rapidly increasing to supply a valuable origin of protein for the increasing worldwide human needs (**Gobi *et al.,* 2016**). Nile tilapia (*Oreochromis niloticus*) is a widely cultivated species as it grows and reproduction in a wide scope of environmental conditions and bears stress induced by handling (**Tsadik and Bart, 2007**). With its characteristics, *O. niloticus* can simply be cultured well in different aquaculture systems like cages, ponds, tanks or raceways (**Gerald *et al.,* 2016**).

Stocking density (SD) is a main factor to realize the perfect production because it is directly correlated with physiological and physical parameters like, water quality, nutrition, biochemical phases and culturing system (**Islam *et al.,* 2006**). On the other hand, several studies showed an opposite relationship between the stocking density and growth rate of tilapia (**Ridha, 2006**). Despite the vital role of the intense rearing systems on the rise the aquaculture industry but it evoked a stressful environment to fish leading to inhibtion of the immune system, decrease feeding, poor growth and low flesh quality (**Harikrishnan *et al.,* 2010**). The Intensive production of tilapia can cause negative implications on fish performance and welfare. Thus, it is necessary to determine a proper density for optimal production **(Costa *et al.,* 2017).**

Special attention has been drawn to stocking density as one of main factors affecting the expected level of stress in fish (**North *et al.,* 2006**). Fish may confronte different types of stress like stocking density (**Lupatsch *et al.,* 2010**), anoxia, hypoxia, chemicals and pesticides (**Vani *et al.,* 2011**). To avert these stressful conditions, interference with immune stimulants, vaccines and probiotic bacteria, either as feed additive or in water, could trigger the defense system and thus improve the harmful effects mediated by various stress factors (**Ringo *et al.,* 2012**).

Biogen is a dehydrated natural product composed of *Allicin*, high unit hydrolytic enzymes, *Bacillus subtilis* and Ginseng extract (**Ali *et al.,* 2010**). Biogen® can enhance the metabolism of fish body cells, raises the palatability of feed, enhances the secretion of digestive fluids and induces the appetite, improve feed utilization, public health and immune responses (**Elam, 2004**). Also, *allicin* enhance the blood circulation in gills result an increasing in fish ability of to employ any little amount of dissolved oxygen. Biogen® also contain ginseng extract that necessary for fish body to preserve its physiological functions and have the ability to promote the natural body resistance by activation of immune cells (**Khalil *et al.,* 2001**).

Bactocell® is a by-product probiotics of lactic acid bacteria which composed of 1×10^{10} CFU g^{-1} Pediococcus acidilactici (**Fuller, 1989**). This product is assumed to enhance the immunity against diseases, environmental stresses, growth performances in shorter time, and decrease mortalities (**Gatesoupe *et al.,* 1997**).

Therefore, the current work aimed to reduce the effects of increasing fish SD as stress factor by using dietary commercial probiotic supplementation (biogen® and Bactocell®).

MATERIALS AND METHODS

The present work was conducted out at the Animal Production department, Agriculture Faculty, Zagazig University, Zagazig, Egypt. Practical work and chemical analysis were carried out at the Abbassa Fish Farm (Sharkia) of the General Authority for Fish Resources and Development (GAFRD), Egypt. The experimental period was 12 weeks (84 days).

Experimental Design and Fish Diet

Healthy Nile tilapia (*Oreochromis niloticus*; mean weight of 4.00 g) were acquired from Abbassa fish hatchery, General Authority for fish Resources and Development (GAFRD). The experimental fish were distributed by random into 18 hapa (1.5 × 2 × 2 m) were installed in earthen pond with the help of rope and clips, representing to 6 treatments (3 replicates per treatment).

In a 2 × 3 factorial arrangement, fish were divided into two main groups. The first group was stocked at 100 fish/m^3 and the second was stocked at 150 fish/m^3, each one was divided

into 3 sub-groups; the 1st sub-group was fed on a diet without supplementation with commercial probiotics, the 2nd sub-group was fed on diet supplemented with 2 g Biogen/kg diet and the 3rd sub-group was fed on diet supplemented with 0.5 g Bactocell/kg diet. All fish groups were fed on basal pelleted diet. The basal diet was composed to coat the recommended nutrient requirements of fish according to **NRC (2011)**. The composition and chemical analysis of the basal diet are shown in Table 1. Fish were fed three times daily (8:00 am, 12:00, and 16:00 pm) by rate of 5% of fish live body weight. During the experiment, feed intake of each replicate was readjusted according to the biomass biweekly.

Experimental Management

Water quality

Water quality measurements were monitored weekly in each hapa during the period of experiment. The total ammonium, nitrite, nitrate and pH levels were determined by using Hach Kit model HI 83205 (Multipurameter Bench Photometer, Hanna Instruments, Romania). Dissolved oxygen and temperature determined by HI 9146 (Oxygen and temperature Meter (Hanna Instruments, Romania). All aforementioned measurements were in the normal levels (**Boyd, 1990)**.

Growth performance

Fish were counted and weighed biweekly to determine growth performance. Specific growth rate (SGR), Feed conversion ratio (FCR) and survival rate (SR) were calculated using the following equations:

SGR = [ln final mean body weight − ln initial mean body weight]/time intervals (days) × 100.

FCR = (feed intake, g) / (weight gain, g).

SR = (final number of fish / initial number of fish) × 100.

Whole-body chemical analysis

At the final of experimental period, samples each of five fish from each hapa were randomly taken and prepared for whole chemical analysis. Proximate analysis was performed on to diet formulation and fish at the final of the experiment. Moisture (loss on drying at 105ºC for 12 hr.). Protein (Kjeldahl, nitrogen × 6.25), lipid (Soxhlet ether extractives), ash (residue after ashing at 550ºC for 12 hr.) and NFE (nitrogen free extractives) being determined, contents were determined using standard **AOAC (2005)** methods.

Physiological parameters

At the end of the experiment, blood samples were taken from the caudal blood vessels by use a disposable 1 cc tuberculin syringe. Whole blood was collected in sterile vial containing EDTA as an anticoagulant. Blood samples were used to measure the hemoglobin (Hb) content using a commercial kit (Diamond Diagnostic, Egypt).The total erythrocyte (RBCs) and leukocyte (WBCs) counts were counted using an Ao Bright–Line Haemocytometer (Neubauer improved, Precicolor HBG, Germany) according to **Jain (1993)**.

Other blood samples for serum separation were collected without the addition of anticoagulants and then centrifuged at 3000 rpm for 20 min and stored at –20°C until further biochemical analyses. Total protein (TP), albumin (**Sundeman, 1964**) and plasma transaminase enzymes (AST; aspartate amino transferase and ALT; alanine amino transferase (**Reitman and Fankel, 1957**) were measured by using the commercial kits from Diamond Diagnostics Company, Egypt.

Bacterial challenge test

At the final of the experiment, response to challenge infection test using a suspension of pathogenic *A. hydrophila* (0.3 ml of 10^7 bacterial cells/ml) *via* I/P route to 20 fish/ treatment/ challenge (**Aly *et al.*, 2008**). Inoculated fish were noticed daily for 15 days after inoculation and mortalities were recorded. The relative level of protection (RLP) among the challenged fish was determined (**Ruangroupan *et al.*, 1986**) by the following equation:

RLP (%) = 100 - (per cent of mortality in treated group / per cent of control mortality) × 100

Profit analysis

Profitable evaluation was calculated as (**Ayyat, 1991**): Final margin (profit) = Income from body gain in weight- Feed cost. Other overhead costs were assumed constant. Price of one kg of diet was 8.00 LE and price of selling of one kg live body weight of fish was 20.0 LE.

Table 1. Formulation and analysed composition of the basal diet

Ingredient	g kg^{-1}
Yellow corn	210
Soybean meal	200
Wheat middlings	150
Fish meal	150
Corn gluten meal	130
Extracted rice brane solvent	110
Soybean oil	30
Vitamin premix[a]	10
Mineral premix[b]	10
Analysed composition (g kg^{-1}) as fed basis	
Crude protein (N × 6.25)	320.8
Crude lipids	45.8
Crude fiber	42.3
Ash	73.3
Nitrogen-free extract (NFE)[c]	517.8
Gross energy (MJ per 100 g)[d]	18.21

[a]Composition of vitamin premix kg^{-1}: vitamin A, 8,000,000 IU; vitamin D_3, 2,000,000 IU; vitamin E, 7,000 mg; vitamin K_3, 1,500 mg; vitamin B_1, 700 mg; vitamin B_2, 3,500 mg; vitamin B_6, 1,000 mg; vitamin B_{12}, 7 mg; biotin, 50 mg; folic acid, 700 mg; nicotinic, 20,000 mg; pantothenic acid, 7,000 mg.
[b]Composition of mineral premix kg^{-1}: manganese, 53 g; zinc, 40 g; iron, 20 g; copper, 2.7 g; iodine, 0.34 g; selenium, 70 mg; cobalt, 70 mg and calcium carbonate as carrier up to 1 kg.
[c]NFE = 100 − (crude protein + Crude lipids + ash + crude fiber).
[d]Calculated using the factors 23.4 kJ $g^{\mu l}$, 39.2 kJ g^{-1} and 17.2 kJ g^{-1} protein, fat and carbohydrate, respectively.

Statistical Analysis

Analysis of variance for data was accomplished using the SAS General Liner Models Procedure **(SAS, 2002)**. The effects of stocking density levels and probiotics treatments were statistically analyzed by factorial analysis of variance according the following statistical model: $Y_{ijk} = \mu + D_i + P_j + DP_{ij} + E_{ijk}$

Where, Y_{ijk} is an observation, μ is the overall mean, D is the fixed effect of density (i=1…2), P is the fixed effect of dietary probiotics level (j=1…3), DP_{ij} is the interaction effect of fish SD and dietary probiotics supplementation and E_{ijk} is random error. Means were tested for significant differences using Duncan's Multiple Range test (**Duncan, 1955**).

RESULTS AND DISCUSSION

Water Quality

All tested water quality measurements were proper for growing Nile tilapia *O. niloticus* fingerlings. Water temperature, oxygen, pH, ammonia and nitrite (overall mean) were 28.4 ± 0.051°C, 5.05±0.033 mg/l, 7.76 ± 0.080, 0.123 ± 0.012 mg/l and 0.012 ± 0.007 mg/l, respectively. Ranges of water quality measurements were within the acceptable ranges wanted for normal growth of tilapia as mentioned by **Boyd (1990)**.

Growth Performance and Feed Efficiency

The non-significant differences between the experimental groups for initial live body weight

indicated that the groups at the starting of the experiment were homogenous (Table 2).

Effect of stocking density

Stocking density is one of the most important factors in aquaculture because it directly affects growth, survival behavior, health, feeding and production of fish under farmed conditions (**Rahman and Rahman, 2003**). Final live body weight (FLBW), daily weight gain (DWG), specific growth rate (SGR) and survival rate (SR) of Nile tilapia fish were significantly ($P<0.001$) affected with the stocking density during all the different experimental period (Table 2). With increasing fish SD the FLBW, DWG and SGR of fish decreased by 7.53, 8.41 and 4.22%, respectively, in fish group stocked at high SD level when compared with fish group stocked at the low density while, SR significantly ($P<0.001$) increased with increasing fish SD (Table 2).

Stress due to reduction in space availability was reported to be the primary factor for growth inhibition in juveniles stocked at high densities (**Diana *et al.*, 2004**). Tilapia is a territorial and aggressive fish **(Halwart *et al.*, 2007)** thus competition for spaces aggravates crowd-related stress, significantly affecting growth. **Gerald *et al.* (2016)** showed that growth performance was favorable in fish stocked at a lower SD as compared with those stocked at higher densities.

Daily feed intake (DFI) was significantly ($P<0.001$) decreased, also feed conversion ratio (FCR) significantly ($P<0.001$) impaired with increasing fish SD. Daily feed intake decreased by 6.04% while FCR impaired by 2.69% at all the experimental period (0-12 weeks) in fish stocked at high density than those stocked at low density (Table 2). The present results were similar with those reported by **Mahmoud (2012)** who recorded that daily feed intake was decreased significantly in tilapia fish group stocked at high density compared with normal density in the same periods. The results of **Ayyat *et al.* (2011)** indicated that the best FLBW, DWG, and FCR were obtained in the fish group stocked at low SD.

Probiotic treatments

The highest significantly ($P<0.001$) value for each of FLBW, DWG and SGR was achieved in fish groups fed diets supplemented with probiotic than the control group (Table 2). Moreover, fish group fed diet supplemented with biogen recorded the highest FLBW, DWG and SGR which increased by 14.36, 16.49 and 6.88%, respectively, followed by the fish group fed diet supplemented with bactocell which increased by 9.12, 10.64 and 5.06%, respectively than the control fish group. Survival rate insignificantly affected with dietary probiotic supplementation (Table 2).

Using probiotics in aquaculture increases the nutrient utilization through providing enzymes capable of transfigure certain components of the diet into more digestible nutrients for the host. In this connection, **Geovany *et al.* (2007)** mentioned that feeding probiotics may enhance appetite and growth performance of the cultured fish species. However, the specific function of probiotics may vary according to the host animal and more on the probiotic characteristics. Also, **Dowidar *et al.* (2018)** examined the effect of three types of probiotics, in commercial names Protexin (T_2), Biogen-S (T_3) and Diamond V (T_4) as well as control diet (without probiotics, T_1). Fish fed probiotics-enriched diets showed higher growth performance than fish fed control diet. There was no significant variation in growth performance within fish fed different probiotics. The enhancement in body weight gain as a result of other various components of Biogen® such as allicin, which is one of the garlic by-product which stimulated growth because of its thyroid activity (**El-Nawawy, 1991**).

Dietary probiotic supplementation was significantly ($P<0.001$) affected DFI and FCR (Table 2). Daily feed intake at the whole of the experiment (0-12 weeks) increased by 6.75 and 5.29%, in fish fed diet supplemented with biogen and bactocell, respectively, while FCR significantly ($P<0.001$) improved by 8.25 and 4.82%, respectively in the same fish groups when compared with the control fish group (Table 2).

The obtained results were similar to those reported by **Soltan *et al.* (2016)** who reported that feed intake (FI) was significantly affected by Biogen® compounds (garlic, ginger, *B. subtilis* and digestive enzymes). Nile tilapia fry fed the

Table 2. Growth performance and feed utilization of Nile tilapia fish ($\overline{X}$ ± SE) as affected by fish stocking density, probiotic treatments and their interactions

Item	Initial weight (g)	Final weight (g)	Daily body weight gain (g/day)	Specific growth rate	Daily feed intake (g/day)	Feed conversion ratio (g food/ g gain)	Survival rate (%)
Stocking density							
Low (LD)	3.96±0.05	39.95±0.87	0.428±0.010	2.750±0.031	0.811±0.010	1.898±0.025	93.44
High (HD)	4.04±0.04	36.94±0.64	0.392±0.008	2.634±0.028	0.762±0.006	1.949±0.025	95.48
Significance	NS	***	***	***	***	***	***
Probiotic treatments							
Control (T1)	4.05±0.06	35.65±0.52^{c}	0.376±0.007^{c}	2.589±0.030^{b}	0.756±0.009^{c}	2.011±0.017^{a}	93.83
Biogen (T2)	3.99±0.03	40.77±0.88^{a}	0.438±0.010^{a}	2.767±0.026^{a}	0.807±0.015^{a}	1.845±0.012^{c}	94.95
Bactocell (T3)	3.96±0.08	38.90±0.67^{b}	0.416±0.008^{b}	2.720±0.035^{a}	0.796±0.010^{b}	1.914±0.014^{b}	94.61
Significance	NS	***	***	***	***	***	NS
Interactions between fish density and probiotic treatments							
LD×T_1	4.00±0.05	36.77±0.22^{d}	0.390±0.003^{d}	2.641±0.021	0.775±0.004^{c}	1.987±0.022	92.33
LD×T_2	3.98±0.04	42.71±0.32^{a}	0.461±0.003^{a}	2.824±0.009	0.839±0.007^{a}	1.821±0.009	94.33
LD×T_3	3.90±0.15	40.37±0.22^{b}	0.434±0.001^{b}	2.784±0.040	0.818±0.002^{b}	1.885±0.006	93.67
HD×T_1	4.10±0.12	34.53±0.21^{e}	0.362±0.003^{e}	2.538±0.036	0.737±0.003^{d}	2.035±0.018	95.33
HD×T_2	3.99±0.04	38.84±0.17^{c}	0.415±0.002^{c}	2.709±0.007	0.776±0.003^{c}	1.869±0.003	95.56
HD×T_3	4.02±0.06	37.44±0.18^{d}	0.398±0.002^{d}	2.656±0.019	0.773±0.002^{c}	1.943±0.013	95.56
Significance	NS	**	**	NS	**	NS	NS

Means in the same column within each classification bearing different letters are significantly different.
***= Highly significant (P ≤ 0.001), **= Significant (P ≤ 0.01) and NS = Not significant.

diet containing 1 g Biogen®/kg diet consumed more feed than groups fed the other experimental diets (0, 2, 3, 4 g Biogen®/kg diet). Biogen® have a response as a palatability enhancer with the best taste that increase the feed intake by fish and hence increase the growth rate which may be due its content of various enzymes that increase feed digestibility, absorbability and intestinal villi activation.

Interaction between stocking density and probiotic treatments

The interaction between fish SD and dietary probiotic supplementation significantly (P < 0.01) affected FLBW and DWG while SGR was insignificantly affected (Tables 2). Fish group stocked at the low level and fed diet supplemented with biogen recorded the highest FLBW and DWG followed by fish group stocked at the same level and fed on diet supplemented with bactocell than other experimental groups.

Interaction between fish SD and dietary probiotic supplementation insignificantly affected SR at the whole of the experimental period (Table 2).

Daily feed intake significantly (P<0.01) affected while FCR was insignificantly affected with the interaction between fish SD and dietary probiotic supplementation (Table 2). The fish group stocked at low density and fed diet supplemented with biogen recorded the highest values of DFI and the best value of FCR when compared with other fish groups (Table 2).

Blood Components

Effect of stocking density

All blood components studied were insignificantly affected with fish SD (Table 3). The concentration of globulin, AST and ALT decreased in blood of fish stocked at high SD as compared with the low density (Table 3).

Table 3. Blood components of Nile tilapia fish ($\overline{X}$ ± SE) as affected by fish stocking density, probiotic treatments and their interactions

Item	Hemoglobin (g/100 ml)	RBCs (10^6 mm^2)	WBCs (10^3 mm^2)	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)	AST (U/L)	ALT (U/L)
Stocking density								
Low (LD)	5.88±0.24	2.42±0.03	75.18±0.54	4.51±0.31	2.27±0.19	2.24±0.14	33.05±0.29	16.01±0.79
High (HD)	5.93±0.21	2.42±0.02	75.39±0.34	4.59±0.36	2.40±0.16	2.19±0.21	31.77±1.04	15.25±0.19
Significance	NS	NS	NS	NS	NS	NS	NS	NS
Probiotic treatments								
Control (T_1)	5.15±0.03^c	2.35±0.01^c	73.59±0.22^c	3.24±0.04^c	1.66±0.12^b	1.58±0.15^b	33.12±0.46	15.98±1.26
Biogen (T_2)	6.59±0.12^a	2.50±0.02^a	76.43±0.16^a	5.33±0.07^a	2.72±0.04^a	2.62±0.06^a	32.87±0.10	15.55±0.11
Bactocell (T_3)	5.97±0.15^b	2.41±0.01^b	75.85±0.09^b	5.09±0.08^b	2.64±0.02^a	2.45±0.07^a	31.24±1.55	15.36±0.22
Significance	***	***	***	***	***	***	NS	NS
Interactions between fish density and probiotic treatments								
LD*T_1	5.17±0.04	2.34±0.00	73.12±0.06^d	3.29±0.06	1.53±0.18	1.76±0.23	33.53±0.89	17.15±2.54
LD*T_2	6.69±0.25	2.50±0.03	76.57±0.32^a	5.23±0.02	2.66±0.05	2.58±0.06	32.87±0.06	15.47±0.15
LD*T_3	5.77±0.26	2.41±0.02	75.87±0.19^b	5.02±0.09	2.63±0.04	2.38±0.07	32.73±0.25	15.42±0.35
HD*T_1	5.12±0.05	2.35±0.02	74.07±0.09^c	3.18±0.04	1.78±0.14	1.40±0.17	32.70±0.29	14.81±0.35
HD*T_2	6.48±0.04	2.50±0.03	76.28±0.12^a	5.43±0.12	2.77±0.01	2.66±0.11	32.87±0.21	15.63±0.18
HD*T_3	6.17±0.04	2.41±0.02	75.83±0.09^b	5.17±0.14	2.64±0.03	2.52±0.11	29.75±3.12	15.30±0.33
Significance	NS	NS	**	NS	NS	NS	NS	NS

Means in the same column within each classification bearing different letters are significantly different.
***= Highly significant (P ≤ 0.001), **= Significant (P ≤ 0.01) and NS = Not significant.

In this respect, **Ayyat *et al*. (2011)** found that blood TP, albumin, ALT and T3 were insignificantly decreased in fish groups stocked at high SD as compared with those stocked at low SD. On contrary, **Mahmoud (2012)** found that the TP and globulin were significantly (P<0.01) affected by fish SD while albumin, AST and ALT were insignificantly affected. The same author showed that the elevation in some blood parameters may due to the crowding effect in groups stocked at higher level which may lead to competition on space, feed and aggression. Crowding on space may results directly on physiological as well as behavioral elevation combined with physiological changes.

Probiotic treatments

Hemoglobin (Hb), RBCs, WBCs, TP, albumin and globulin of Nile tilapia fish were significantly (P<0.001) affected with probiotic treatments, but AST and ALT were insignificantly affected (Table 3).

The highest value fore each of Hb, RBCs, WBCs, TP, albumin and globulin was recorded with fish group fed diet supplemented with biogen followed by fish group fed diet supplemented with bactocell as compared with control group (Table 3).

Abd Elaziz *et al.* (2007) noticed significant increases in RBCs count, WBC_S count, Hb concentration and PCV (%) in Nile tilapia received two kinds of probiotics (*Bacillus subtilis and saccharomyces cerevisiae*). The enhancement of the erythrogram parameters may be attributed to the hepato-stimulatory and hepato-protective effects of probiotics (**Sarma *et al.*, 2003**). In this respect, **Soltan *et al.* (2016)** showed that supplementation of *O. niloticus* diet with Biogen® (0, 2, 3, 4 g Biogen®/kg diet) significant (P<0.05) increase in hemoglobin, hematocrite, RBCs and WBCs. This could be associated to the necessity of transport more oxygen in blood to meet the rising energy demand of fish promoted by high levels of glucose (**Nikinmaa *et al.*, 1983**). Also, all dietary

Biogen® levels significantly (P<0.05) decreased serum transferase enzymes (ALT and AST). These results indicated that dietary probiotic Biogen® play a role in removing the toxic factors presented in the diets and therefore improved liver function. Garlic (one component of Biogen®) inhibits the fatty acids synthesis and other lipid components in liver and reduces the level of fat accumulation in liver leading to a decrease in liver weight (**Ibrahim *et al.*, 2004**). Garlic contains a variety of organosulphur compounds, amino acids, minerals and vitamins (**Block, 1985**). Sulphur compounds of garlic are responsible for inhibition of cholesterol synthesis (**Liu and Yeh, 2000)**.

Interaction between stoking density and probiotic treatments

All blood components studied insignificantly affected with the interaction between fish SD and dietary probiotic supplementation except WBCs significantly (P<0.01) affected (Table 3). Within each SD levels, dietary probiotic supplementation increased Hb, RBCs, WBCs, TP, albumin and globulin; while, AST and AST were decreased (Table 3).

Body Composition

Effect of stocking density

Approximate analysis of body composition of Nile tilapia fish showed that, ash percentage significantly (P<0.05) affected with SD, while moisture, crude protein and ether extract were insignificantly affected (Table 4). Ash percentage were significantly (P<0.01) increased in fish group stocked at high density than those reared under low SD, but ether extract slightly decreased (Table 4). Also, **Mahmoud (2012)** found that ether extract and ash percentages of the whole fish body were significantly (P<0.05 or 0.01) affected by fish SD while crude protein and dry matter were not significantly affected. With increasing fish SD the dry matter and ether extract percentages decreased by 2.2 and 2.3%, respectively while ash percentage increased by 7.9% as compared with lower fish SD.

Probiotic treatments

Approximate analysis of body composition of Nile tilapia showed that, crude protein, ether extract and ash percentages significantly (P < 0.001) affected with probiotic treatments while, moisture was insignificantly affected (Table 4). Ether extract and ash percentages significantly (P<0.001) decreased in fish group fed diet supplemented with biogen or bactocell while, crude protein significantly (P< 0.001) increased in fish group fed diet supplemented with biogen as compared with the control group (Table 4). These results are similar with the results of **Abd El-Rhman *et al.* (2009)** they found that, feeding Nile tilapia on basal diet with *M. luteus* (bacterial supplement) significantly affected (P<0.05) the moisture-content only. These results mean that the *M. luteus* supplemented diet promoted the feed intake hence increased body weight gain. Meanwhile, the changes in lipid and protein contents of fish-body could be linked to the changes in their synthesis and deposition-rate in muscles of fish (**Soivio *et al.*, 1989 and Abdel-Tawwab *et al.*, 2006**).

Interaction between stoking density and probiotic treatments

Approximate analysis of body composition of Nile tilapia insignificantly affected with the interaction between fish SD and probiotic treatments (Table 4).

Infection with *Aeromonas hydrophila* Bacteria

After the challenge infection test using *A. hydrophila,* the mortality rate was higher in each of fish group stocked at low or high density and fed diet without probiotic supplementation (control fish group) than that of fish groups treated with dietary probiotics (Table 5). Within low stocking density the percents level of protection (RLP) in fish groups fed diet augmented with biogen or bactocell were 100 and 60%, respectively. While, with high stocking density the RLP was 100 and 75% in fish groups fed diet augmented with biogen and bactocell, respectively as compared by the control group in the two levels of stocking density which recorded 0% of RLP (Table 5).

The use of probiotics for disease preventing and improved nutrition in aquaculture has gained prominence in recent times due to an increasing demand for environment-friendly aquaculture (**Vine *et al.*, 2006**). Common probiotic products used in aquaculture, such as

Table 4. Whole body composition (%) of Nile tilapia fish ($\overline{X}$ ±SE) as affected by fish stocking density, probiotic treatments and their interactions.

Item	Moisture	Protein	Fat	Ash
Stocking density				
Low (LD)	74.39±0.21	61.45±0.88	20.43±0.68	15.41±0.11
High (HD)	74.19±0.31	61.42±0.85	20.50±0.68	15.53±0.10
Significance	NS	NS	NS	*
Probiotic treatments				
Control (T_1)	74.10±0.37	60.30±0.09^{b}	22.78±0.18^{a}	15.76±0.03^{a}
Biogen (T_2)	74.26±0.22	64.60±0.35^{a}	18.16±0.19^{c}	15.07±0.03^{c}
Bactocell (T_3)	74.52±0.37	59.40±0.62^{b}	20.45±0.12^{b}	15.58±0.07^{b}
Significance	NS	***	***	***
Interactions between fish density and probiotic treatments				
LD×T_1	74.71±0.55	60.39±0.14	22.78±0.36	15.75±0.05
LD×T_2	73.98±0.04	64.80±0.42	18.18±0.39	15.01±0.00
LD×T_3	74.48±0.32	59.15±0.48	20.32±0.07	15.48±0.06
HD×T_1	73.49±0.15	60.21±0.11	22.78±0.19	15.77±0.04
HD×T_2	74.53±0.40	64.40±0.62	18.14±0.18	15.13±0.04
HD×T_3	74.55±0.76	59.65±1.28	20.59±0.21	15.68±0.09
Significance	NS	NS	NS	NS

Means in the same column within each classification bearing different letters are significantly different.

***= Highly significant ($P \leq 0.001$), *= Significant ($P \leq 0.05$) and NS = Not significant.

Table 5. Relative level of protection (RLP) of Nile tilapia fish at the end of the experimental period

Treatment	Total number	The number of dead fish	Survival (%)	Mortality (%)	RLP (%)
LD×T_1	20	5	75	25	0
LD×T_2	20	0	100	0	100
LD×T_3	20	2	90	10	60
HD×T_1	20	4	80	20	0
HD×T_2	20	0	100	0	100
HD×T_3	20	1	95	5	75

Bacillus species, can improve water quality by decreasing the number of microbial pathogens in ponds (**Wang *et al.*, 2008**). Inclusion of probiotic in fish feed can also composition the beneficial bacterial flora in skin and intestine while they grow competitively over pathogenic bacteria (**El-Rhman *et al.*, 2009**).

Aeromonas hydrophila, one of the major bacterial pathogens which causes a variety of diseases in fish like hemorrhagic septicemia, infectious dropsy, and tropical ulcerative disease and fin rot leading to high mortality in aquaculture (**Karunasagar *et al.*, 1997**). In this respect, **Mahmoud (2012)** found that the mortality rate was higher in control fish group than that of fish groups treated with dietary probiotics after the challenge infection using *A. hydrophila*. Also, **Dowidar *et al.* (2018)** reported that fish prophylacted by probiotics then challenged with *A. hydrophila* showed a statistical improvement in survival percent between treated groups than control one when fed Nile tilapia on three types of probiotics, in commercial names Protexin, Biogen-S and Diamond V.

Profit Analysis

Effect of stocking density

Total body gain, return from body gain and final profit margin decreased with increasing fish stocking density. Fish group stocked at high density recorded lower return from body gain and final profit margin by 8.61 and 16.57%, respectively as compared with low density (Table 6).

Probiotic treatments

Total body gain, feed cost, return from body gain and final profit margin increased with dietary probiotic. Fish group fed diet contained Biogen and Bactocell recorded higher final profit margin by 55.65 and 32.26%, respectively, as compared with fish group fed diet without probiotic (Table 6).

Table 6. Profit analysis of Nile tilapia as affected by fish stocking density, probiotic treatments and their interactions

Item	Total body gain (g)	Total feed intake (g)	Feed cost (LE)	Return (LE)	Final profit margin (LE)
Stocking density					
Low (LD)	35.99	68.12	0.545	0.720	0.175
High (HD)	32.90	63.99	0.512	0.658	0.146
Probiotic treatments					
Control (T_1)	31.60	63.52	0.508	0.632	0.124
Biogen (T_2)	36.79	67.83	0.543	0.736	0.193
Bactocell (T_3)	34.94	66.83	0.535	0.699	0.164
Interactions between fish density and probiotic treatments					
LD×T_1	32.77	65.11	0.521	0.655	0.135
LD×T_2	38.73	70.51	0.564	0.775	0.211
LD×T_3	36.47	68.75	0.550	0.729	0.179
HD×T_1	30.43	61.92	0.495	0.609	0.113
HD×T_2	34.85	65.14	0.521	0.697	0.176
HD×T_3	33.42	64.92	0.519	0.668	0.149

Interaction between stocking density and dietary probiotic treatments

Within the interaction between fish SD and dietary probiotic supplementation, supplemented diet with probiotic achieved increasing in total body gain, feed cost, return from body gain and total profit margin (Table 6). The best final profit margin (increased by 56.30%) recorded in fish group stocked at low density and fed diet augmented with biogen, Final profit margin increased by 30.37 and 10.37%, in fish group stocked at high density and fed diet augmented with biogen or bactocell, respectively when compared with fish group stocked at low density and fed diet without probiotic (Table 6).

Conclusion

Based on the obtained results, it recommended that dietary probiotic (biogen or bactocell) supplementation to Nile tilapia (*Oreochromis niloticus*) diets reduce the effects of increasing stocking density as stress factor and achieve better growth performance, high disease resistance and high margin.

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تأثير البروبيوتيك التجارى على أداء النمو، مقاومة الأمراض والربحية للبلطى النيلى (*Oreochromis niloticus*) تحت إجهاد كثافة التسكين

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أجريت هذه الدراسة بهدف تقليل تأثيرات زيادة كثافة تسكين أسماك البلطي النيلي (*Oreochromis niloticus*) كعامل إجهاد باستخدام إضافات من البروبيوتيك التجاري (البيوجين أو الباكتوسيل) للعليقة، تم توزيع أسماك البلطي النيلي (بمتوسط ٤,٠٠ جم) عشوائياً في ١٨ hapa (١,٥ × ٢ × ٢ م)، ممثلين في ٦ معاملات (٣ مكررات لكل معاملة)، في ترتيب عاملي ٢ × ٣، تم تقسيم الأسماك إلى مجموعتين رئيسيتين، سكنت المجموعة الأولى بمعدل ١٠٠ سمكة/م^3 وسكنت المجموعة الثانية بمعدل ١٥٠ سمكة/م^3، تم تقسيم كل مجموعة من المجموعتين الرئيسيتين إلى ٣ مجموعات فرعية، غذيت المجموعة الفرعية الأولى على عليقة أساسية بدون إضافة البروبيوتيك التجاري وغذيت المجموعتين الفرعيتين الثانية والثالثة على عليقة مضاف لها ٢ جرام بيوجين و ٠,٥ جرام باكتوسيل/كجم عليقة على التوالي، أظهرت النتائج المتحصل عليها أن جميع قياسات جودة المياه التي تم اختبارها كانت مناسبة لتربية إصبعيات البلطي النيلي، بزيادة كثافة تسكين الأسماك، انخفض معنويا (٠,٠٠١) كل من الوزن النهائي للجسم، الزيادة اليومية للوزن، معدل النمو النوعي والغذاء المأكول يوميا للأسماك وتدهور معامل تحويل الغذاء معنويا (٠,٠٠١) بينما زاد معدل البقاء معنويا (٠,٠٠١)، حققت مجموعات الأسماك التي غذيت علي علائق مضاف لها البروبيوتك أعلى قيم معنوية (٠,٠٠١) في كل من الوزن النهائي للجسم، الزيادة اليومية للوزن، معدل النمو النوعي والغذاء المأكول يوميا وتحسن معامل تحويل الغذاء معنويا (٠,٠٠١) مقارنة بمجموعة الكنترول، أثر التداخل بين كثافة تسكين الاسماك واضافة البروبيوتك للعليقة علي الوزن النهائي للجسم، الزيادة اليومية للوزن والغذاء المأكول يوميا معنويا (٠,٠٠١) بينما لم يتأثر معنويا كل من معدل النمو النوعي ومعامل تحويل الغذاء، لم تتأثر معنويا جميع مكونات الدم التي تمت دراستها بكثافة تسكين الاسماك. تأثر معنويا (٠,٠٠١) كل من الهيموجلوبين، عدد كرات الدم الحمراء، عدد كرات الدم البيضاء، البروتين الكلي، الألبومين والجلوبيولين باضافة البروبيوتك للعليقة، أثر التداخل بين كثافة تسكين الاسماك واضافة البروبيوتك للعليقة معنويا (٠,٠١) على عدد كرات الدم البيضاء فقط، أظهر التحليل الكيماوي لمكونات جسم أسماك البلطي النيلي أن كثافة تسكين الاسماك أثرت معنويا (٠,٠١) في نسبة الرماد فقط، بينما أثرت أضافة البروبيوتك للعليقة معنويا (٠,٠٠١) علي نسبة البروتين الخام والمستخلص الأثيري ونسبة الرماد، داخل المستوى المنخفض من كثافة تسكين الاسماك، كان المستوى النسبي للحماية من الأمراض (RLP) بعد اجراء اختبار التحدي للعدوي باستخدام ميكروب *A. hydrophila* ١٠٠ و ٦٠% في مجموعات الأسماك التي تم تغذيتها علي عليقة مضاف لها البيوجين أو الباكتوسيل علي التوالي، بينما مع المستوي العالي من كثافة تسكين الأسماك كان RLP ١٠٠ و ٧٥% في مجموعات الأسماك التي تم تغذيتها علي عليقة مضاف لها البيوجين أو الباكتوسيل علي التوالي عند مقارنتهم بالكنترول والتي قد سجلت مستوي صفر% من الـ RLP، ارتفع هامش الربح النهائي بنسبة ٣٠,٣٧ و١٠,٣٧%، في مجموعة الأسماك المسكنة بمستوي كثافة عالي ومغذاة على عليقة مضاف لها البيوجين أو الباكتوسيل، على التوالي عند مقارنتها بمجموعة الأسماك التي سكنت بمستوي كثافة منخفض وغذيت علي عليقة بدون اضافة البروبيوتيك، بناء على النتائج المتحصل عليها من هذه الدراسة، يوصى باضافة البروبيوتيك التجاري (biogen أو bactocell) الي علائق أسماك البلطي النيلي (*Oreochromis niloticus*) لتخفيف أثار الإجهاد الناتج عن كثافة التسكين المرتفعة وتحقيق أداء نمو أفضل، مقاومة عالية للأمراض وهامش ربح عالي.

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