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Effect of Dietary Supplementation of Biogen® (Commercial Probiotic) on Mono-Sex Nile tilapia *Oreochromis niloticus* under Different Stocking Densities

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Abstract: The present study was carried out to evaluate the effects of dietary supplementation of a commercial probiotic Biogen® on growth performance, carcass composition, blood hematological and biochemical parameters, histometric characteristics of fish dorsal muscles and economic efficiency of mono-sex Nile tilapia *Oreochromis niloticus* under different stocking densities. Therefore, fish with similar body weight (12.71±0.17 g) were distributed randomly into seven treatments at different stocking densities, being 10 fish m^{-3} which fed a basal diet without Biogen® (T_1), 10 fish m^{-3} (T_2), 20 fish m^{-3} (T_3), 30 fish m^{-3} (T_4), 40 fish m^{-3} (T_5), 50 fish m^{-3} (T_6) and 60 fish m^{-3} (T_7), which were fed the basal diet but supplemented with 3 g Biogen® kg^{-1} diet for 14 weeks. The obtained results indicated that T_4 was the best treatment which realized significantly ($p \leq 0.05$) increases of all growth performance parameters (final weight, AWG, ADG and SGR), hematological parameters (hemoglobin, RBCs count, PCV, blood platelets and WBCs count), plasma proteins (total protein, albumin, globulin and albumin/globulin ratio), improved FCR, blood indices (MCV, MCH and MCHC), differentiation of leukocytes, carcass composition, histometric characteristics of fish dorsal muscles and best economic efficiency. There were no adverse effects on water quality criteria among all experimental treatments. Consequently, from the obtained results, it could be concluded that the inclusion of the commercial probiotic Biogen® at a level of 3 g kg^{-1} diet at stocking density rate of 30 fish m^{-3} of mono-sex Nile tilapia *O. niloticus* is useful to get the best fish performance with friendly effects on the environment.

Key words: Nile tilapia, Biogen®, probiotic, stocking density, fish physiology

INTRODUCTION

Tilapia species are used in commercial farming systems in almost 100 countries and are developed to be the most important fish for aquaculture in this century (Fitzsimmons, 2000). Nile tilapia, *O. niloticus* is currently considered to be the most important and commonly cultured tilapia species around the world and constitutes over 70% of the cultured tilapia (Fitzsimmons, 2000, 2004). In Egypt, tilapia production surpassed the production of common carp and thus tilapia has become the preeminent cultured fish species. Tilapias are often cultured in freshwater ponds without supplemental feeding. Intensification of culture practices necessitates the use of external feed input (Essa and Salama, 1994). Tilapia production in developing countries occurs primarily in semi-intensive ponds with fertilization and/or supplementary feeding (Tacon, 1990). Supplemental feeds are applied to increase fish yields above those produced with fertilization alone (Knud-Hansen and Batterson, 1994). In fish farming practices, stocking density is considered to be one of the important factors that affects fish growth, feed utilization and fish yield (Liu and Chang, 1992). Furthermore, Ellis *et al*. (2002) reported that Stocking Density (SD) is a key factor in determining the productivity and profitability of commercial fish farms. In tilapia, experiments on the effect of stocking density have been conducted on different fish sizes including fry and juveniles (El-Sayed, 2002; Khattab *et al*., 2004b; Abdelhamid *et al*., 2007), sub-adults (Yi *et al*., 2004; Bakeer *et al*., 2007) and large tilapia (Diana *et al*., 2004; Rakocy *et al*., 2004).

The use of probiotics as farm animal feed supplements dates back to the 1970s. They were originally incorporated into feed to increase the animal's growth and improve its health by increasing its resistance to disease (Fuller, 1992).Today, probiotics are quite commonplace in health promoting functional foods for humans, as well as therapeutic, prophylactic and growth supplements in animal production and human health (Abdelhamid *et al*., 2000; Mombelli and Gismondo, 2000; Ouwehand *et al*., 2002; Sullivan and Nord, 2002; Senok *et al*., 2005). Many studies on probiotics in aquaculture have been used in *in vitro* models of specific bacteria as antagonists of pathogens (Vine *et al*., 2004, 2006; Wang *et al*., 2008b). Other studies have been focused on growth promotion of fish by probiotic supplements (Gatesoupe, 2002; Lara-Flores *et al*., 2003; EL-Haroun *et al*., 2006; Eid and Mohamed, 2008; Marzouk *et al*., 2008b) as well as on physiological and immune responses of fish by probiotic supplements (Khattab *et al*., 2004a; EL-Gohary *et al*., 2005; Marzouk *et al*., 2008a).

Commercial probiotic Biogen® consists of *Bacillus licheniformis* and *Bacillus subtilis*. The advantage of these spore-forming bacteria is that they are able to survive the pelletization process. Biogen® can enhance the metabolism and energy of fish body cells, raise the efficiency of feed utilization and balance the secretion of various secretory glands. Moreover, it increases the vitality of cells by supplying oxygen to whole body, improves the immune responses, helps to excrete heavy metals, inhibits aflatoxin and maintains the normal endocrine system. Biogen® has bactericidal effects and increases the palatability of feed, promotes the secretion of digestive fluids and stimulates the appetite (Mehrim, 2001). Moreover, Wang *et al*. (2008a) reported that with increasing demand for environment friendly aquaculture, the use of probiotics in aquaculture is now widely accepted. Therefore, the present study aimed to determine the effects of dietary supplementation of commercial probiotic Biogen® on growth performance, carcass composition, blood hematological and biochemical parameters, histometric characteristics of fish dorsal muscles and economic efficiency of mono-sex Nile tilapia *O. niloticus* under different stocking densities for 14 weeks.

MATERIALS AND METHODS

This study was conducted during the summer season 2007 in Fish Research Unit, Faculty of Agriculture, Mansoura University, Al-Dakahlia Governorate, Egypt. Mono-sex Nile tilapia *O. niloticus* fingerlings were gifted from the private hatchery in Tolombat 7 at Al-Reiad belonging to Kafr El-Sheikh Governorate. Fish were stocked into a rearing tank for two weeks as an adaptation period, during which they were fed on a basal experimental diet. Thereafter, apparently-healthy fish with similar body weight (12.71±0.17 g) were distributed randomly into seven treatments (as three replicates per treatment) with different stocking densities, being 10 fish m^{-3} which fed the basal diet without Biogen® (T_1) and 10 fish m^{-3} (T_2), 20 fish m^{-3} (T_3), 30 fish m^{-3} (T_4), 40 fish m^{-3} (T_5), 50 fish m^{-3} (T_6) and 60 fish m^{-3} (T_7), which were fed the basal diet but supplemented with 3 g Biogen® kg^{-1} diet. Each tank (1 m^3 in volume) was constructed with an upper irrigation open, an under drainage, an air stone connected with electric compressor. Dechlorinated tap water was used to change one third of the water in each aquarium every day. Some fish were kept frozen at the start of the experiment for chemical analysis.

A basal diet (89.89% dry matter, 30.13% crude protein, 4.42% ether extract, 53.54% carbohydrates, 11.91% ash, 431.71 Kcal/100 g DM gross energy and 69.79 mg CP/Kcal GE, P/E ratio), which was calculated according (Macdonald *et al*., 1973) in form of; GE (Kcal/100 g DM) = CP×5.64 +EEx9.44+Carbohydrates×4.11. It was formulated from the commercial ingredients (fish meal 22%, soybean meal 27%, yellow corn 21%, wheat bran 20%, corn oil 3%, vit. and min. mixture 2% and molasses 5%). The dietary ingredients and Biogen® supplement were bought from the local market. Feed ingredients were ground and the different ingredients were mixed manually by warm water and molasses. The big part from the ingredients was milled and mixed well with adding 3 g probiotic Biogen® kg^{-1} diet according to Mehrim (2001) and the other part of feed ingredients (without Biogen®) also was milled and mixed well. The two parts of the diet were pressed by manufacturing machine (pellets size 1 mm). During the experimental period (14 weeks), the fish were fed the experimental diets at a rate of 5% of the live body weight daily, six days a week during the first 7 weeks and 4% feeding rate of the live body weight at the other 7 weeks of the experiment. Experimental diets were introduced by hand twice daily, at 8 a.m. and 2 p.m. The amount of food was adjusted bi-weekly based on the actual body weight changes. Light was controlled by a timer to provide a 14 h light: 10 h dark as a daily photoperiod. Commercial probiotic Biogen® is a natural non-antibiotic feed supplement comprised of allicin (aged garlic extract) not less than 0.247 m mol g^{-1}, *Bacillus subtilis* nato (6×10^7 cells g^{-1}), high unit hydrolytic enzymes not less than 3690 units g^{-1} (amylolitic, lipolytic, prolytic and cell separating enzymes), germanium (ginseng 41.98 ppm of Ge. element) and organic selenium (Mehrim, 2001).

At the end of the experiment, the remained fish were sampled from each tank and kept frozen for chemical analysis. The chemical analyses of the basal diet and whole fish body were carried out according to the AOAC (2000). Water quality parameters were measured weekly (Abdelhamid, 1996) including temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter). Body weight of individual fish was measured bi-weekly to point feed quantity and to calculate growth performance according to Abdelhamid (2000) in form of: Average weight gain (g/fish) AWG = average final weight (g) – average initial weight (g), Average daily gain, (g/fish/day) ADG = AWG (g)/experimental period (days), Specific growth rate (SGR %/day) = [ln final weight-ln initial weight]×100/experimental period (d), Feed conversion ratio (FCR) = feed intake (g)/live weight gain (g) and Survival rate (SR%) = end number of the alive fish/the beginning number of the fish×100.

At the end of the experiment, blood samples were collected from the fish caudal peduncle of the different groups. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by using commercial kits (Diamond Diagnostic, Egypt). Also, total erythrocytes count (RBCs) and total leukocytes count (WBCs) were measured on an A_o Bright-Line Haemocytometer model (Neubauer improved, Precicolor HBG, Germany). Other blood samples were collected and transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for determination of total protein according to Gornall *et al.* (1949), albumin according to Weichsebum (1946), globulin by difference according to Doumas and Biggs (1972). As well as, at the end of the experiment some fishes from all treatments were sacrificed and fish dorsal muscles were sampled. Samples were fixed in 10% neutralized formalin solution to histometric examination according to Pearse (1968). The data collected were statistically analyzed using one-way ANOVA adapted by SAS (1997). Means were statistically compared for the significance ($p\leq0.05$) using Duncan (1955) multiple range test.

RESULTS AND DISCUSSION

Rearing Water Quality Properties

As shown from Table 1, all tested water quality criteria (temperature, °C; pH value and dissolved oxygen, mg L^{-1}) were suitable for rearing the experimental mono-sex Nile tilapia *O. niloticus* fingerlings. Although, there were no significant ($p\geq0.05$) differences in temperature °C and pH values by increasing the stocking densities of fish among all treatments; yet, dissolved oxygen (mg L^{-1}) of T_4 increased significantly ($p\leq0.05$) compared with other treatments, except T_1. While, by increasing the

Table 1: Effect of different stocking densities and dietary supplementation of Biogen® on quality parameters of rearing water of mono-sex Nile tilapia during the experimental period (Means±SE)

Treatment	Temperature (°C)	pH value	Dissolved oxygen (mg L^{-1})
T_1	25.00±0.20	8.03±0.16	7.83±0.11a
T_2	25.00±0.20	7.94±0.13	7.60±0.09ab
T_3	25.00±0.20	7.94±0.12	7.21±0.12c
T_4	25.00±0.20	7.99±0.14	7.88±0.10a
T_5	25.00±0.20	8.01±0.08	7.30±0.19bc
T_6	25.00±0.20	8.19±0.11	7.28±0.13bc
T_7	25.00±0.20	7.90±0.09	7.29±0.11bc

a-c: Mean in the same column having different letters are significantly different ($p \leq 0.05$)

Table 2: Effect of different stocking densities and dietary supplementation of Biogen® on growth performance of mono-sex Nile tilapia (Means±SE)

	Body weight (g $fish^{-1}$)						
Treatment	Initial	Final	AWG	ADG	SGR% day^{-1}	FCR	SR %
T_1	12.67±0.17	78.13±4.37e	65.47±4.35e	668.17±44.55e	1.90±0.06d	1.77±0.12a	100±0.00
T_2	12.83±0.17	95.40±3.88cd	82.57±3.93cd	842.63±40.35cd	2.03±0.07cd	1.63±0.09ab	100±0.00
T_3	12.67±0.17	101.83±5.97bc	89.17±5.86bc	910.10±59.77bc	2.13±0.07bc	1.43±0.09bc	100±0.00
T_4	12.83±0.17	132.17±7.34a	119.33±7.42a	1217.67±75.76a	2.37±0.09a	1.17±0.07d	100±0.00
T_5	12.67±0.17	115.00±2.93b	102.33±2.95b	1044.33±29.96b	2.23±0.03ab	1.27±0.03cd	100±0.00
T_6	12.83±0.17	86.90±3.72de	74.07±3.88de	756.03±39.66de	1.97±0.07cd	1.57±0.09ab	100±0.00
T_7	12.67±0.17	81.00±2.40de	68.33±2.24de	697.50±22.89de	1.90±0.00d	1.73±0.07a	100±0.00

a-e: Means in the same column having different letters are significantly different ($p \leq 0.05$). AWG: Average weight gain (g $fish^{-1}$), ADG: Average daily gain (mg/fish/day), SGR: Specific growth rate (% day^{-1}), FCR: Feed conversion ratio, SR: Survival rate (%)

stocking densities of fish the dissolved oxygen was decreased significantly ($p \leq 0.05$), nevertheless this significant decrease was in the acceptable limits for rearing mono-sex *O. niloticus* fingerlings. These positive findings in water quality criteria related with good growth performance since there were no mortalities among all treatments (Table 2). These results are in agreement with those of Gall and Bakar (1999) they reported that Dissolved Oxygen (DO) decreased with increasing density of tilapia, being 6.7 mg L^{-1} at 20 fish L^{-1} to 3.2 mg L^{-1} at 200 fish L^{-1}. On the other side, Abdelhamid *et al*. (2007) and Bakeer *et al*. (2007) reported that there were no significant differences in all water quality criteria measured by increasing the stocking densities of *O. niloticus*. Also, in the present study water quality criteria were suitable for rearing mono-sex *O. niloticus* fingerlings as cited by Abdelhamid (2000) and Abd El-Hakim *et al*. (2002). Moreover, Abdelhamid *et al*. (2007) tested water quality criteria and reported similar values which were suitable for rearing Nile tilapia fish.

Growth Performance

Results in Table 2 indicated that fish of T_4 at 30 fish m^{-3} and 3 g Biogen® kg^{-1} diet had significantly ($p \leq 0.05$) increases in all growth performance parameters (final weight, average weight gain, average daily gain and specific growth rate) compared with the control group (T_1, stocked at 10 fish m^{-3} without Biogen®). Also, the same fish group (T_4) had significantly ($p \leq 0.05$) improved feed conversion ratio compared with the other fish groups. However, by increasing the stocking density of fish (50 and 60 fish m^{-3} and fed diet supplemented with 3 g Biogen® kg^{-1} diet, T_6 and T_7) there were no significant ($p \geq 0.05$) differences in all above growth performance parameters compared with the control group (T_1). Yet, there were no mortalities among all fish treatments. These positive effects in fish growth performance may be related with supplementation of commercial and natural probiotic Biogen®, which can enhance the metabolism and energy of fish body cells, raise the efficiency of feed utilization, increase the palatability of feed, promote the secretion of digestive fluids and stimulate the appetite. In this trend, Abdelhamid *et al*. (2007) reported that raising the stocking density (2, 3 and 4 g fish L^{-1}) of the experimental Nile tilapia *O. niloticus* resulted in a significantly ($p \leq 0.05$) decrease of the growth performance parameters (final weight, weight gain, average daily gain, relative growth rate and specific growth rate) of fish. However, they added that increasing the stocking density rate of fish led to significantly ($p \leq 0.05$) increased feed conversion ratio of the experimental fish, but survival rate of fish was not influenced by raising the stocking density. Yet, they added that increasing dietary Betafin® (betaine) level caused a significant improve in this picture. As well as, Bakeer *et al.* (2007) found that body weight and length were negatively correlated to the stocking density of tilapia fish. Moreover, results of the present study are in agreement with those of Khattab *et al.* (2004b) and Srour (2004) for tilapia and EL-Haroun (2007) for catfish. Also, EL-Haroun *et al.* (2006) reported that the growth performance and nutrient utilization of Nile tilapia were significantly ($p \leq 0.01$) higher in the treatment receiving probiotic (Biogen®) than the control diet. Yet, Mohamed *et al.* (2007) reported that *O. niloticus* fingerlings fed on diets supplemented with probiotics exhibited greater growth than those fed the control diet. Also, they added that the diet containing 30% protein and supplemented with Biogen® at level of 0.1% produced the best growth performance. Recently, Eid and Mohamed (2008) revealed that using Biogen® at level of 0.1% was the best in terms of growth performance of mono-sex *O. niloticus* fingerlings. Moreover, Wang *et al.* (2008b) mentioned that tilapia (*O. niloticus*) supplemented with the probiotic bacterium, *Enterococcus faecium* ZJ4 showed significantly ($p<0.05$) better final weight and daily weight gain (DWG) than those fed the basal diet (control). Recently, Marzouk *et al.* (2008b) reported that both fish groups fed on diet supplemented with probiotics (dead *Saccharomyces cerevisae* yeast and both of live *Bacillus subtilis* and *Saccharomyces cerevisae*) revealed significant ($p<0.05$) increases in the body weight gain, specific growth rate and condition factor (K). While, they added that a significant ($p<0.05$) decreased in feed conversion ratio was recorded comparison with the control group fed on probiotic-free diet. On the other hand, Abdelhamid *et al.* (2002) found that Biogen® supplementation (2 and 4 g kg^{-1} diet) did not significantly improve fish growth performance. Also, Diab *et al.* (2002) reported that Biogen® addition to fish diet at 0.5, 1.0 and 1.5% gave insignificant increase in fish growth performance.

Carcass Composition of the Experimental Fish

There were no significant ($p \geq 0.05$) differences in dry matter, crude protein and ash of *O. niloticus* between T_4 and T_1. While, there were significant ($p \leq 0.05$) decreased in both of ether extract and energy content in T_4 compared with the control group (T_1). However, by increasing the stocking densities of fish in treatments no significant ($p \geq 0.05$) differences were recorded in crude protein and ash comparing with T_1. While, increasing the stocking densities of fish led to significant ($p \leq 0.05$) decreased in both of ether extract and energy content compared with the control group (Table 3). These positive effects in carcass composition of experimental fish may be due to the dietary supplementation with Biogen® which caused the good growth performance compared with the control

Table 3: Effect of different stocking densities and dietary supplementation of Biogen® on carcass composition of mono-sex Nile tilapia (Means±SE)

		On dry matter basis (%)			
Treatment	DM (%)	CP	EE	Ash	EC (Kcal 100 g^{-1})
At the start of the experiment					
	13.10	60.83	17.73	21.44	510.45
At the end of the experiment					
T_1	16.95±0.25ab	63.45c±1.95c	25.65±0.75a	10.95±1.25bc	599.85±4.15a
T_2	18.30±1.40a	65.40±1.20bc	24.55±1.15a	10.10±0.00c	600.15±4.25a
T_3	16.00±0.40abc	65.60±0.60bc	21.50±0.30b	12.90±0.30ab	572.95±0.75c
T_4	18.05±0.05ab	66.10±0.30bc	21.50±0.30b	12.40±0.60abc	575.85±4.25bc
T_5	13.80±0.60cd	67.65±0.55ab	18.45±0.05c	13.85±0.55a	555.90±2.90d
T_6	13.35±0.55d	70.00±0.90a	18.45±0.15c	11.55±0.75bc	569.15±3.85c
T_7	15.75±0.35bc	66.00±0.30bc	22.65±0.05b	11.35±0.25bc	586.20±1.10b

a-d: Means in the same column having different letters are significantly different ($p \leq 0.05$). DM: Dry matter (%), CP: Crude protein (%), EE: Ether extract (%) and EC: Energy content (Kcal 100 g^{-1}), calculated according to Macdonald *et al.* (1973)

group (Table 2). Since, Biogen® can enhance the metabolism and energy of fish body cells and raise the efficiency of feeds (Mehrim, 2001).

In this topic, Khattab *et al*. (2004b) reported that crude protein, total lipids and ash were significantly ($p<0.01$) affected by protein level and increasing stocking density rate of tilapia fish. Yet, Abdelhamid *et al*. (2007) added that increasing the stocking density rate of fish was responsible for increased percentage of DM, leading to increases in CP and ash, but EE percentages of the whole fish body decreased. Yet, they added that increasing dietary Betafin® (betaine) level caused a significant improve in this picture. On the other side, results in the present study are in close agreement with those of Khattab *et al.* (2004a), Srour (2004), EL-Haroun *et al.* (2006) and Mohamed *et al.* (2007) for tilapia and EL-Haroun (2007) for catfish. Moreover, Eid and Mohamed (2008) found that no statistical differences were observed in whole body moisture, crude protein, ether extract and ash of mono-sex *O. niloticus* fingerlings fed diets containing different levels of commercial feed additives (Biogen® and Pronifer®), compared with the control treatment.

Blood Hematological and Biochemical Parameters

Results in Table 4 and 5 showed that hemoglobin concentration, RBCs count, PCV%, blood platelets (thrombocytes) count and WBCs count in the experimental fish of T_4 were increased significantly ($p\leq 0.05$) compared with the control. While, no significant ($p\geq 0.05$) differences were recorded in blood indices (MCV, MCH and MCHC) and differentiation of leukocytes among all experimental treatments. On the other side, by increasing the stocking densities of fish, no significant ($p\geq 0.05$) differences were recorded in all above mentioned blood parameters compared with the control (T_1). However, T_7 (stocking density of 60 fish m^{-3} plus feeding diet supplemented with 3 g Biogen® kg^{-1} diet) recorded the worst values in the leukocytes count among all experimental treatment. The promising positive results obtained in hematological blood parameters led to increase the immune status of fish and prevent mortality among all the treated fish by increasing the stoking densities of fish

Table 4: Effect of different stocking densities and dietary supplementation of Biogen® on blood hematological parameters of mono-sex Nile tilapia (Means±SE)

				Blood indices			
Treatment	Hb (g dL^{-1})	RBCs×10^6 mm^{-3}	PCV (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	Platelets×10^3 mm^{-3}
T_1	3.45±0.45b	1.35±0.15b	10.15±0.85b	75.50±2.50	25.00±1.00	33.50±1.50	202.50±12.50ab
T_2	3.95±0.25b	1.50±0.20b	11.75±0.35b	79.50±8.50	26.00±1.00	33.50±1.50	125.00±20.00b
T_3	4.15±0.35b	1.70±0.30ab	12.05±0.35b	73.00±11.0	25.00±2.00	34.00±2.00	147.50±22.50b
T_4	5.95±0.35a	2.40±0.30a	16.90±0.40a	71.50±10.5	24.50±1.50	35.00±3.00	312.50±32.50a
T_5	5.30±0.20a	1.95±0.05ab	15.05±0.75a	77.50±5.50	27.00±1.00	35.00±3.00	297.50±77.50a
T_6	3.80±0.40b	1.60±0.30ab	11.25±0.65b	72.50±9.50	23.50±1.50	33.50±1.50	110.00±15.00b
T_7	3.85±0.15b	1.55±0.25ab	12.00±0.60b	79.00±9.00	25.00±3.00	32.00±0.00	97.50±12.50b

a-b: Means in the same column having different letters are significantly different ($p\leq 0.05$). Hb: Hemoglobin, RBCs: Red blood cells (Erythrocytes), PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration and Platelets: Blood platelets (Thrombocytes)

Table 5: Effect of different stocking densities and dietary supplementation of Biogen® on leukocytes count and its differentiation of mono-sex Nile tilapia (Means±SE)

		Lymphocytes	Monocytes	Neutrophils	Eosinophils
Treatment	WBCs×10^3 mm^{-3}	(%)			
T_1	560.50±12.50cd	89.00±4.00	3.00±0.99	6.00±2.99	2.00±0.00
T^2	509.00±21.00de	91.00±2.00	3.00±0.99	5.00±0.99	1.00±0.00
T_3	464.00±28.00de	93.50±1.50	2.50±0.49	2.50±0.49	1.50±0.49
T_4	827.50±32.50a	96.00±1.00	1.00±0.00	2.00±0.99	1.00±0.00
T_5	672.50±17.50b	88.50±2.50	3.00±0.00	7.00±1.99	1.50±0.49
T_6	652.50±32.50bc	89.50±5.50	2.50±1.49	6.50±3.49	1.50±0.49
T_7	422.50±42.50e	88.00±3.00	3.00±0.99	7.50±1.49	1.50±0.49

a-e: Means in the same column having different letters are significantly different ($p\leq 0.05$). WBCs: White blood cells (Leukocytes)

(Table 2). These findings were related to Biogen®, since both of allicin and ginseng (active ingredients of Biogen®) improved the physiological function of fish and immune response which in turn increased the ability of exposed fish to resist the stress effect of different stoking densities.

The present findings confirm those reported by Khattab *et al.* (2004a) they revealed that the blood hematological parameters (hemoglobin, erythrocytes count and packed cell volume percentage) in fish fed diets containing Biogen® were significantly higher than that of the control. Also, EL-Gohary *et al.* (2005) found the same positive effects on blood hematological parameters of *O. niloticus* fed the diet supplemented with Biogen® and exposed to metrifonate (as a broad spectrum insecticide). They added that the use of Biogen® as a growth promoter in fish diet minimized the toxicity of metrifonate. Moreover, Marzouk *et al.* (2008a) illustrated that there were significant ($p<0.05$) increases in RBCs count, Hb value, PCV%, WBCs count and differential of leukocytic count in the two fish groups fed the diets supplemented with probiotics (dead *Saccharomyces cerevisae* yeast and both of live *Bacillus subtilis* and *Saccharomyces cerevisae*) in comparison with the control group fed on probiotic-free diet. On the other hand, Abdelhamid *et al.* (2002) found that Biogen® reduced blood hemoglobin of aflatoxicated *O. niloticus* fish.

Total plasma protein, albumin, globulin and albumin/globulin ratio of the experimental fish of T_4 increased significantly ($p\leq 0.05$) compared with the control treatment (T_1). However, increasing the stocking densities of fish (T_5, T_6 and T_7) led to insignificant ($p\geq 0.05$) decreases of total protein, albumin and globulin and to insignificant ($p\geq 0.05$) increase of albumin/globulin ratio compared with the control (T_1) as shown from Table 6. These findings mean that the addition of Biogen® led to significantly ($p\leq 0.05$) increases of plasma proteins which indicates the improvement of the nutritional values of the diet, the growth performance, carcass composition of crude protein, physiological functions and the healthy status of the experimental fish fed on this commercial probiotic Biogen® against the high stocking density of fish. Whereas, Biogen® increases the vitality of cells by supplying oxygen to whole body, improves the immune responses and maintains the normal endocrine system (Mehrim, 2001). Moreover, Raa (1996) and Sakai (1999) revealed that some responses that are routinely reported by using immunostimulants are macrophage activation, increased phagocytosis by neutrophils and monocytes, increased lymphocyte numbers, increased serum immunoglobulins and increased lysozyme.

These results are in agreement with those obtained by Mohamed (2007) revealed the increase in plasma total protein of *O. niloticus* fingerlings fed on probiotic and yeast. On the other hand, Diab *et al.* (2002) reported that there were no significant differences in serum total protein in fish fed diets containing 0.5%, 1.0% and 1.5% of Biogen®. Moreover, Eid and Mohamed (2008) found no significant differences ($p>0.05$) in plasma total protein, albumin and total globulins of fish fed the experimental diets containing different levels of probiotics (Biogen® and Pronifer®) in comparison with the control diet. Also, Wang *et al.* (2008b) found that there was no remarkable difference ($p>0.05$) in the total serum protein, albumin and globulin concentrations and albumin/ globulin ratio between the *O. niloticus* supplemented with the probiotic bacterium, *Enterococcus faecium* ZJ4 and the control fed the basal diet.

Table 6: Effect of different stocking densities and dietary supplementation of Biogen® on plasma proteins of mono-sex Nile tilapia (Means±SE)

Treatment	Total protein (g/%)	Albumin (g dL^{-1})	Globulin (g dL^{-1})	*AL/GL ratio
T_1	7.90±0.30c	5.05±0.25c	2.85±0.05bc	1.75±0.05c
T_2	8.10±0.20c	5.25±0.25c	2.85±0.05bc	1.85±0.15bc
T_3	9.95±0.45b	6.80±0.40b	3.15±0.05b	2.20±0.10ab
T_4	11.90±0.50a	8.25±0.45a	3.65±0.05a	2.30±0.10a
T_5	7.75±0.15c	5.05±0.15c	2.70±0.00c	1.85±0.05bc
T_6	7.85±0.15c	5.15±0.05c	2.70±0.20c	1.95±0.15abc
T_7	7.65±0.15c	4.95±0.05c	2.70±0.20c	1.85±0.15bc

a-c: Means in the same column having different letters are significantly different ($p\leq 0.05$). *AL/GL ratio: Albumin /Globulin

Histometric Examination of Fish Dorsal Muscles

There were insignificant ($p \geq 0.05$) increases of smallest diameter, mean diameter (µm), smallest/largest ratio, intensity of muscular bundles mm^{-2} and the percentage of muscular bundles area mm^{-2} of dorsal muscles of fish in T_4 compared with the control (T_1), but the percentage of interstitial connective tissue mm^{-2} decreased insignificantly ($p \geq 0.05$) in T_4 compared with the control. No significant differences were recorded in largest diameter (µm) among all experimental treatments. It is of interest to note that, T_4 treatment realized the best growth performance and carcass composition of fish compared with the control and other treatments. On the other side, there were no adverse effects recorded on histometric measurements of fish dorsal muscles by increasing the stocking density of fish probably for the addition of the natural probiotic Biogen®. Compared with the control treatment (T_1), fish in T_7 reflected significantly ($p \leq 0.05$) increase in smallest diameter and smallest/largest ratio, but these increases appeared not significantly ($p \geq 0.05$) for mean diameter (µm), intensity of muscular bundles mm^{-2}, the percentage of muscular bundles area mm^{-2} and the percentage of connective tissue mm^{-2} of fish dorsal muscles (Table 7, Fig. 1a-g). This means that supplementation of natural and commercial probiotic Biogen® at level of 0.3% to fish diets under high stocking densities led to improvement of most histometric characteristics of the dorsal muscles of fish compared with the control fish group (T_1) which fed diet without addition of Biogen®.

These results agree with those reported by Abdelhamid *et al.* (2004), they found that the *O. niloticus* group fed diet containing 1 kg Betafin® ton^{-1} and 600 mL Biopolym® ton^{-1} was the best treatment among all treatments concerning the muscular bundles and total surface area occupied by the muscular bundles mm^{-2}. Also, they added that the superiority of the histological structure of dorsal muscles in this treatment among all treatments was related with the high growth performance, feed and nutrients utilization and characteristics of fish production. Moreover, recently Khalil *et al.* (2009) revealed that mono-sex *O. niloticus* fed diet containing 25% replacement of fish meal by supplemented jojoba meal *Simmondsia chinensis* with methionine and Biogen® at level of 0.6 and 2.0 g kg^{-1} diet respectively (T_2), led to improved significantly ($p \leq 0.05$) the histometric characteristics of the dorsal muscles of mono-sex *O. niloticus* compared with the control treatment (T_1).

Table 7: Effect of different stocking densities and dietary supplementation of Biogen® on histometric characteristics of dorsal muscles of mono-sex Nile tilapia (Means±SE)

	Treatment			
	T_1	T_2	T_3	T_4
Smallest diameter (µm)	10.40±0.87b	12.20±0.97b	11.40±0.51b	13.40±1.50ab
Largest diameter (µm)	21.00±2.65	18.80±1.20	17.20±1.77	22.00±1.76
Mean diameter (µm)	15.70±1.17ab	15.50±1.01ab	14.30±0.97b	17.70±1.37ab
Smallest/Largest ratio	0.55±0.11b	0.65±0.03ab	0.69±0.07ab	0.61±0.07ab
Intensity of muscular bundles (mm^{-2})	5466.67±933.33cd	4533.33±933.33cd	3600.00±0.00d	3600.00±0.00d
% of muscular bundles area* (mm^{-2})	72.30±0.00ab	59.30±6.67b	72.30±0.00ab	91.60±0.00a
% of connective tissue** (mm^{-2})	27.70±0.00ab	40.70±6.67a	27.70±0.00ab	8.40±0.00b

	Treatment		
	T_5	T_6	T_7
Smallest diameter (µm)	11.40±1.40b	12.80±1.16b	17.20±2.27a
Largest diameter (µm)	18.80±1.28	19.20±1.53	21.00±1.87
Mean diameter (µm)	15.10±1.21b	16.00±0.72ab	19.10±1.78a
Smallest/Largest ratio	0.61±0.05ab	0.69±0.09ab	0.83±0.10a
Intensity of muscular bundles (mm^{-2})	11466.67±1466.67a	8800.00±1200.00ab	7600.00±1200.00bc
% of muscular bundles area* (mm^{-2})	76.43±2.07ab	74.60±10.31ab	71.97±12.68ab
% of connective tissue** (mm^{-2})	23.57±2.07ab	25.40±10.31ab	28.03±12.68ab

a-d: Means in the same row having different letters are significantly different ($p \leq 0.05$). * % of muscular bundles area mm^{-2} = ([3.14×(mean diameter/2)^2]×Intensity of muscular bundles mm^{-2})×100, whereas: the muscular bundles were considered as approximately circularity shape,** % of connective tissue mm^{-2} = (1-muscular bundles area, mm^{-2})×100

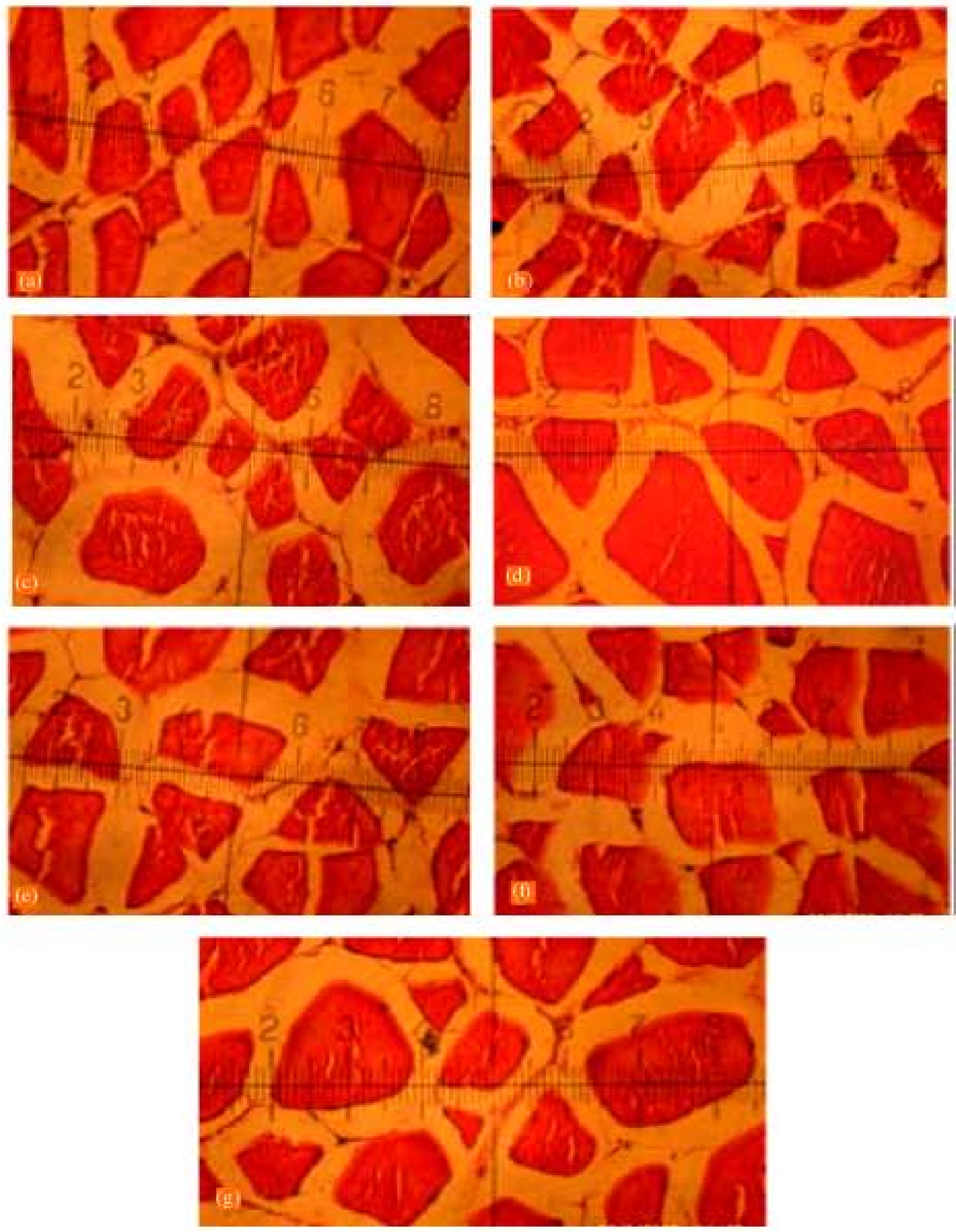

Fig. 1: Showing cross-section of muscular bundles and interstitial connective tissue of the dorsal muscles of mono-sex Nile tilapia in the (a) 1st, (b) 2nd, (c) 3rd, (d) 4th, (e) 5th, (f) 6th and (g) 7th treatments, respectively. (X 280 E and H stains)

Economic Efficiency

Results in Table 8 showed that T_4 realized significant ($p \le 0.05$) increases in all economic parameters (total feed cost, total outputs, net return and economic efficiency) and high relative economic efficiency compared with the control treatment (T_1) and other treatments. However, increasing stocking densities (T_5, T_6 and T_7) led to significant ($p \le 0.05$) increases in economic parameters compared with the control (T_1). From economic point of view, these positive effects due to the dietary supplementation of commercial probiotic Biogen® which alleviated the stocking density stress and significantly improved the growth performance, carcass composition, blood hematological and biochemical parameters and histometric characteristics of fish dorsal muscles in the present study. Additionally, Biogen® can enhance the metabolism and energy of fish body cells, raise the efficiency of feed utilization, increase the vitality of cells by supplying oxygen to whole body and improve the immune responses. Moreover, Biogen® has bactericidal effects and increases the palatability of feed, promotes the secretion of digestive fluids and stimulates the appetite (Mehrim, 2001).

Table 8: Economic efficiency (%) of different stocking densities and dietary supplementation of Biogen® of mono-sex Nile tilapia (Means±SE)

Treatment	Total feed cost[1]	Total outputs[2]	Net return[3]	Economic efficiency[4]	Relative economic efficiency
T_1	0.31±0.93g	2.62±0.18d	2.30±0.17d	732.47±55.48d	100.00
T_2	0.38±1.85b	3.30±0.16d	2.92±0.16d	761.33±41.22d	126.69
T_3	0.36±1.85d	8.92±0.59c	8.56±0.59c	2355.23±161.26c	371.26
T_4	0.39±1.85a	21.48±1.34a	21.09±1.34a	5475.70±346.91a	915.37
T_5	0.37±1.85c	20.47±0.59a	20.10±0.59a	5384.83±157.37a	872.02
T_6	0.33±0.93f	14.82±0.78b	14.49±0.78b	4413.60±236.72b	628.75
T_7	0.34±0.93e	16.41±0.54b	16.06±0.54b	4686.17±157.01b	697.01

a-g: Means in the same column having different letters are significantly different ($p \leq 0.05$). 1. Total feed costs (LE) : Feed costs per one kg diet×feed intake. 2. Total outputs (LE kg^{-1}): Fish price X total fish production*, * Total fish production: Final number of fish×fish weight gain. 3. Net return per treatment (LE): Total outputs-total feed costs. 4. Economic efficiency (%): (net return/total feed costs)×100. The price of 1 kg ingredients used was 7.00 LE for fish meal, 2.50 LE for soybean meal, 1.50 LE for yellow corn, 1.50 LE for wheat bran, 7.50 LE for corn oil, 7.00 LE for vit. and min. premix,1.50 LE for molasses and 45.00 LE for Biogen® according to local market price at the time of study (2007) in Egypt

CONCLUSION

In conclusion, from the above mentioned results in the present study, it could be recommended the useful dietary supplementation of commercial probiotic Biogen® at a level of 0.3% with stoking density of 30 fish m^{-3} of mono-sex Nile tilapia *O. niloticus* fingerlings (T_4).This treatment realized the best growth performance, carcass composition, blood hematological and biochemical parameters, histometric characteristics of fish dorsal muscles and economic efficiency without any adverse effects on water quality criteria. Yet, it could be required a lot of scientific efforts to maximize the commercial benefits from the environmentally-friendly commercial or natural probiotics with the local fish species.

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