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IMPACT OF COMMERCIAL AND ISOLATED *Bacillus amyloliquefaciens* PROBIOTIC BACTERIA AND / OR OVERCROWDING ON GROWTH PERFORMANCE AND IMMUNE STATUS OF *Oreochromis niloticus*

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ABSTRACT

A total number of two hundreds and seventy apparent healthy Oreochromis. niloticus (30.0±2.0 g) was used to evaluate the effect of dietary supplementation with probiotic bacteria (commercial and isolated Bacillus amyloliquefaciens) at a level of 0.1x10^{10}g/ kg diet and/or overcrowding stress on fish growth performance, immune status and economic efficiency.

Fish were divided into three groups. The first group (G1) was fed on basal diet (D1) without probiotic supplementation. The second group (G2) was fed on basal diet supplemented with commercial probiotic (D2). The third group (G3) was fed on basal diet supplemented with isolated probiotic (D3). Each group was subdivided into two subgroups, subgroup A was maintained at density of 10 fish/ aquaria (considered as optimum density), and subgroup B was maintained at density of 20 fish/aquaria (considered as high density).

***The obtained results showed that** the difference in initial and final body weight (IBW & FBW), total body weight gain (TWG), feed conversion ratio (FCR), and specific growth rate percentage SGR(%) values of Nile tilapia fed non supplemented or supplemented with probiotic (commercial and isolated) were not significant. Total feed intake (TFI) and survivability values were decreased significantly (P<0.01) when Nile tilapia was fed diet supplemented with probiotic bacteria (Bacillus* *amyloliquefaciens) sources. Differences in plasma total protein (TP), plasma albumin (ALB) and plasma anti-protease, bacteria count and survivability (%) were significant (P <0.05 or P <0.01). However, the other immunological parameters were not significant.*

The differences in stocking density (10 or 20 fish / aquarium) on IBW, FBW and TFI values were not significant. However, there were significant differences (P<0.05) in TWG, FCR and SGR values of Nile tilapia. Stocking density 10 fish /aquarium improved significantly (P<0.05) the TWG, FCR and SGR values as compared to stocking density of 20 fish /aquarium. Feed cost required to produce 1kg weight gain for stocking density at 10 fish / aquarium) decreased as compared to stocking density at 20 fish / aquarium).

The interaction effect between probiotic sources and stocking density revealed that there were significant difference (P <0.05) in FBW and TWG of Nile tilapia, while IBW value was not significant. The highest values of FBW and TWG values were obtained when Nile tilapia was fed diet supplemented with probiotic and stocking density at 10 fish /aquarium. On the other hand, the lowest values of FBW and TWG values were observed when Nile tilapia was fed diet non-supplemented with probiotics sources and 20 fish / aquarium.

***Conclusively,** from the previous results, it could be concluded that diet supplemented with isolated probiotic and stocking density 10 fish /aquarium was the best values in growth performance, immunity and economical efficiency of Nile Tilapia (Oreochromis niloticus).*

Keywords: Commercial and isolated *Bacillus amyloliquefaciens,* probiotic, overcrowding, growth, immune status, economic efficiency, *Oreochromis niloticus.*

INTRODUCTION

Aquaculture is an important and valuable source of easily digestible animal protein with high nutritional value. It could be considered the cheapest animal protein that can cover the world's increase demand. Tilapia are one of the most cultured fish species because of their tolerance to environmental changes such as; wide pH fluctuations, high ammonia and nitrite levels and low dissolved oxygen levels (Ardjosoediro and Ramnarine, 2002). Nile tilapia (*Oreochromis* *niloticus*) has an excellent faster growth and tolerance even in bad environmental water condition (Zahidah *et al.,* 2012).

Nevertheless, the most important stressor that affects the fish farming is the exposure of fish to overcrowding during the intensification**.** This was resulting in a set of adverse changes to the health and growth of fish by influence of blood corticosteroid levels, hematological, physiological responses. Besides, damage and erosion that leads to aggressive interactions, which may increase susceptibility to secondary infection (Barton, 2002; Ellis *et al*., 2002).

Probiotics is a live microbial supplement, which are now used in aquaculture as a simple and safe additive that promote the growth performance of the host (Newaj-Fyzul *et al*., 2014). Probiotics also have been used to enhance the immune system, hinder pathogen, and induce stress tolerance (Wang *et al.,* 2008).

Moreover, several studies have revealed possible amelioration of crowding stress by dietary supplementation with probiotics in gilthead seabream (Varela *et al.,* 2010), and Senegalese sole (Tapia-Paniagua *et al.,* 2014).

Therefore, the aim of this study was to determine the effects of dietary supplementation with commercial and isolated probiotic bacteria (*Bacillus amyloliquefaciens*) and / or overcrowding stress on fish growth performance, immunology status and economic efficiency.

MATERIALS AND METHODS

1. Probiotics and diet preparation

Two type of *Bacillus amyloliquefaciens* one isolated and other commercial products were used in the present study. The first *B. amyloliquefaciens* (7HN) was previously isolated from the intestine of *Calarias gariepinus*, which identified by 16 SrRNA gene sequencing and was submitted to the Gene bank database (accession numbers: Kx015882) through the project No 5589, which applied at the Department of Fish Diseases and Management (Selim *et al*, 2019). The second was commercial *B. amyloliquefaciens*, which known commercially as Ecobiol, Norel Animal Production that manufactured at Attaka industrial zone- Suez Gulf, Egypt.

Three diets were formulated. The first was a basal control diet (D1) in form of pellets and formulated from ground yellow corn, Soya bean meal, fish oil, meat meal, fishmeal, mineral, and vitamin mixture to meet the experiment of

Nile tilapia according to NRC (2011) as shown in Table 1. The second (D2), was supplemented with commercial *B. amyloliquefaciens* probiotic (Ecobiol) at 10^{10} CFU/kg of diet depending on the manufacturer's recommended dosage. The third diet (D3) was supplemented with 10^{10} CFU of isolated *B. amyloliquefaciens* (7HN) probiotic/ kg of diet (Reda *et al.*, 2018).

All ingredients were thoroughly mixed and manufactured into pellets at 1.5 mm in diameter using pelleting machine. Every two weeks, the required diet was prepared and stored at refrigerator at 4°C for daily use. In addition, chemical analysis of basal diet was determined according to the method described in the AOAC (1990).

2. Fish and experimental design

Two hundred and seventy apparent healthy *O. niloticus* (30.0±2.0 g, initial body weight) fish was used. Fish were acclimated for fourteen days for the experimental conditions.

Water quality criteria was maintained at acceptable level (APHA, 1998). Fish were divided into three groups. The first group (G1) was fed basal diet (D1) without probiotic supplementation. The second group (G2) was fed on diet supplemented with commercial probiotic (D2) at level of $0.1\text{x}10^{10}$g/ kg diet**.** The third group (G3) was fed on diet supplemented with isolated probiotic at level of $0.1\text{x}10^{10}$g/ kg diet (D3). Each group was subdivided into two subgroups (A and B; each subgroup had triplicates). Subgroup A was maintained at density 10 fish/ aquaria (considered as optimum density). Subgroup B was maintained at density 20 fish/aquaria (considered as high density).

3. Growth performance traits

The final body weight (FBW), total weight gain (TWG), total feed intake (TFI), feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR) were calculated as described by Stuart and Hung (1989) and Hevrøy *et al.* (2005) as the follows**:**

FCR = Total feed intake (g) / Total weight gain (g)

SGR = ln (Final weight) - ln (Initial weight) / Culturing days x100

Weight gain (WG) = Final weight (g) – Initial weight (g)

SR (%) = Number of fish harvested / Number of fish stocked x100

Table 1. Ingredients and chemical analysis of the basal diet.

Ingredients	**%**
Ground yellow corn	26.5
Soya bean meal	22
Fish oil	5
Meat meal	20
Fish meal	25
Mineral and vitamin mixture(premix)*	1.5
Total	**100.0**
Chemical analysis	
Moisture	0.22±9.49
Crude protein, CP %	31.61±1.21
Crude fibers, CF %	2.80±0.21
Ether extract, EE %	2.58±0.12
Ash, %	5.85±0.04
NFE**	47.67±0.36
Gross energy***	409.9
Cost of kg basal diet, LE	15 LE

* **Each Kg vitamin & mineral mixture premix contained** Vitamin A, 4.8 million IU, D_3, 0.8 million IU; E, 4 g; K, 0.8 g; B_1, 0.4 g; Riboflavin, 1.6 g; B_6, 0.6 g, B_{12}, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

** Nitrogen free extract (NFE) =100- (CP+ CF+ EE + Ash)

***Gross energy. Based on 5.65 Kcal/g protein, 9.45 Kcal/g fat and 4.1 carbohydrate Kcal/g (NRC, 2004).

4. Blood sample collection for immunological analysis

At the end of 60 days of feeding regime, from the caudal blood vessels for monitoring without anticoagulants and centrifuged at 3000 rpm/15 minutes.

Estimation of total protein was carried out according to the Biuret method using biochemistry kit using spectrophotometer (Model M70; Bausch & Lomb Pharma NV, Brussel, Belgium) at 540 Nm described by Weichselbaum, (1946) and Gornal *et al*., (1949). Albumin was determined spectophotometerically as implied by the method of Reinhold (1953).Serum globulin was calculated as difference between total proteins and albumin. The level of nitric oxide in serum samples was determined as the methods described by Rajaraman *et al.* (1998) Lysozyme activity was determined in serum by turbidometric assay utilizing lyophilized *Micrococcus lysodeikticu* according to Ellis (1990). The commercial kits (Shanghai Focus Medical Science Company, China) were used for determination of the complement component 3. The ability of serum to inhibit trypsin activity to determine antiprotease activity was assayed according to Hanif *et al,* (2004).

5*. Total gut bacterial count*

At the end of experimental period (60 days), fish (3 fish/ replicate) was fasten for 24 h then was anesthetized. Under aseptic condition, the entire intestine was extirpated, pooled, homogenized, and diluted with phosphate buffer saline then one microliter from this mixture was spread on triplicate tryptic soya agar (TSA) plates. The plates were incubated at 28 ^{0}C for 24 h. the colony was counted from variable plates to calculate the colony forming units per gram (CFU/g) (Al-Harbi and Uddin, 2004)**.**

6. Economic efficiency (%)

The economic efficiency was determined through the following formula:
Economic efficiency (%) = (A – B) / B x 100
Where, A= Selling cost of obtained gain, B= Feeding cost of this gain.

7. *Statistical analysis:*

This experiment was conducted as factorial design (2 probiotic sources x 2 stoking density). A factorial Analysis of Variance (ANOVA) was statistically analyzed according to Snedecor and Cochran (1982) using general linear model (GLM) of SPSS software (SPSS Institute, 2014) as the following model:

$$Y_{ijk} = \mu + P_i + D_j + PD_{ij} + e_{ijk}$$

Where: Y_{ijk} = An observation, μ = Overall mean, P_i = Probiotic sources supplementation (i= 1 and 2), Stocking density (1and 2), PD_{ij} = Interaction effect between them and e_{ijk} = Experimental errors. Significant differences between treatment means were determined using Duncan's New Multiple Ranges- Test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance

The main values of the initial and final body weight ((IBW and FBW), total weight gain (TWG), feed conversion ratio (FCR), specific growth rate (SGR, %) and survivability (%) of Nile tilapia as affected by probiotic sources, stocking density and their interaction are shown in Table 2.

Table 2. Effect of Commercial and isolated probiotics and /or overcrowding and interaction on growth performance traits of Nile tilapia after 60 days of feeding.

Items		Growth performance traits, g						
		IBW (g)	**FBW (g)**	**TWG (g)**	**TFI (g)**	**FCR**	**SGR (%)**	**Survivability (%)**
Probiotics sources (p)								
G1: Control		30.88 ±0.11	60.61 ±2.07	29.72 ±1.10	52.81 ±1.00[b]	1.81 ±0.10	1.21 ±0.06	95.0 ±2.24[a]
G2: Probiotics product		30.80 ±0.12	62.06 ±1.13	31.26 ±1.05	51.41 ±0.96[b]	1.62 ±0.04	1.25 ±0.03	94.17 ± 2.1[a]
G3: Probiotics Isolated		30.80 ±.029	65.66 ±1.66	34.86 ±1.79	58.0 ±0.93[a]	1.69 ±0.07	1.35 ±0.06	84.17 ± 2.39[b]
Sig. test		**NS**	**NS**	**NS**	**	NS	**NS**	**
Density (D), Fish /aquarium								
10 Fish / aquarium		30.71 ±.140	64.46 ±0.96	33.75 ±.97[a]	54.07 ±1.32	1.60 ±0.02[b]	1.32 ±0.03[a]	91.11 ±2.00
20 Fish / aquarium		30.94 ±.150	61.08 ±1.72	30.14 ±1.69[b]	53.46 ±1.41	1.80 ±0.08[a]	1.21 ±0.05[b]	91.11 ± 2.96
Sig. test		**NS**	**NS**	*	**NS**	*	*	**NS**
Interaction effect (P x D)								
G1: Control	10	31.17 ±0.13	65.15 ±0.17[a]	34.03 ±.22[a]	57.90 ±0.64[a]	1.70 ±0.01[b]	1.32 ±0.01[a]	93.33 ±3.33[a]
	20	30.70 ±0.12	56.11 ±1.11[b]	25.41 ±1.15[b]	51.08 ±1.34[b]	2.02 ±0.11[a]	1.08 ±0.04[b]	96.67 ±3.33[a]
G2: Probiotic product	10	30.73 ±0.20	62.04 ±2.54[ab]	31.30 ±1.93[ab]	49.78 ±1.95[b]	1.56 ±0.012[b]	1.25 ±0.05[ab]	93.33 ±3.33[a]
	20	30.87 ±0.18	62.08 ±1.50[ab]	31.22 ±1.33[ab]	51.03 ±0.70[b]	1.67 ±0.05[b]	1.25 ±0.03[ab]	95.0 ±2.89[a]
G3: Probiotics Isolated	10	30.33 ±0.019	66.2 ±1.47[a]	35.92 ±1.45[a]	54.54 ±0.1[ab]	1.51 ±0.07[b]	1.39 ±0.04[a]	86.67 ±3.33[ab]
	20	31.27 ±0.39	65.06 ±3.34[a]	33.79 ±3.58[a]	58.27 ±1.98[a]	1.75 ±0.14[b]	1.30 ±0.11[a]	81.67 ±3.33[b]
Sig. test		**NS**	*	*	*	*	*	*

a,b and c--- Means in the same column in each classification are bearing different superscripts differ significantly ($P<0.05$) .

NS: Not significant, *: $P<0.05$, **: $P<0.01$

IBW: Initial body weight, FBW: Final body weight, TWG: Total weight gain, TFI: Total feed intake, FCR: Feed conversion ratio, SGR: Specific growth rate (%).

Results obtained showed non-significant differences on initial (IBW), (FBW), (TWG), (FCR) and (SGR) values of Nile tilapia fed basal diet or supplemented with probiotic (commercial and isolated) groups. However, total feed intake (TFI) and survivability decreased significantly ($P < 0.01$) when basal diet was supplemented with probiotic. The highest TFI and lowest survivability (%) were obtained when isolated probiotic was added to the diet. These results are in agreement with those obtained by Mehrim (2001), Diab *et al*. (2002). Moreover, the same results were obtained by Gatesoupe, (1991) and Newaj-Fyzul *et al*., (2014) who reported that probiotics is a live microbial supplement (Zootechnical additives), which are used in aquaculture as simple and safe additive that promote growth performance of the host. Probiotics might have likely stimulated fish appetite and increased palatability of the food offered as indicated by the higher feed intake (Elam 2004). Gatesoupe (1999) stated that, the most efficient probiotics for aquaculture may be different from those of terrestrial animals, and recommended to develop commercial probiotics specifically for aquaculture use. Azad and Al-Marzouk (2008) reported that unlike the commercial probiotics, the autochthonous gut bacteria would have more persistence in the gut; and therefore, it would be more effective than the commercial bacteria. Studies with an autochthonous probiotic isolated from the yellow fin bream of Kuwait showed that it persists much longer than the commercial probiotic destined for use in veterinary animals (Azad & Al-Marzouk 2008).

The effects of stocking density (10 or 20 fish / aquarium) on IBW, FBW, TFI and survivability rate values were not significant (Table 2). However, there were significant differences ($P <0.05$) in TWG, FCR and SGR values of Nile Tilapia. TWG, FCR and SGR were significantly ($P<0.05$) improved in groups 10 fish/ aquarium stocking density, these results were in agreement with Hasan *et al*. (2010) who reported higher growth in weight (g) at lower stocking densities and the growth rate decreased gradually with increasing densities. Moreover**,** Imani Kapinga *et al.* (2014) revealed that the results of low density (LD) reared fish had better individual growth performance than high density (HD) reared fish. They also, added that stocking density had no significant effect on survival and FCR of Nile tilapia (*Oreochromis niloticus*). The same authors added that, the lower growth performance of tilapia exhibited at higher stocking density could be attributed to the intense antagonistic behavioral interaction, competition for food and living space and increased stress.

Moreover, Shourbela *et al.* (2017) revealed that stocking density had no influence on survival rate while, the final weight markedly reduced with increased density. They also, added that fish at high density had a higher feed intake (%) and feed conversion ratio.

The interaction effect between probiotic sources and stocking density revealed significant differences (P <0.05) on FBW, TWG, FCR and survivability (%) of Nile tilapia, while IBW value was non- significant (Table 2). The highest values of FBW and TWG values were obtained by of Nile tilapia fed diet supplemented with isolated probiotic and stocking density of 10 fish /aquarium (Table 2). On the other hand, the lowest values of FBW and TWG values were observed on Nile tilapia fed non supplemented diet with probiotics sources at 20 fish / aquarium as showed in Table 2. Varela *et al.*, (2010) found that *Sparus auratus* fed on Pdp11, a bacterial probiotic for 116 days either under low density (3 kg/m^3), or high density (30 kg/m^3) are not significantly different in immunological and metabolic parameters besides, the groups fed on probiotic are significantly higher in growth performance than control group even at low or high density. Reda and Selim (2015) pointed that in case of the addition of probiotics to fingerlings of tilapia fish were fed at concentrations of 1×10^4 and 1×10^6 for 30 days showed that significant improvements in body weight and weight gain as well as improved conversion factor *amyloliquefaciens* spores.

Immune parameters

Significant differences (P <0.05 or P <0.01) were detected in serum total protein (TP), albumin (ALB), anti-protease and total gut bacterial count of Nile tilapia fed diet supplemented with probiotic (commercial and isolated). However, the other traits of immunological parameters were non- significant. Mohamed (2007) noted that increasing plasma total protein indicates an improvement of the nutritional value of the diet. These results were disagreement with Eid and Mohamed (2008) who revealed that blood measurements had no significant differences in plasma total protein, plasma albumin and plasma total globulins of *O. niloticus* fingerlings fed probiotic as growth promoters in commercial diets.

Stocking density (10 or 20 fish / aquarium) had a significant effects (P <0.05 or <0.01) on globulin and bacteria count. The other traits of mmunological parameters were non-significant (Table 3). Nevertheless, the most important

Table 3. Effect of Commercial and isolated probiotics and /or overcrowding and interaction on immunological parameters and total gut bacterial count of Nile tilapia after 60 days of feeding.

Items		TP	ALB	NO	LYZ	C3	Anti-protease	Glob ulin	Bacteria count
Probiotics sources (p)									
G1: Control		4.43 ±0.36^{b}	2.27 ±0.36^{b}	51.83 ±11.75	17.83 ±2.71	106.67 ±14.49	0.49 ±0.04^{b}	2.16 ±0.52	7.19 ±0.07^{a}
G2: Probiotics product		5.93 ±0.17^{a}	2.85 ±0.13^{ab}	37.33 ±2.88	18.67 ±2.16	89.00 ±10.53	0.62 ±0.03^{a}	3.083 ±0.25	6.93 ±0.06^{b}
G3: Probiotics Isolated		5.40 ±0.22^{a}	3.26 ±0.37^{a}	51.50 ±12.19	18.83 ±2.16	111.17 ±14.95	0.47 ±0.05^{b}	2.14 ±0.51	6.88 ±0.09^{b}
Sig. test		**	*	NS	NS	NS	*	NS	*
Density (D), Fish/aquarium									
10Fish/aquarium		5.51 ±0.25	2.55 ±0.29	48.33 ±6.78	20.44 ±1.51	107.33 ±9.64	0.55 ±0.03	2.96 ±0.32^{a}	7.14 ±0.06^{a}
20Fish/aquarium		5.00 ±0.21	3.03 ±0.29	45.44 ±10.51	16.44 ±1.81	102.28 ±7.67	0.49 ±0.04	1.97 ±0.36^{b}	6.86 ±0.06^{b}
Sig. test		NS	NS	NS	NS	NS	NS	*	**
Interaction (P x D)									
G1: Control	10	4.88 ±0.59^{ab}	1.85 ±0.38^{b}	54.67 ±8.35	22.00 ±11.7	115.00 ±14.11	0.44 ±0.05^{b}	3.03 ±0.73^{a}	7.33 ±0.04^{a}
	20	3.97 ±0.31^{b}	3.01 ±0.18^{a}	49.0 ±24.7	11.67 ±4.05	98.33 ±27.95	0.53 ±0.08^{ab}	1.29 ±0.28^{b}	7.04 ±0.04^{b}
G2: Probiotics product	10	5.89 ±0.33^{a}	3.01 ±0.18^{a}	30.07 ±13.61	21.00 ±2.65	80.0 ±16.74	0.64 ±0.03^{a}	2.88 ±0.48^{b}	7.04 ±0.06^{b}
	20	5.97 ±5.20^{a}	2.69 ±0.16^{ab}	44.0 ±8.96	16.33 ±3.28	98.00 ±13.89	0.59 ±0.05^{a}	3.29 ±0.23^{a}	6.68 ±0.06^{c}
G3: Probiotics Isolated	10	5.75 ±0.11^{a}	2.79 ±0.67^{ab}	59.67 ±7.53	18.33 ±3.71	127.00 ±6.64	0.58 ±0.01^{ab}	2.96 ±0.70^{a}	7.04 ±0.08^{b}
	20	5.06 ±0.32^{ab}	3.73 ±0.14^{a}	43.33 ±24.89	19.33 ±2.03	95.33 ±28.75	0.36 ±0.03^{ab}	1.32 ±0.34^{b}	6.70 ±0.09^{c}
Sig. test		*	*	NS	NS	NS	**	*	**

a,b and c: Means in the same column in each classification are bearing different superscripts significantly difference ($P<0.05$)
NS: Not significant, *: $P<0.05$, **: $P<0.01$
Tp: Total protein, Alb: albumen, NO: Nitric oxide, LYZ: Lysozyme activity, c3: Complementary- 3

stressor that affects fish farming is the exposure of fish to overcrowding during the intensification. This intensification was resulting in a set of adverse changes to the health and growth of fish by influence of blood corticosteroid levels and hematological, physiological responses (Barton, 2002; Ellis *et al*., 2002). However, Jia *et al*. (2016) reported that the activities of lysozyme (LZM), alkaline phosphatase (ALP) and esterase in high density (HD) treatment were lower than in low density (LD) or medium density (MD) treatments. Moreover, Shourbela *et al.* (2017) revealed that fish at LD showed considerable increase in total protein, albumin and globulin as compared with those reared at HD. The plasma glucose and cortisol levels elevated in HD-raised fish contrast to LD and MD groups. LD-raised fish had more elevated RBCs and WBCs parallel to MD and HD. Similarly, lymphocytes, neutrophils, and monocytes (%) decreased with increasing density. In addition, serum antioxidant and lysozyme enzymes were considerably ($P<0.05$) higher in LD compared with MD and HD groups. Moreover, the interaction effect between probiotic sources and stocking density revealed that, there were significant differences ($P <0.05$ or $P <0.01$) on total protein, albumen, anti-protease, globulin and bacteria count of Nile tilapia, while the other traits of immunological parameters were non-significant (Table 3). Cordero *et al.* (2016) recorded that some fish in the case of high density occurs stress, but in the use of probiotics led to improve the immune status and proteins responsible for immune processes such as through the killer cells lysozyme and c3. The elevated levels of lysozyme activity observed in juveniles fed the probiotics, although non statistically significant, but indicates better ability to kill pathogenic bacteria by breaking down the cell wall. Shelby *et al.* (2006) and Ferguson *et al*. (2010), reported similar results.

Economic efficiency

In economical point of view, feed cost required to produce 1Kg weight gain was reduced by using dietary supplementation with commercial and isolated probiotic bacteria (*B. amyloliquefaciens*) as shown in Table 4. These results revealed that using probiotic commercial and isolated bacteria (*B. amyloliquefaciens*) at level of $0.1x10^{10}$ mg/ kg diet was the best in terms of feeding cost of gain weight and economic evaluation. The reduction of feed costs was easily observed for the feed cost/Kg weight gain, which decreased with the increasing incorporation levels of $0.1x10^{10}$ probiotic for mono sex

Table 4. Effect of Stocking density and/or probiotic type and their interaction on economic efficiency after 60 days of feeding.

Items		Economic efficiency, %			
		Feed cost, LE	**FCR**	**B**	**EE (%)**
Probiotics sources (p)					
G1: Control		15.00 ±0.00 [b]	1.81 ±0.10	24.36 ±1.54	30.89 ±7.01
G2: Probiotics product		15.0 6±0.00 [b]	1.65 ±0.03	24.78 ±0.49	44.13 ±3.54
G3: Probiotics Isolated		15.01 ±0.0.00	1.71 ±0.07	25.63 ±1.03	39.62 ±5.62
Sig. test		**	**NS**	**NS**	**NS**
Stocking density (D), Fish /aquarium					
10 Fish /aquarium		15.02 ±0.01	1.64 ±0.02[b]	24.60 ±0.27[b]	45.42 ±1.50[a]
20 Fish /aquarium		15.02 ±0.01	1.80 ±0.08[a]	27.09 ±1.14[a]	31.03 ±5.38[b]
Sig. test		**NS**	*	*	*
Interaction effect (P x D)					
G1: Control	10	15.00 ±0.00[b]	1.70 ±0.01[b]	25.50 ±0.19[b]	37.25 ±1.17[a]
	20	15.00 ±0.00[b]	2.02 ±0.11[a]	30.26 ±1.44[a]	16.17 ±5.29[b]
G2: Probiotics	10	15.06 ±0.00[b]	1.65 ±0.012[b]	24.86 ±0.15[b]	45.98 ±0.4.45[a]
product	20	15.06 ±0.00[b]	1.67 ±0.05[b]	24.70 ±1.09[b]	42.28 ±6.29[a]
G3: Probiotics	10	15.01 ±0.00[a]	1.51 ±0.05[b]	22.66 ±0.75[b]	54.40 ±5.74[a]
Isolated	20	15.01 ±0.00[a]	1.75 ±0.14[b]	26.31 ±2.07[ab]	34.56 ±9.98[ab]
Sig. test		**	*	*	*

a,b and c: Means in the same column in each classification are bearing different superscripts significantly (P<0.05).
NS: Not significant, * P<0.05, **P<0.01.
FCR: Feed conversion ratio, B= Feeding cost of this gain, EE: Economic efficiency (%), Price of kg fish: 35 L.E

fingerling Nile tilapia diets (Khattab *et al*., 2004 and Mohamed *et al*., 2007). The effect of stocking density (10 or 20 fish / aquarium) on feeding cost of gain and economic efficiency (%) values were significant ($P < 0.05$) as shown in Table 4. The interaction effects between probiotic sources and stocking density were significant ($P < 0.05$) on feeding cost of gain and economic efficiency (%) of Nile tilapia (Table 4). The highest values of economic efficiency (%) were obtained with Nile tilapia fed diet supplemented with probiotic isolated and commercial and stocking density of 10 fish /aquarium. On the other hand, the lowest values of economic efficiency (%) were observed with Nile tilapia fed basal diet with stocking density of 20 fish / aquarium as shown in Table 4.

Conclusively, from the previous results, it could be concluded that diet supplemented with isolated probiotic and stocking density 10 fish /aquarium was the best values in growth performance, immunity and economical efficiency of Nile Tilapia (*Oreochromis niloticus*).

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تأثير البروبيوتيك لبكتيريا ***Bacillus amyloliquefaciens*** التجارية والمعزولة مع أو إلغاء الكثافة على أداء النمو وقياس المناعة للبلطى النيلى ***Oreochromis niloticus***

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اجريت التجربة على عدد مائتان وسبعون من زريعة اسماك الباطى النيلى *niloticus Oreochromis* بمتوسط وزن (2.0 ± 30.0جم) كتجربة عاملية (2x3) لتقييم تأثير الاضافات الغذائية من البكتيريا بروبيوتيك *Bacillus amyloliquefaciens*) ا لتجارية

والمعزولة) عند مستوى $0.1 \ x10^{10} g / kg$ مع كثافة السمك فى الحوض على أداء نمو الأسماك والحالة المناعية والكفاءة الاقتصادية .
تم تقسيم الأسماك إلى ثلاث مجموعات. تم تغذية المجموعة الأولى (G1) على عليقة كنترول (D1)دون اضافات بروبيوتيك. تم تغذية المجموعة الثانية (G2) على نظام غذائي أساسي باضافة بروبيوتيك تجاري.(D2) ، تم تغذية المجموعة الثالثة (G3) على نظام غذائي أساسي باضافة بروبيوتيك معزول (D3) ، ثم تم تقسيم كل مجموعات التجريبية السابقة إلى مجموعتين فرعيتين لكثافة السمك فى الحوض، تم الاحتفاظ بالمجموعة الفرعية A بكثافة 10 سمكة / حوض أسماك (تعتبر الكثافة المثلى) ، وتم الاحتفاظ بالمجموعة الفرعية B بكثافة 20 سمكة / سمكة (تعتبر كثافة عالية.
أظهرت النتائج التي تم الحصول عليها أن الفرق في وزن الجسم الأولي والنهائي IBW) و FBW)، زيادة الوزن الكلي للجسم (TWG) ، نسبة تحويل العلف (FCR) ، وقيم معدل الحيوية (SGR) ٪لاسماك البلطي النيلي تغذى غير مكمل أو تستكمل بروبيوتيك (التجارية والمعزولة) كانت غير معنوية . انخفض مجموع استهلاك العلف (TFI) وقيم البقاء بشكل ملحوظ (P <0.01) عندما تم تغذية البلطي النيلي حمية المضاف اليها مصدر البكتيريا بروبيوتيك .(Bacillus amyloliquefaciens) المعزولة كانت الاختلافات في البروتين الكلي للبلازما (TP) ، وألبومين البلازما (ALB) والبلازما المضادة للبروتياز ، وعدد البكتيريا ومعدل قيد الحياة (٪) كبيرة P <0.05) أو .(P <0.01 ومع ذلك ، القياسات ا_لاختلافات في كثافة التخزين (10 أو 20 سمكة / حوض) على قيم IBW و FBW و TFI لم تكن مهمة. ومع ذلك ،
كانت هناك فروق ذات دلالة إحصائية (P <0.05) في قيم TWG و FCR و SGR لسمك البلطي النيلي. تم تحسين كثافة تخزين 10 سمكة / حوض أسماك بشكل ملحوظ (P <0.05) من قيم TWGو FCR و SGR مقارنة بكثافة تخزين 20 سمكة / سمكة. انخفضت تكلفة الأعلاف المطلوبة لإنتاج زيادة الوزن 1 كجم لكثافة التخزين في 10 سمكة / حوض السمك مقارنةً بكثافة التخزين عند 20 سمكة / سمكة.
وكشف تأثير التداخل بين مصادر بروبيوتيك و الكثافة السمك فى الاحواض أنه كان هناك فرق كبير (P <0.05) في FBW و TWG من البلطي النيل ، في حين أن قيمة IBW ليست كبيرة. تم الحصول على أعلى قيم FBW وقيم TWG عندما تم تغذية البلطي النيلي على نظام غذائي معزز بكثافة البروبيوتيك والتخزين في 10 سمكة / حوض. من ناحية أخرى ، لوحظت أدنى قيم لقيم FBW و TWG عندما تم تغذية البلطي النيلي بنظام غذائي غير مستكمل بمصادر البروبيوتيك و 20 سمكة / حوض أسماك.
التوصية: من النتائج السابقة ، يمكن أن نستنتج أن اضاف البروبيتك المعزولة بكثافة الحوض 10 سمكة / حوض، كانت أفضل القيم في أداء النمو وقياسات المناعة والكفاءة الاقتصادية لنيل البلطي ..(*Oreochromis niloticus*).