Effect of Using Probiotic and Yeast as Growth Promoters in Commercial Diet of Tilapia (*Oreochromis niloticus*) Fingerlings

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Abstract: An experiment was conducted to evaluate the effect of two different commercial feed additives as growth promoters of monosex Nile tilapia fingerlings. Seven treatments were applied, three levels of Biobuds $^{\circ}2$ -X (0.05, 0.075 and 0.1%), Yeast (0.05, 0.075 and 0.1%) and the control: The tested diets were applied in 21 fiberglass tanks each was stocked randomly with 20 Nile tilapia fingerlings with an average initial body weight of 10.0 ± 0.20 g. The experiment lasted for 90 day. Generally, growth performance, feed conversion ratio, protein efficiency ratio and apparent protein digestibility were improved for tilapia fingerlings fed the diets with commercial feed additives compared to fish fed 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 the experimental diets. By using commercial feed additives (biobuds $^{\circ}2$ -X or yeast) feed cost required to produce 1Kg weight gain compared to fish fed the control diet was reduced. These results revealed that the Yeast 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

Forty percent of world aquatic product (including capture fisheries) derives from aquaculture. Importance of aquaculture product is set to increase dramatically as a result of over fishing of the world's waters and an increasing demand for seafood (FAO, 2007). In Egypt, the production of fish coming from aquaculture represented about 60% of total fish production sources (GAFRD, 2006). 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 environmentfriendly 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 and Streptococcus lactis (FAO)

2004. Yeasts Saccharomyces cerevisiae also are promising candidates as probiotics in aquaculture abilities to produce polyamines and to adhere and grow in the intestinal mucus of fish (Buts et al., 1994; Va'zquez-Jua'rez et al., 1997; Andlid et al., 1998; Tovar et al., 2002 and 2004). From the other hand, yeast (Saccharomyces cerevisiae) is a natural product from the brewing industry that contains various immune stimulating compounds such as h-glucans, nucleic acids as well as mannan oligosaccharides, and has been used as a diet additive for various animals. It has been observed to be capable of enhancing immune responses (Siwicki et al., 1994; Ortun o et al., 2002) as well as growth (Lara-Flores et al., 2003) of various fish species and thus may serve as an excellent health promoter for fish culture. Thus, this study was conducted to determine the effect of using graded levels of probiotic (Biobuds®2-X) and yeast on growth performance, feed utilization, body composition and economic evaluation of feed costs of Nile tilapia (O. niloticus) fingerlings.

MATERIALS AND METHODS

Experimental fish

420 mono sex Nile tilapia fingerlings with an average body weight ($10g \pm 0.2g$) which hatched and reared at 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 (each treatment had three replicate tanks, 20 fish each) 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

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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. Water temperature was maintained at (27 $\pm 1^{\circ}\text{C}$) and water quality parameters were monitored weekly where the average range of dissolved oxygen was above 5.8 mg/l. The total ammonia was 0.025-0.032 mg/l and pH was in range of 7.2 \pm 0.5 during the experiment.

Experimental diets

Seven isonitrogenous diets were formulated from practical ingredients (Table 1) where the control basal

diet was without additives and the other diets were supplemented by 0.05, 0.075 and 0.1% Biobuds®2-X for diets 1.2 and 3 and 0.05, 0.075 and 0.1% yeast for diets 4, 5 and 6, respectively. The experimental diets were formulated to contain almost 30% 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	Experimental Diet										
Ingredients	Basal diet (control)	1	2	3	4	5	6				
Fish meal	15	15	15	15	15	15	15				
Soybean meal	18.5	18.5	18.5	18.5	18.5	18.5	18.5				
Corn gluten	15	15	15	15	15	15	15				
Wheat bran	22	22	22	22	22	22	22				
Yellow corn	22	21.95	21.925	21.9	21.95	21.925	21.9				
Soy & fish oil	4	4	4	4	4	4	4				
Vitamin & Mineral Mix ¹	3	3	3	3	3	3	3				
Biobuds®2-X2	-	0.05	0.075	0.10	<u>-</u>	· -	_				
Yeast ³	_	-	-		0.05	0.075	0.10				
$Cr_2O_3^4$	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	8.9	8.9				
Crude protein	30	30.9	30.8	30.2	30.1	30.7	30.3				
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	5.7	6.1				
Ash	7	7.1	6.8	6.5	6.8	6.7	6.7				
N.F.E ⁵	40.3	40	40.1	40.3	41.2	41.4	41.5				
Metabolizabae energy Kcal/ 100g ⁶	332.7	335.7	334.7	333.6	335.5	338.8	336.5				

1- Each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D₃, 0.8 million IU; E, 4 g; K, 0.8 g; B₁, 0.4 g; Riboflavin, 1.6 g; B₆, 0.6 g, B₁₂, 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 Biobuds[®] 2 X contained: Active dried yeast culture, 200 million live cell yeast (CFU)/gm, dried saccharomyces Cerevisiae, fermentation product, roughage products, calcium carbonate, soybean oil.

- 3- Each kg yeast (DCL) contained: (saccharomyces Cerevisiae), Emulisifiante and Sorbitan Monostearte.
- 4- Cr2O3: Chromic Oxide
- 5-Nitrogen free extract
- 6-Metabolizabae energy based on 4.5 Kcal/g protein, 8.51 Kcal/g fat & 3.49 Kcal/g carbohydrate (Pantha, 1982).

Experimental Methodology

The tested diets as well as fish whole body samples were analyzed for crude protein (CP %), ether extract (EE %), crude fiber (CF %), (ash %) and moisture according to the procedures described by A.O.A.C. (1995). The nitrogen free-extract (NFE %) was calculated by differences. Blood 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 feces 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 of the data were subjected to one way analysis (P<0.05) of variance using the SPSS version 10/PC

(Statistical Package Computer Software, Chicago, Illinois, USA, 2001). The treatment means were compared according to method of Duncan new multiple range test (Duncan., 1955).

The used model was Yij = μ +Ti +eij

Where

 μ = over all mean.

Yij =the observation of the individual from T

Ti= the fixed effect of T the diet.

Eij= the experimental random error associated with individual J.

RESULTS AND DISCUSSION

The growth performance parameters of Nile tilapia Oreochromis niloticus fingerlings which fed diets either feed additives supplemented with or (Yeast) are shown in Table (Biobuds[®]2X) (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 basal diet. Whereas the final body weight of the fish groups fed on diets 6 and 3 had significantly (P<0.05) higher final body weight than the rest of the experimental groups. However, the lowest final body weight (91g) was achieved by the group of fish fed the basal diet. Analysis of variance for weight gain followed the same trend as in final body weights. On the other hand the groups of fish fed on diets land 6 had a significantly (P<0.05) higher SGR than the rest of experimental groups. However at the end of the trial, SGR values were 2.45 (basal diet), 2.64, 2.68, 2.74, 2.67, 2.67 and 2.74 %/d for groups of fish fed on diets containing 0.05, 0.075 and 0.1% (Biobuds2-X) and 0.1,0.075 and 0.1% (Yeast), respectively. These results are in agreement with the results of Lara-Flores et al. (2003) who reported that the Nile tilapia (O. niloticus) fry fed on diets supplemented by probiotics exhibited greater growth than those fed with the control diet. Also, they reported that the diet contain 40% protein supplemented with yeast produced the best growth performance and feed efficiency, suggesting that yeast is an appropriate growth-stimulating additive in tilapia cultivation. Similar results were reported by Vazquez-Juarez el al., (1993) for rainbow trout, Oliva-Teles and Goncalves (2001) for sea bass Dicentrarchus labrax, Li and Gatlin (2003) for hybrid striped bass (Morone chrysops X M. saxatilis), and Carnevali et al., (2006) for sea bass Dicentrarchus labrax. Also, Yeast provide very important non-nutritive compounds that may benefit fish health and improve growth performance, including mannose polymers covalently linked to peptides (mannoprotein), glucose polymers(glucans), chitin in minor amounts (Cabib, et al., 1982) as well as nucleic acids (Rumsey, et al., 1992). Recently, mannan oligosaccharides define as a prebiotic which are defined as a non digestible food ingredient which beneficially affects the host by selectively stimulating the growth and /or activating of the metabolism of one or limited number of health-promoting bacteria in the intestinal tract, thus improving the host's intestinal balance. Furthermore, the cell wall of Yeast includes mannan oligosaccharides improved growth performance and health promoter (White et al., 2002 and Li and Gatlin, 2004).

These results may be explained by the greater adaptive capacity of yeasts in aquatic environments in contrast to bacteria such as Bacillus sp with common carp (Yanbo and Zirong 2006). It is also necessary, to consider the possibility of interspecies differences. Results of feed utilization terms of FCR, PER and FE are presented in (Table2). The average of feed conversion ratio (FCR) of groups of fish fed on diets 6 and 3 followed by groups of fish fed on diets 5 and 2 were significantly (P<0.05) improved in comparison with the other groups and better than the basal diet. The FCR values were found to be 1.70 (Basal diet), 1.50, 1.38, 1.35, 1.45, 1.39 and 1.33, respectively. These results indicated that the best (P<0.05) FCR values were obtained by groups of fish fed on diet 6 followed by diets 3, 2 and 5. The best FCR values observed with probiotics-supplemented diets suggest that addition of probiotics improved feed utilization, with yeast being the most effective of the supplements tested in the present study. Similar results have been reported for probiotics and yeast used in diets for Nile tilapia (O.niloticus) fry by Lara-Flores et al. (2003). In the way of applicable, using of probiotics or yeast can decrease the amount of feed necessary for animal growth which results in reductions of production cost. Similar trend was observed in PER where the groups of fish fed on diets 6 and 3 showed better (P<0.05) PER values compared with the other groups. The PER was found to be 1.96 (Basal diet), 2.16, 2.36, 2.45, 2.29, 2.34 and 2.48, respectively. The protein efficiency ratio results indicate that supplementing diets with probiotics and Yeast significantly (P<0.05) improve protein utilization in tilapia. Also, the results of feed efficiency followed the same trend as FCR and PER which was found to be 0.75 for diet 6 followed by 0.74 for diet 3, 0.72 for diet 5 and 2. In the present study, the probiotic and yeast used in the experimental significantly (P<0.05) enhanced feed efficiency and the results are in agreements with the findings of Oliva-Teles and Goncalves (2001), Li and Gatlin (2005) and Yanbo and Zirong (2006). Table (2) showed that apparent protein digestibility were improved for tilapia fingerlings fed the diets with commercial feed additives compared to fish fed the control diet. The better digestibility obtained with the addition of probiotic or Yeast 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 De-Schrijver and Ollevier (2000) and Lara-Flores et al. (2003).

Also, the results of blood measurements showed no significant differences 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) who noted that increasing the Plasma total protein indicates the improvement of the nutritional value of the diet. While, Table (3) explored that average of whole

body composition including crude protein, ether extracts crude fiber and ash estimated as wet weight basis. No statistical differences were observed in whole body moisture, crude protein, ether extracts, crude fiber and ash. These results are in close agreement with the results of Diab et al. (2002) and Lara-Flores et al. (2003). In addition, Survival rate (%) shown in Table (2) illustrated that mortalities, in general, were not effected with treatments supplemented by the application of commercial feed additives (Biobuds[®]2-X and Yeast).

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 basal diet (4.79 L.E) and gradually decreased with increasing the levels of feed additives (Biobuds*2-X and Yeast). The lowest relative percentage of feed cost/ kg fish being to be 91, 84, 83, 88, 84 and 81 for diets 1, 2, 3, 4, 5 and 6 respectively. Moreover, the relative percentage of feed cost/ kg gain was found to be 4.21, 3.90, 3.83, 4.04, 3.89 and 3.72 (L.E) for diets 1, 2, 3, 4, 5 and 6, respectively. These results indicate the effect of

Biobuds[®]2-X and Yeast 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 Biobuds[®]2-X in mono sex Nile tilapia fingerlings diets seemed to be economic at incorporation level 0.1% but decreasing its level to 0.075 and 0.05% sharply increased feed cost by 3.90 and 4.21 L.E. Moreover the incorporation of Yeast at level 0.05, 0.075% seemed to be more 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% Yeast for mono sex fingerling Nile tilapia diets in agreement with Lara-Flores et al. (2003).

CONCLUSION

From the previous results, it could be concluded that the positive influence of additions (Biobuds®2-X and Yeast) 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% Yeast was the best treatment.

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

Parameters	Experimental Diets									
	Basal diet (control)	1	2	3	4	5	6			
Initial avg. wt. (g)	10.0	10	10.2	9.8	9.9	10.1	10			
final avg. wt. (g)	91.0°	108 ^b	113 ^a	116 a	109 ^ь	111ª	118 a			
Weight gain (g)	81 ^d	98°	102.8 ^b	106.2 a	99.1°	100.9 ^b	108 a			
SGR%/d	2.45°	2.64 ^b	2.68^{b}	2.74 a	2.67 ^b	2.67 ^b	2.74 a			
FCR	1.70°	1.50 b	1.38 ^a	1.35 a	1.45 ^b	1.39 a	1.33 a			
PER	1.96 ^e	2.16 ^d	2.36 b	2.45°	2.29°	2.34 ^b	2.48 a			
FE	0.59 °	0.67^{b}	0.72 a	0.74 a	0.69 ^b	0.72 a	0.75 °			
Feed intake (g)	137.7	147	141.86	143.37	143.69	140.25	143.64			
APD (%)	75.3 ^b	79.3 ^a	80.5 a	81.5 a	80.1 a	80.4 a	82 a			
PTP (g/dl)	5.01	5.27	5.55	5.75	5.35	5.50	5.78			
PA (g/dl)	2.04	2.15	2.00	2.17	2.04	2.13	2.12			
PTG (g/dl)	2.97	3.12	3.55	3.58	3.31	3.37	3.66			
Survival rate (%)	95	98	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): individual 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)
- 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 fishX100

Table (3): Chemical composition of whole body O. niloticus fingerlings fed the experimental diets.(as weight basis)

Chemical composition	Initial	Experimental diets								
		Basal diet (control)	1	2	3	4	5	6		
Moisture (%)	76.40	71.00°	71.1ª	70.98ª	71.00ª	71.17ª	71.12	71.13ª		
Crude protein (%)	13.55	15.15ª	15.22ª	15.24ª	15.21*	15.02ª	15.23ª	15.25*		
Ether extract (%)	4.5	6.17 ^a	6.00 ^a	6.07ª	6.09ª	6.09ª	6.02ª	6.01ª		
Crude fiber (%)	0.58	1.03ª	1.05ª	1.06ª	1.03ª	1.07ª	1.01ª	1.01		
Ash (%)	4.97	6.65ª	6.63 ^a	6.65ª	6.67ª	6.65ª	6.62ª	6.60°		

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	Basal	Experimental Diets						
·	diet (control)	1	2	3	4	5	6	
Cost /kg diet (LE)	2.78	2.81	2.83	2.84	2.79	2.80	2.80	
Consumed feed to produce 1kg fish (kg)	1.51	1.36	1.25	1.23	1.32	1.26	1.22	
Feed cost per kg fresh fish (LE)	4.19	3.82	3.53	3.49	3.68	3.53	3.42	
Relative % of feed cost/ kg fish	100	91	84	83	88	84	81	
Feed cost /1Kg gain(LE)	4.73	4.21	3.90	3.83	4.04	3.89	3.72	
Relative % of feed cost of Kg gain	100	89	82	81	85	82	78	

- 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

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^{*}Cost of 1 kg ingredients used were 6.5L.E for fish meal, 2.25 L.E for soybean meal, 3.15 L.E for corn gluten, 1.20 L.E for wheat bran, 1.30 L.E for corn,5.5 L.E for oil, 5L.E for Vit & Min, 55 L.E for Biobuds 2-X and 18 L.E for Yeast. Feed Ingredients Price end of 2006.

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تأثير أستخدام البروبيوتك و الخميرة كمنشط للنمو في العلائق التجارية لأصبعيات أسماك البلطي النيلي وحيد الجنس

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أجريت تجربة لتقييم تأثير نوعين مختلفين من الإضافات الغذائية التجارية على آداء النمو في إصبعيات أسماك البلطي النيلي وحيد الجنس. تم تطبيق سبع معاملات: ثلاث مستويات من بيوبلاز ٢ لكس (٠,٠٥، ٥,٠٠٠ و ٢٠,٠٪)، ومن الخميرة الجافة (٥٠,٠٠ من ١٠٠٠، و ١٠) بالإضافة الى معاملة الكنترول. تم تطبيق العلائق المختبرة في ٢١ حوض من الفيبرجلاس حيث تم التخزين بمعدل ٢٠ من أصبعيات أسماك البلطي موزعه عشوائيا بمتوسط وزن ١٠ ± ٢٠,٠٠ جرام. استمرت التجربة لمدة ٩٠ يوما. وقد كان أفضل أداء للنمو و معدل تحويل الغذائي وكفاءة تمثيل البروتين و معامل هضم البروتين في أصبعيات أسماك البلطي التي غذيت على العلائق المضاف اليها الإضافات الغذائية التجارية بالمقارنة بالمقارنة بمجموعة الاسماك التي غذيت على العلائق المضاف اليها الإضافات الغذائية التجارية بالمقارنة بالعليقة الكنترول. أظهرت هذه النسائج أن استخدام الخميرة التي غذيت على العلائق المعاملات من حيث القيمة الإقتصادية وأداء النمو.