EFFECT OF USING PROBIOTIC AS GROWTH PROMOTERS IN COMMERCIAL DIETS FOR MONOSEX NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS

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Abstract

An experiment was conducted to evaluate the effect of two different commercial feed additives on growth performance of monosex Nile tilapia fingerlings. Seven treatments were applied, three levels of Biogen® (0.1, 0.2 and 0.3%) and Pronifer® (0.1, 0.2 and 0.3%) in addition to control. The tested diets were applied in 21 fiberglass tanks each was stocked randomly with 20 monosex Nile tilapia fingerlings with an average initial body weight 10.0 ± 0.20g. The experiment lasted for 90 day. Generally, growth performance, feed conversion ratio, protein efficiency ratio and apparent protein digestibility were improved for monosex tilapia fingerlings fed on diets with commercial feed additives compared to fish fed on the control diet. In terms of blood measurements no significant differences were detected in plasma total protein, plasma albumin and plasma total globulins of fish fed on the experimental diets. Feed cost required to produce 1Kg weight gain compared to fish fed the control diet was reduced by using commercial feed additives (Biogen® or Pronifer®). These results revealed that using Biogen® at level of 0.1% was the best in terms of growth performance and economic evaluation.

Keywords: feed additives, tilapia, growth performance, feed utilization.

INTRODUCTION

The global aquaculture industry currently accounts for over 45% of all seafood consumed. That figure has been projected to increase to 75% over the next 20 years (FTU, 2007). In Egypt, the production of fish coming from aquaculture represented about 60% of total fish production sources (GAFRD, 2007). This activity requires high-quality feeds, which should contain not only necessary nutrients but also complementary feed additives to keep organism's healthy, favor growth and environment-friendly aquaculture. Feed additives are substances which added in trace amounts provide a mechanism by which such dietary deficiencies can be addressed which benefits not only the nutrition and thus the growth rate of the animal concerned, but also its health and welfare in modern day fish farming. Some of the most utilized growth-promoting feed additives include hormones, antibiotics, ionospheres and some salts (Fuller, 1992; Go´ngora, 1998; Klaenhammer and kullen, 1999). Probiotics Also feed additives (Zootechnical additives) which are defined as live microbes that may serve as dietary supplements to improve the host intestinal

microbial balance and growth performance (Gatesoupe, 1999). The Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Thompson *et al.*, 1999; Verschuere *et al.*, 2000 and Carnevali *et al.,* (2006). Thus, the use of probiotics in aquaculture has received some of attention (Diab *et al.,* 2002, Irianto and Austin, 2002, Li and Gatlin, 2004, 2005, Yanbo and Zirong 2006 and El-Dakar, *et al.,* 2007). Some common strains used as probiotics products such as *Lactobacillus acidophilus, L. bulgaricus, L.plantariu, Streptococcus lactis* and *Saccharomyces cerevisiae* (FAO) 2004. Thus, this study was conducted to determine the effect of using graded levels of probiotics (Biogen®) and Pronifer® on growth performance, feed utilization, body composition and economic evaluation of feed costs of Nile tilapia (*O. niloticus)* fingerlings.

MATERIALS AND METHODS

Experimental fish

Four hundred and twenty monosex Nile tilapia fingerlings with an average body weight (10g ±0.2g) were obtained from Fish Research Center, Faculty of Agriculture, Suez Canal University. Fish were acclimated to laboratory conditions for 2 weeks before being randomly divided into seven equal experimental groups (20 fish each treatment, three replicate/tanks,) representing seven nutritional groups. One group served as control and six groups represented the feed additives tested. The experimental fish were weighted every 15 days in order to adjust the daily feed rate which was 3 %of the total biomass at three times/ day (8.30, 12.30, and 4.30 pm) for 90 days.

Experimental unit

The present study was conducted in the Fish Research Center, Faculty of Agriculture, Suez Canal University. The experimental fish were stocked in 21 circle fiber glass tanks (380L) supplied with fresh water through a closed recycling system. Tank water was aerated continuously by using an air compressor. Water flow rate was maintained at approximately 1.5L/min. Photoperiod was 12h light/ 12h dark. Water temperature was maintained at (27 ±1°C) by using a 250- watt immersion heater with thermostat. Water temperature and dissolved oxygen were recorded daily (by metteler Toledo, model 128.s/No1242) where the average range of dissolved oxygen was above 5.8 mg/l. Other water quality parameters including pH and ammonia were measured every two days by pH meter (Orion model 720A,s/No

13062) and ammonia meter (Hanna ammonia meter),where the average range of total ammonia was 0.12 - 0.23 mg/l and pH was in range of 7.2 ± 0.5 during the experiment.

Experimental diets

Seven isonitrogenous diets were formulated from practical ingredients (Table1) where the control basal diet was without feed additives and the other diets were supplemented by 0.1, 0.2 and 0.3% Biogen® for diets 1, 2 and 3 and 0.1, 0.2 and 0.3% Pronifer® for diets 4, 5 and 6, respectively. The experimental diets were formulated to contain almost 25% crude protein. The experimental diets were prepared by individually weighing of each component and by thoroughly mixing the mineral, vitamins and additives with corn. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making granules. The wet mixture was passed through CBM granule machine with 2mm diameter. The produced pellets were dried at room temperature and kept frozen until experimental start.

Table 1. Composition and proximate analysis of the experimental diets

Feed Ingredients	Experimental Diets						
	Control	1	2	3	4	5	6
Fish meal (60% CP)	10	10	10	10	10	10	10
Corn gluten (60% CP)	12	12	12	12	12	12	12
Soybean meal (44% CP)	22	22	22	22	22	22	22
Wheat bran	18	18	18	18	18	18	18
Yellow corn	29.5	29.4	29.3	29.2	29.4	29.3	29.2
Soy & fish oil	4	4	4	4	4	4	4
Vitamin & Mineral Mix[1]	3	3	3	3	3	3	3
Di Calcium phosphate	1	1	1	1	1	1	1
Biogen®[2]	-	0.1	0.2	0.3			
Pronifer®[3]					0.1	0.2	0.3
Cr_2O_3[4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
TOTAL	100	100	100	100	100	100	100
Chemical composition (%)							
Moisture	9.4	9.4	9.6	9.5	9.3	9.9	9.6
Crude protein	25	25.3	25.5	25.8	25.1	25.3	25.4
Ether extract	6.7	6.7	6.6	6.7	6.4	6.6	6.5
Crude fiber	6.6	5.9	6.1	6.8	6.2	6.1	6.2
Ash	7	7.1	6.8	6.5	6.8	6.7	6.8
N.F.E[5]	45.3	45.6	45.4	44.7	46.2	45.4	45.5
Gross energy Kcal/ 100g[6]	390.3	393.2	392.6	392.3	391.7	391.5	391.5

1- 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.

2- Each kg Biogen® contained: Allicin 0.247 micromil/gm, high-unit hydrolytic enzyme 3690 units/gm, (proteolytic- lipolytic- amylolytic and cell separating enzymes), *Bacillus subtilis* Nato 6x 107 cells/gm, Ginseng extract. Manufactured by China Way Corporation 16- 4 No. 424 Chung Ming Road. Taichung Taiwan.

3- Each kg Pronifer® contained: Viable lactic acid bacteria approx. 10^6 colony forming units (CFU)/gram, being *Lactobacillus plantarum, L. fermentum, L. brvis, L. casei* and *Pediococcus acidilacticcii*), Lactic acid fermentation metabolites and enzymes (organic acid, glucosidase and peptidase enzymes), Free (soluble) amino acids and short-chain peptides (protein predigesting as a result of lactic acid fermentation).Manufactured by P.G.E Company (EGGER), Austria.

4- Cr2O3: Chromic Oxide

5- Nitrogen free extract

6- Gross energy. Based on 5.65 Kcal/g protein, 9.45 Kcal/g fat and 4.1 carbohydrate Kcal/g (NRC, 1993)

Experimental Methodology

The tested diets were analyzed for crude protein (CP %), ether extract (EE %), crude fiber (CF %), ash (%) and moisture while whole body composition of fish samples were also analyzed except crude fiber (CF %) according to the procedures described by standard A.O.A.C. methods (1995). The nitrogen free-extract (NFE %) was calculated by differences. Blood sample was collected using heparinized syringes from caudal vein of the experimental fish at the termination of the experiment. Blood was centrifuged at 3000rpm for 5 minutes to allow separation of plasma which was subjected to determination of plasma total protein (Armstrong and Carr, 1964) and plasma albumin (Doumas, *et al.*, 1977). Apparent protein digestibility was determined using the method of Furukawa and Tuskahara (1966). For determination of protein digestibility the diets and faeces were collected during the last 15 days of the experimental period. Any uneaten feed or faeces from each tank was carefully removed by siphoning about 30 min after the last feeding. Faeces were collected by siphoning separately from each replicate tank before feeding in the morning. Collected faeces were then filtered, dried in an oven at 60°C and kept in airtight containers for subsequent chemical analysis.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using the general linear models procedure of statistical analysis system (SAS) version 8.02.

Duncan's multiple range test (Duncan, 1955) was used to resolve differences among treatment means at 5% significant level

RESULTS AND DISCUSSION

Growth performance

The growth performance parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings which fed diets supplemented with either feed additives of (Biogen®) or (Pronifer®) are shown in Table (2). Average of initial body weight of Nile tilapia fingerlings fed the experimental diets at the start did not differ, indicating that groups were homogenous. At the end of the experimental period (90 days), the group of fish fed the supplemented diets grew as well or better than the group of fish fed the control diet. Whereas, the final body weight of the fish groups fed on diets 1, 2 and 3 had significantly ($P<0.05$) higher final body weight than the rest of the experimental groups. However, the lowest final body weight (72g) was achieved by the group of fish fed the control diet. Analysis of variance for weight gain followed the same trend as in final body weights. On the other hand, the fish groups fed on diets 1, 2, and 3 had significantly ($P<0.05$) higher SGR than the rest of experimental groups. However at the end of the trial, SGR values were 2.20 (control diet), 2.42, 2.41, 2.43, 2.22, 2.23 and 2.29 %/d for fish groups fed on diets containing 0.1, 0.2 and 0.3% (Biogen®) and 0.1,0.2 and 0.3% (Pronifer®), respectively. These results are in agreement with the results of Mehrim (2001), and Diab, *et al.* (2002) for tilapia. Khattab *et al.* (2004) and Mohamed *et al.* (2007) reported that the Nile tilapia (*O. niloticus)* fingerlings fed on diets supplemented by probiotics exhibited greater growth than those fed with the control diet. Also, they reported that the diet contain 30% protein supplemented with Biogen® at level of 0.1% produced the best growth performance and feed efficiency, moreover they also reported that Biogen® is an appropriate growth-stimulating additive in tilapia cultivation. Similar results were reported using bacteria as a probiotics by Kozasa (1986) for yellowtail (*Seriola lalandei*), Gatesoupe (1989 and 1991) and Gatesoupe *et al.* (1989) for Turbot (*Psetta maxima*) and Japanese flounder (*Paralichthys olivaceus*), in addition Carnevali *et al.* (2006) for sea bass *Dicentrarchus labrax*, Decamp and Moriarty (2006) for shrimp (*Litopenaeus vannamei, L. stylirostris* and *Penaeus monodon*) and Yanbo and Zirong (2006) for common carp (*Cyprinus carpio*). Similar trend was found, in this respect with Noh *et al.* (1994) and Bogut *et al.* (1998) who studied the effect of supplementing common carp feeds with different additives, including antibiotics, yeast (*S. cerevisiae*) and bacteria (*S. faecium*). They observed better growth with probiotic-supplemented diets but obtained the best growth with the bacterium. It is also necessary, to consider the possibility of

interspecies differences with the use of the probiotics. In contrary with these findings Abdelhamid *et al.* (2002) who found that Biogen® supplementation did not significantly improve growth performance in tilapia fish. In addition, the supplementation of probiotics (Biogen® and Pronifer®) led to 100% Survival rate which shown in Table (2).

Feed Utilization

Results of feed utilization in terms of FCR, PER and FE are presented in (Table2). The average of feed conversion ratio (FCR) in fish groups fed on diets 6 and 5 followed by groups of fish fed on diets 3 and 2 were significantly ($P<0.05$) improved in comparison with the other groups and better than the basal diet. The FCR was found to be 1.75 (control diet), 1.45, 1.44, 1.46, 1.71, 1.66 and 1.61, respectively. These results indicated that the best ($P<0.05$) FCR values were obtained for group of fish fed on diet 2, 1, 3, 6 and 5 respectively. The best FCR values observed with probiotics Biogen® supplemented diets suggested that addition of probiotics improved feed utilization. Similar results have been reported for probiotics use in diets for tilapia fingerling by Khattab *et al.* (2004) and Mohamed *et al.* (2007). In practical terms, this means that the use of probiotics can decrease the amount of feed necessary for animal growth which could result in reductions of production cost. The same trend was observed in PER where the fish groups fed on diets 1 and 2 showed better ($P<0.05$) PER values compared with the other groups. The PER was found to be 2.28 (control diet), 2.72, 2.72, 2.66, 2.33, 2.38 and 2.49, for group of fish fed diets 1, 2, 3, 4, 5, and 6 respectively. The protein efficiency ratio results indicate that supplementing diets with probiotics significantly ($P<0.05$) improved protein utilization in commercial diets of tilapia. Also, the results of feed efficiency followed the same trend of FCR and PER which was found to be 0.69 for group of fish fed diets 1, 2 and 0.68 for group of fish fed diet 3. In the present study, the commercial feed additives (probiotics) used significantly ($P<0.05$) enhanced feed efficiency. These results are in agreements with the findings of Bomba *et al.* (2002), Khattab *et al.* (2004) and Mohamed *et al.* (2007).

Digestibility Study

Results of apparent protein digestibility are presented in (Table 2) showed that the apparent protein digestibility were improved for tilapia fingerlings fed on the diets supplemented by commercial feed additives compared to group of fish fed the control diet. The better digestibility obtained with the addition of probiotics improved diet and protein digestibility, which may in turn explain the better growth and feed efficiency noticed with the supplemented diets. These results are in conformity with Lara-Flores *et al.* (2003), De-Schrijver and Ollevier (2006) and Mohamed (2007).

Blood measurements

Results of blood measurements showed no significant differences (P> 0.05) in plasma total protein, plasma albumin and plasma total globulins of fish fed the experimental diets in comparison with the control diet. These findings are in agreement with Soliman (2000) and Mohamed (2007) they noted that increasing the Plasma total protein indicates the improvement of the nutritional value of the diet.

Body composition

Table (3) explored that average of whole body composition including crude protein, ether extracts and ash estimated as wet weight basis. No statistical differences were observed in whole body moisture, crude protein, ether extracts and ash. These results are in close agreement with the results of Diab *et al.* (2002), Lara-Flores *et al.* (2003) and Mohamed *et al.* (2007).

Economic evaluation

Calculations of economical efficiency of the tested diets based on the cost of feed, costs of one Kg gain in weight and its ratio with the control group are shown in Table (4). As described in this Table feed costs and cost per kg gain (L.E) were the highest for the control diet (5.74 L.E) and gradually decreased with the increasing levels of feed additives (Biogen® and Pronifer®). The lowest relative percentage of feed cost/ kg fish being to be 85, 87, 88, 98, 96 and 95 for diets 1, 2, 3, 4, 5 and 6, respectively. Moreover, the relative percentage of feed cost/ kg gain was found to be 4.59, 4.63, 4.73, 5.38, 5.26 and 5.13 (L.E) for diets 1, 2, 3, 4, 5 and 6, respectively. These results indicate that the effect of Biogen® and Pronifer® for improving growth and feed utilization parameters of mono sex Nile tilapia fingerlings as noted in Table (2). On the other hand, the incorporation of Biogen® in mono sex Nile tilapia fingerlings diets seemed to be economic at incorporation level 0.1% but increasing its level to 0.2 and 0.3% sharply increased feed cost by 4.63 and 4.73 L.E. Moreover the incorporation of Pronifer® at level of 0.1, 0.2 and 0.3% seemed to be not economic. The reduction of feed costs was easily observed for the feed cost/Kg weight gain which decreased with the increasing incorporation levels of 0.1% Biogen® for mono sex fingerling Nile tilapia diets in agreement with Khattab *et al.* (2004) and Mohamed *et al.* (2007).

CONCLUSION

From the previous results, it could be concluded that the positive influence of additions (Biogen® and Pronifer®) on growth performance of monosex fingerlings Nile tilapia diets showed positive effects. From feed utilization data and the economical point of view the diet supplemented with 0.1% Biogen® was the best treatment.

Table 2. Growth Performance and feed utilization of *O. niloticus* fingerlings fed the experimental diets

Parameters	Experimental Diets						
	Control	1	2	3	4	5	6
Initial avg. wt. (g)	10.0	10.0	10.2	9.8	9.9	10.1	10.0
final avg. wt. (g)	72.0^{c}	88^{a}	89^{a}	87^{a}	73^{c}	75^{b}	78^{b}
Weight gain (g)	62^{d}	78^{a}	78.8^{a}	77.2^{a}	63.1^{d}	64.9^{c}	68^{b}
SGR%/d	2.20^{b}	2.42^{a}	2.41^{a}	2.43^{a}	2.22^{b}	2.23^{b}	2.29^{b}
FCR	1.75^{c}	1.45^{a}	1.44^{a}	1.46^{a}	1.71^{c}	1.66^{b}	1.61^{b}
PER	2.28^{e}	2.72^{a}	2.72^{a}	2.66^{b}	2.33^{d}	2.38^{d}	2.49^{c}
FE	0.57^{c}	0.69^{a}	0.69^{a}	0.68^{a}	0.58^{c}	0.60^{b}	0.62^{b}
Feed intake (g)	108.5	113.1	113.5	112.7	107.9	107.7	109.5
APD (%)	74.3	79.3	79.5	78.5	75.1	76.1	77.3
PTP (g/dl)	4.98	5.11	5.20	5.15	5.01	5.10	5.20
PA (g/dl)	2.06	2.18	2.14	2.17	2.08	2.13	2.16
PTG (g/dl)	2.92	2.93	3.06	2.98	2.93	2.97	3.04
Survival rate (%)	95	100	100	100	100	100	100

Value in the same row with a common superscript are not significantly different ($P<0.05$)

1- Body weight (BW): fish were weighted every 15 day to the nearest g.

2- Weight gain (WG) = average final weight (g) - average initial weight (g)

3- Specific growth rate (SGR) = (Ln. Final body weight- Ln. Initial body weight) x 100/ experimental period (days)

4- Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g)

5- Protein efficiency ratio (PER) = weight gain (g) / protein intake (g)

6- Feed efficiency = (Body weight gain (g)/ feed intake (g)

7- Apparent protein digestibility. APD (%)

8- Plasma Total protein. PTP(g/dl)

9- Plasma albumin. PA(g/dl)

10- Plasma total globulins= plasma total protein- plasma albumin. PTG (g/dl)

11- Survival rate =No of survive fish/total No. of fish at the beginning X100

Table 3. Chemical composition of whole body *O. niloticus* fingerlings fed the experimental diets. (As Wet weight basis)

Chemical composition	Initial	Experimental diets						
		(control)	1	2	3	4	5	6
Moisture (%)	76.40	71.00[a]	71.1[a]	70.98[a]	71.00[a]	71.17[a]	71.12[a]	71.13[a]
Crude protein (%)	13.55	15.15[b]	15.26[a]	15.29[a]	15.27[a]	15.22[a]	15.23[a]	15.25[a]
Ether extract (%)	4.5	6.17[a]	6.00[a]	6.07[a]	6.09[a]	6.09[a]	6.02[a]	6.01[a]
Ash (%)	5.55	7.68[a]	7.64[a]	7.66[a]	7.64[a]	7.52[a]	7.63[a]	7.61[a]

Values in the same row with a common superscript are not significantly different (P< 0.05).

Table 4. Cost of feed required for producing one Kg gain of *O. niloticus* fingerlings fed the experimental diets.

ITEM	(control)	Experimental Diets					
		1	2	3	4	5	6
Cost /kg diet (LE)	3.13	3.17	3.22	3.24	3.15	3.17	3.19
Consumed feed to produce 1kg fish (kg)	1.51	1.28	1.27	1.29	1.47	1.43	1.40
Feed cost per kg fresh fish (LE)	4.72	4.05	4.09	4.18	4.63	4.53	4.47
Relative % of feed cost/ kg fish	100	85	87	88	98	96	95
Feed cost /1Kg gain(LE)	5.74	4.59	4.63	4.73	5.13	5.26	5. 13
Relative % of feed cost of Kg gain	100	80	81	82	94	92	89

1- Cost per Kg diet L.E.

2- Feed intake per fish per period/ final weight per fish Kg/Kg

3- Step 1X step 2

4- Respective figures for step 3/ highest figure in this step

5- Feed intake per Kg gain X step 1

6- Respective figures for step 5/ highest figure in this step

*Cost of 1 kg ingredients used were 8 L.E for fish meal, 2.25 L.E for soybean meal, 3.50 L.E for corn gluten, 1.50L.E for wheat bran, 1.80 L.E for corn,6.5 L.E for oil, 5L.E for Vit & Min, 45 L.E for Biogen. 20 L.E for Pronifer®

Egypt Feed Ingredients Price at start of 2008.

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تأثيرأستخدام البروبيوتك كمنشط للنمو فى العلائق التجارية لأصبعيات سمك البلطى النيلى وحيد الجنس

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أجريت تجربة لتقييم تأثير نوعين مختلفين من الإضافات الغذائية التجارية على آداء النمو فى إصبعيات أسماك البلطي النيلى وحيد الجنس. تم تطبيق سبع معاملات: ثلاث مستويات من البيوجين (٠,١ ، ٠,٢ و ٠,٣٪) ، ومن البرونيفير (٠,١ ، ٠,٢ و ٠,٣٪) بالإضافة الى معاملة الكنترول. تم تطبيق العلائق المختبرة فى ٢١ حوض من الفيبرجلاس حيث تم التخزين بمعدل ٢٠ من أصبعيات أسماك البلطي موزعه عشوائياً بمتوسط وزن ١٠ ± ٠,٢٠ جرام. استمرت التجربة لمدة ٩٠ يوما. وقد كان أفضل أداء للنمو و معدل تحويل الغذائي وكفاءة تمثيل البروتين·و معامل هضم البروتين فى أصبعيات أسماك البلطي التى غذيت على العلائق المضاف اليها الإضافات الغذائية التجارية بالمقارنة بمجموعة الأسماك التى غذيت على عليقة الكنترول. انخفضت تكاليف التغذية اللازمة لأنتاج واحد كيلو جرام من الاسماك التى غذيت على العلائق المضاف اليها الإضافات الغذائية التجارية بالمقارنة بالعليقة الكنترول. أظهرت هذه النتائج أن استخدام البيوجين بتركيز ٠,١% كان أفضل المعاملات من حيث القيمة الإقتصادية وأداء النمو.