Effect of Probiotics on performance and nutrients digestibility of Nile tilapia *(Oreochromis niloticus*) Fed Low Protein Diets

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Abstract: This study was carried out at fish laboratory of Animal Production Department, National Research Center, Dokki ,Egypt using two commercial probiotics (Premalac and Biogen) to study their effects on growth the performance of Nile Tilapia fed diets with slightly lower levels of crude protein. Premalac is a dried fermented product of *Lactobacillus acidophilus, Aspergillus oryzae* extract , *Bifedobacterium bifedum , Streptococcus faecium* , Torula yeast , skim milk , vegetable oil and $CaCo_3$. Biogen is a dried natural product composed of Allicin , high unit hydrolytic enzymes, *Bacillus subtilis* and Ginseng extract. The basal diets were formulated to contain 30, 27.5 and 25.0% crude protein (CP), each was either supplemented or not with either Premalac or Biogen at 2g /kg diet. The experiment was conducted in 3x3 factorial design and included nine treatments each in three replicates (aquaria) in which 20 fingerlings mono sex Nile tilapia of the same size and weight (1 gram) were stocked in each aquarium. The actual experimental feeding trials durated for four months. Results indicated that the lowest CP level (25%) in tilapia diets without or with the tested probiotics recorded the worst values of growth performance parameters and nutrients digestibility. The use of either Premalac or Biogen at 2g/kg diet in diets with 27.5% crude protein was more efficiently than those containing 30% CP,. in addition, it gave best values of economic efficiency. However, Biogen was more superior than Premalc. In other words, these probiotics spared nearly 2.5% CP of the recommended level for tilapia. This result would be effective from the economical point of view, since protein is the most expensive feed nutrient in all live stock feeding, particularly fish. [Nature and Science. 2010;8(5):46-53]. (ISSN: 1545-0740).

Key words: Probiotics; Oreochromis niloticus; nutrients digestibility.

1. Introduction

Nile tilapia is an economically important cultured species in several areas of the world **(El-Husseny *et al.*, 2007 and El-Saidy and Gaber, 2005)**. Egypt made an impressive increase in aquaculture tilapia production, from 24 916 mt in 1990 to 487000 mt in 2005m accounting for 55% of Egyptian total fish production (879000, mt $year^{-1}$). **(FAO 2004 and GAFRD, 2006)**.

As the principal and most expensive component in tilapia diets, protein has received the most attention in nutritional requirement studies. Tilapia need continuous supply of protein which is necessary for maintenance, growth and reproduction (**NRC, 1993)**. A study evaluating the least –cost dietary protein level for four species of tilapia (*Oreochromus mossambicus, O. niloticus, O. aureus and Tilapia Zillii)* showed that the dietary protein level from 34% to 36% provided maximum growth of young tilapia (1.5 g) , but the most cost-effective protein level was 25% to 28% (**De Silva *et al.*, 1989)**.

Practical feeds for grow out of tilapia usually contain 25 to 35% CP. However, it has been reported that the dietary CP requirements of fish vary with species, size or age , protein quality, dietary energy level, water quality, feeding and culture management. **Ogunji and Wirth (2000)** found that the diet containing 33.32% dietary protein DM with a protein: energy ratio of 16.26mg/kJ appeared optimal for the protein requirement of tilapia. **Meyer and Pera (2001)** indicated that tilapia efficiently utilize dietary protein at level between 25 and 35%. The same authors added that , the fish were less efficiently at utilizing 45% crude protein in the diet for growth.

Ogunji and Wirth (2002) concluded that a dietary deficiency in protein results not only in a deficiency of essential amino acids in the body but also affects transport and storage of lipids within the fish body

The probiotics of live microbes have shown their effectiveness to mitigate the effects of stress , resulting in a greater production. **Olvera *et al.*, (2001)** concluded that yeast have a positive effect on fish performance when cultured under stress condition of lowering dietary protein, leading to improving growth and feed efficiency. In contrast , **Hidalgo *et al.*, (2006)** found that growth and feed conversion of juvenile dentex were not significantly influenced by probiotics which is in agreement with the findings, **Shelby *et al.* (2006)** who found that the probiotic used with juvenile channel catfish diet had lack effect on specific growth promoting or immune stimulating aspects.

On the other hand, many studies concluded the positive effect of using viable microorganisms in probiotic mixtures into diets of fish (**Li. and Gatlin 2004; Brunt and Austin, 2005; Pangrahi *et al.*, 2005; Barnes *et al.*, 2006**; **Abo-State *et al.*, 2009).**

Regarding the effect of interaction between dietary CP and probiotics, **Lara- Flores *et al.*, (2003)** evaluated the effects of probiotics on growth performance in Nile tilapia under two stress factors. These stressors were dietary protein level and stocking density. They found that the fry fed diets with a probiotic supplement exhibited greater growth than those fed the control diet without probiotic.

The aim of this study is to find out the effect of Premalac and Biogen as commercial probiotics on growth and protein utilization of Nile tilapia fingerlings fed on diets with different levels of CP.

2. Material and Methods

The experiment was conducted for four months, using a total number of 540 Nile tilapia (*Oreochromis niloticus)* all male fingerlings with one gram average weight obtained from Kafr El-Sheikh fish hatchery Egypt.

The fingerlings were distributed at random into nine experimental dietary treatments , each in three aquaria (60x30x40 cm^3) as replicates in which fingerlings were stocked at a rate of 20 fish/ aquarium. Water temperature in the experiment of aquaria ranged between 27.4-27.6ºC with photo period of 12h light and 12h darkness, the dissolved oxygen level was 6.6 mg/L, and the pH value was about 7.5 representing the alkali media needed for tilapia. Water samples were weekly taken for analysis of total ammonia nitrite, nitrate and pH levels using standard methods (**APHA , 1992**) to adjust the appropriate water quality parameters for tilapia cultivation .

Experimental diets and design

Two tested commercial probiotics being Premalac and Biogen were used to study their effects on growth performance of Nile tilapia fingerlings fed diets with slightly decreasing levels of crude protein (30, 27.5 and 25%). Premalac is a dried fermentation product of *Lactobacillus acidophilus* , *Aspergillus oryzae* extract , *Bifedobacterium bifedum*, *Streptococcus faecium* , Torula yeast , skim milk , vegetable oil and $CaCO_3$. Biogen is a dried natural product composed of Allicin , high unit hydrolytic enzymes, *Bacillus subtilis* and Ginseng extract . Three basal diets were formulated to contain the recommended 30% and two lower 27.5 and 25% crude protein levels (Table 1). Each of the tested diets was either supplemented or not with either Premalace or Biogen at 2g /kg diet level. Therefore , The experiment was conducted in 3x3 factorial design and included nine treatments . Feeding level of all experimental diets was 4% of the total biomass of the fish per day. The amount of feed was divided into three equal portions and distributed by hand in one side of the aquaria three times daily at 9 a.m, 1p.m. and 5 p.m. Every fourteen days, the fish in each aquarium were weighed and the amount of feed was readjusted according to the new fish biomass **(El-Banna, 1991).** The performance parameters included weight gain, average daily gain (ADG), feed conversion ratio (FCR) and protein utilization efficiency (PUE). After running the feeding experiment, digestibility trails were conducted using 8 fish /aquarium and fish were offered the same experimental diets at a rate of 2% of the total biomass/day.

During the digestibility trail the daily meal was offered one time at 9.00 a.m. and the siphoning method was undertaken to collect the settled feces from the bottom of the aquarium. The collection of feces was made for three weeks, one time daily in the morning before the meal was provided. Consequently, the collected feces were directly spread with 10% sulfuric acid and 10% formalin and kept in a deep freezer at -4C till analysis to avoid the fermentation.

Analysis of protein, ether extract, crude fiber, and crude ash in the collected feces were carried out in pooled –dried samples. Proximate chemical analysis were made according to **A.O.A.C (1990)** methods.

Apparent Digestion Coefficients (ADC%) of the nutrients were calculated using crude fiber as an inert marker as described by **Tacon and Rodringus (1984)** as follows:

$$\%\ ADC = 100 - 100 \times \left[\frac{\%\ Marker\ in\ feed}{\%\ Marker\ in\ feaces} \times \frac{\%\ Nutrient\ in\ feces}{\%\ Nutrient\ in\ feed} \right]$$

The economical efficiency of dietary treatments were calculated to estimate the cost of feed needed to produce one kg of fish weight gain. The cost of experimental diets has been calculated in L.E according to the local market prices at year 2004.

Growth performance and feed utilization efficiency parameters were statistically compared using the **SAS programme (1992)** SAS/STAT users guide, release 6.03 Edition SAS Inst. INC. Cary, NC. USA.

Considering the interaction effect between dietary CP level and added probiotics , the used model was :

$$Xijk = M + Pi + Lj + Pi\,Lj + Eijk$$ where ,

- M the overall mean
- Pi the effect of probiotic supplementation
- Lj the effect of cp level

- Pi Lj the effect of interaction between p and l
- Eijk the experimental error .

Duncans New Multiple Range Test was conducted to determine the significant differences between means (**Duncan, 1955)**.

3. Result Analysis

Growth and feed utilization:

The effects of both dietary CP, probiotic supplementation and their interaction on growth and feed utilization of Nile tilapia fingerlings are summarized in Tables (2 and 3). The initial live body weight of all fish used was almost similar, which confirmed appropriate randomization process . Meanwhile, it created suitable condition to appraise the effect of dietary treatments on the performance of fish .

Results showed that the diet contained 27.5% CP irrespective of probiotic supplementation gave better ($P<0.05$) values for final body weight, FCR, PUE of fish when compared with those containing 30% and 25% CP. Regarding the tested probiotics, results showed that adding either Premalac or Biogen at 2g/kg recorded significantly ($P<0.05$) higher values compared to the control without supplementation .However , The best values of live body weight, FCR and PUE were obtained with Biogen followed by Premalac.

The combined effect of dietary CP with the tested probiotics showed that the diet contained 27.5% CP and supplemented either Premalac or Biogen (T5 , T6) had recorded best values for growth performance and improved the feed utilization when compared to diets with the same probiotics but contained the recommended level of CP as 30% (T_2, T_3). Moreover, the diets with 27.5% CP (T_5, T_6) were superior than the corresponding diet with the same CP level but without probiotic supplementation (T_4). This may be due to the effect of the tested probiotics which improved absorption of nutrients and depressed harmful bacterial affects that causes growth depression. These results are in agreement with those obtained by **Hoyos and Cruz (1990)** who stated that the positive effect of probiotcs may be due to their beneficial effects since its microbial constituents produce natural lactic acid that helps in maintaining an optimum low pH in the digestive tract which inhibit growth of undesirable bacteria leading to optimum enzyme activity. Similar results were observed by **Fernandes and Shahani (1990)**, who indicated that probiotic preparations contain multiple strains of *Lactobacillus* which are highly active against a wide range of stress conditions in the fish gastrointestinal tract, resulting in higher immunity and higher rate of utilizing nutrients and accordingly higher growth rate.

In this connection, **Noh *et al.* (1994)**; **Bogut *et al.* (1998)** and **Nikoskelainen *et al.* (2001)** obtained better growth response with diets supplemented with probiotics containing bacteria.

Although , diets with the high CP (30 %) and supplemented with any of the tested probiotics had recorded significantly better values than the corresponding diet without supplementation, nevertheless, diets with 27.5 % CP supplemented with probiotics were superior. These results may be explained as these probiotics (Premalac or Biogen) spared nearly 2.5% crude protein so, optimizing protein utilization for growth. These findings are in good agreement with those obtained by **Ringø and Gatesoupe (1998)** , who found that probiotics performed more efficiently in case of stress conditions like that of lowering dietary CP.

Nutrients digestibility:

Results of the effect of either dietary CP, probiotic supplementation or their interaction on nutrients digestibility are listed in table (4). Results indicated that tilapia fingerlings utilized the nutrients content of diets with 30% or 27.5% CP at equal rate. While, that contained 25% CP failed to obtain similar findings. Seamingly, Premalac and Biogen both have the same effect on nutrients digestibility. Regarding the interaction effect of dietary CP and probiotics supplementation on nutrients digestibility (Table 4), results revealed that either Premalace or Biogen when supplemented to diet containing 30% or 27.5% CP recorded significantly ($P<0.05$) higher values of nutrient digestibility than the non supplemented diets, with no significant differences among those treatments. However, the use of the same probiotics with diets containing 25% CP recorded significantly the lowest values.

Similar results were obtained by **De Schrijver and Ollevier (2000)** who found positive effect on apparent protein digestion when turbot (*Scophthalmus maximus*) diets were supplemented with bacteria *Vibrio proteolyticus*. They attributed this effect to the proteolytic activity of bacteria.

Economical evaluation:

Data in Table (5) show that the best values of economical efficiency expressed as feed cost /kg gain in weight and relative economic efficiency were for diets containing 27.5% CP and supplemented with either Premalac or Biogen. Meanwhile, the worst values were recorded with diets containing the lowest CP (25%) level .

By comparison, the results obtained confirmed the higher adequacy of Biogen for tilapia than Premalac,

which is commonly used in poultry feeding. It is obvious that the bacterial strain *Bacillus subtilis* mainly present in Biogen is more convenient to the digestive system of fish than *Lactobacillus* strain in Premalac. Moreover, Biogen contains ginseng extract that needed by fish to keep their internal biological functions at sufficient levels. In addition, Biogen characterized by the presence of Allicin which helps to increase the activity of endocrine glands, therefore, secretion of various hormones especially growth hormone are increased. It contained also high-unit hydrolytic enzymes such as photolytic, biolytic and amylolytic that have the capacity to increase the digestibility and utilization of nutrients and decrease ammonia production by fish, which results in higher growth with lower feed consumption, otherwise healthy environment.

Table (1): Composition and calculated analysis of the experimental diets.

Ingredient , %	**Recommended CP**	**Lower CP**	
	30%	**27.5%**	**25.0%**
Yellow corn	48.0	52.0	56.0
Soy bean meal (44%)	18.5	18.5	18.5
Fish meal (72%)	26.0	22.0	18.0
Corn oil	5.0	5.0	5.0
Vit. Min. Mix*	2.5	2.5	2.5
Total	100	100	100
Calculated analysis , %			
Moisture	6.30	7.00	7.60
DM Composition:			
CP	32.13	29.62	27.11
EE	11.01	10.34	10.34
CF	1.83	1.89	1.94
Ash	5.59	5.07	4.55
NFE	49.44	52.75	56.06
GE kcal / kg **	4906.6	4867.4	4828.8
E : P ratio	152.7	164.3	178.1
Price / ton (L . E)	2932.2	2708.6	2485.0

* Each 1 kg. Contains vitamin A, 4.8 I.U.; vit D_2 , 0.8 m I.U; vit E, 4.0 g; vit. K, 0.8 g; vit B1, 0.49, vit. B2, 1.6 g; vit. B6, 0.6 g; vit. B12, 4 mg; Pantothenic acid 49; Nicotinc acid 8 g; Folic acid, 400 mg; Biotin, 20 mg; Choline chloride, 200 mg; Copper, 4.0 g ; Iodine,0.4g ; Iron, 12 mg ; Manganese, 22 g; Zinc 22 g and Selenium 0.04 g.

** Gross energy value was calculated from their chemical composition, using the factors 5.65, 9.45, 4.00 and 4.00 (k cal/g) for protein, fat, fiber and NFE respectively **(Jobling, 1983).**

Table (2): Effect of dietary protein level, probiotics supplementation and their interaction on growth parameters of Nile tilapia.

Treatment			**Initial wt. g/fish**	**Final wt. g/fish**	**Total gain g/fish**	**ADG g/fish**
Tr No.	**CP %**	**Probiotic**				
(1)	**30**		1.12	17.73^{b}	16.61^{b}	0.138^{b}
	27.5		1.15	19.87^{a}	18.72^{a}	0.156^{a}
	25		1.13	14.98^{c}	13.85^{c}	0.115^{c}
(2)		_____	1.14	16.38^{b}	15.24^{b}	0.127^{b}
		Premalac	1.13	18.03^{a}	16.90^{a}	0.141^{a}
		Biogen	1.14	18.55^{a}	17.41^{a}	0.145^{a}

T1	**30**	**Control**	1.14	17.95^{bc}	16.81^{bc}	0.140^{bc}
T2	**30**	**Premalac**	1.13	17.64^{c}	16.51^{c}	0.138^{bc}
T3	**30**	**Biogen**	1.14	18.65^{bc}	17.51^{bc}	0.146^{bc}
T4	**27.5**	**_____**	1.16	17.05^{bc}	15.89^{bc}	0.132^{bc}
T5	**27.5**	**Premalac**	1.12	21.10^{a}	19.98^{a}	0.167^{a}
T6	**27.5**	**Biogen**	1.18	21.60^{a}	20.42^{a}	0.170^{a}
T7	**25**	**_____**	1.12	14.15^{d}	13.03^{d}	0.109^{d}
T8	**25**	**Premalac**	1.14	15.36^{d}	14.22^{d}	0.118^{d}
T9	**25**	**Biogen**	1.12	15.40^{d}	14.28^{d}	0.119^{d}
SEM			0.028	1.98	1.82	0.020
Prob.			0.3014	0.0008	0.0007	0.0029

a, b .... Means in the same column within each factor with different superscripts are significantly different (P<0.05).

(1) Effect of CP level regardless to probiotics

(2) Effect of probiotic regardless to CP level

Table (3): Effect of dietary protein level, probiotics supplementation and their interaction on feed conversion ratio (FCR) and protein utilization efficiency (PUE) by Nile tilapia during whole period.

Treatment			**FCR**	**PUE**
Tr. No.	**CP %**	**Probiotic**	**feed / gain**	**gain / protein intake**
(1)	**30**		1.746^{a}	1.782^{b}
	27.5		1.695^{a}	1.991^{a}
	25		2.695^{b}	1.368^{c}
(2)		**_____**	2.185^{b}	1.556^{b}
		Premalac	1.926^{a}	1.765^{a}
		Biogen	1.934^{a}	1.756^{a}
T1	**30**	**Control**	1.812^{a}	1.717^{c}
T2	**30**	**Premalac**	1.723^{a}	1.806^{bc}
T3	**30**	**Biogen**	1.717^{a}	1.812^{bc}
T4	**27.5**	**_____**	1.919^{a}	1.759^{c}
T5	**27.5**	**Premalac**	1.634^{a}	2.066^{a}
T6	**27.5**	**Biogen**	1.623^{a}	2.079^{a}
T7	**25**	**_____**	2.986^{c}	1.235^{d}
T8	**25**	**Premalac**	2.570^{b}	1.434^{d}
T9	**25**	**Biogen**	2.648^{b}	1.393^{d}
SEM			0.324	0.203
Prob.			0.2487	0.0001

a, b .... : Means in the same column within each factor with different superscripts are significantly different (P<0.05).

(1) Effect of CP level regardless to probiotic

(2) Effect of probiotic regardless to CP level

Table (4): Effect of dietary protein level, probiotics supplementation and their interaction on apparent digestion coefficient (ADC) of dietary nutrients of Nile tilapia.

Treatment			ADC , %			
Tr. No.	**CP %**	**Probiotic**	**DM**	**CP**	**EE**	**NFE**
(1)	**30**		86.07[a]	85.23[a]	91.45[a]	82.21[a]
	27.5		86.13[a]	85.69[a]	92.00[a]	83.02[a]
	25		76.99[b]	76.28[b]	85.33[b]	77.87[b]
(2)		**_____**	78.20[b]	77.79[b]	86.71[b]	78.09[b]
		Premalac	84.30[a]	83.06[a]	90.85[a]	82.54[a]
		Biogen	86.55[a]	85.92[a]	90.92[a]	82.71[a]
T1	**30**	**Control**	80.85[b]	79.60[c]	89.45[b]	79.70[c]
T2	**30**	**Premalac**	86.95[a]	86.05[b]	92.11[a]	83.50[ab]
T3	**30**	**Biogen**	89.66[a]	89.61[a]	92.62[a]	84.21[a]
T4	**27.5**	**_____**	80.15[b]	79.82[c]	88.73[b]	78.45[c]
T5	**27.5**	**Premalac**	87.53[a]	87.15[ab]	92.50[a]	85.64[a]
T6	**27.5**	**Biogen**	89.74[a]	89.70[a]	93.04[a]	84.87[a]
T7	**25**	**_____**	73.60[c]	73.95[d]	81.95[c]	76.12[c]
T8	**25**	**Premalac**	78.42[bc]	75.98[d]	87.94[b]	78.48[c]
T9	**25**	**Biogen**	80.25[b]	78.45[c]	87.10[b]	79.05[c]
SEM			3.44	3.17	2.97	2.65
Prob .			0.0011	0.0024	0.0130	0.0260

a, b .... : Means in the same column within each factor with different superscripts are significantly different ($P<0.05$).
(1) Effect of CP level regardless to probiotic
(2) Effect of probiotic regardless to CP level

Table (5): Effect of dietary protein level and probiotics supplementation on the economic efficiency of the experimental treatments.

Treatment			**Cost /ton L.E ***	**Feed intake g/ fish**	**Total gain g/fish**	**Feed cost / kg gain L.E**	**Relative feed cost / kg gain % ****
Tr No.	**CP %**	**Probiotic**					
T1	**30**	**Control**	2932.2	30.47	16.81	5.31	100.00
T2	**30**	**Premalac**	3086.2	28.45	16.51	5.31	100.00
T3	**30**	**Biogen**	3042.2	30.07	17.51	5.22	98.30
T4	**27.5**	**_____**	2708.6	30.50	15.89	5.20	97.92
T5	**27.5**	**Premalac**	2862.6	32.66	19.98	4.68	88.13
T6	**27.5**	**Biogen**	2818.6	33.16	20.42	4.58	86.25
T7	**25**	**_____**	2485.0	38.92	13.03	7.42	139.73
T8	**25**	**Premalac**	2639.0	36.55	14.22	6.78	127.68
T9	**25**	**Biogen**	2595.0	37.82	14.28	6.87	129.37

* The price of Premalac = 77 L.E / Kg , of Biogen = 55 L.E / Kg
** Relative to T1 which represents the recommended CP level

4. Conclusions

Ether Premalac or Biogen spared nearly 2.5 % CP of the recommended level for tilapia. This result would be effective from the economical point of view, since protein is the most expensive feed nutrient in all livestock feeding , particularly in fish feeding .

References

Abo-State, H.A.; El-Kholy, Kh. F. and Al-Azab, A.A. (2009): "Evaluation of probiotic (EMMH) as a growth promoter for Nile tilapia (*Oreochromis niloticus*) fingerlings. Egyptian J. Nutrition and Feeds. Vol 12(2): 347-358

A.O.A.C. (1990): "Association of Official Agricultural chemists" official methods of analysis. 15th Ed. Published by the A.O.A.C., Benjamin Franklin Station, Washington. D.C.

APHA, (1992): Standard methods for the examination of water and wastewater. American Public Health Association Washington, D.C.

Barnes, M.E.; Durben, D.J.; Reeves, S.G. and Sanders, R. (2006): Dietary yeast culture supplementation improves initial rearing of Mc conaughy strain rainbow trout. Aqua. Nutrition Vol. 12(5): 388-394.

Bogut, I.; Milakovie, Z.; Bukvic, Z.; Brikie, S. and Zimmer, R. (1998): Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microlora in carp (*Cyprinus carpio*). Zivocisna-vyrob, 43; 2312-235.

Brunt, J. and Austin, B. (2005): Use of probiotic to control Lactococcosis and strptococcosis in rainbowtrout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 28, 693-701.

De Silva, S.S.; Gunasekera, R.M. and Atapatta, D. (1989): The dietary requirements of young tilapia and an evaluation of the least coast dietary protein levels. Aquaculture, 80: 271-284.

De-Schrijver, R. and Ollevier, F. (2000): Protein digestion in Juvenile turbot (*Scophthalmus maximus*) and effects of dietary administration of *Bivrio proteolyticus*. Aquaculture, 186: 107-116.

Duncan, D. (1955): Multiple range tests and multiple F. tests. Biometrics, 11: 1-42.

El-Banna,R. A. A. (1991) .Studies on some nutrients requirements for tilapia and its effect on performance . Ph. D . Thesis, Fac. Vet., Cairo Univ.

El-Haroun, E.R.; Goda, A.M. and Kabir Chowdhury, M.A. (2006): Effect of dietary probiotic biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.) Aquaculture Research 37(14): 1473-1480.

El-Husseiny, O. M.; Goda, A.M.; Abdul-Aziz, G.M. and El-Haroun, E.R. (2007): Fish meal free diets for Nile Tilapia *Oreochromis niloticus* (L.),. Mugill cephalous and liza Ramada in Semi-intensive ppolyculture system in earthen ponds. Egyptian J. Nutrition and Feeds 10(1): 179-203.

El-Saidy, D.M.S. and Gaber, M.M.A. (2005): Effect of dietary protein levels and feeding rates on growth performance, production trains and body composition of Nile tilapia, *Oreochromis niloticus* (L.) cultured in concrete tanks. Aquaculture Research, 36(2): 163-171.

FAO (Food and Agricultural Organization) (2004): Fishery statistics . Aquaculture production at: http:www.faostat.fao.org/faostat./ notes/units.e.html.

Fernandes, C.F. and Shahani, K.M. (1990): Anticarcinogeni and immunological properties of dietary lactobacilli. J. Food Porotect, 53: 704-710.

GAFRD (Gernal Authority for Fish Resources Development) (2006): Statistical analysis of total aquaculture production in Egypt, Ministry of Agriculture, Cairo, Egypt (Arabic edition).

Hidalgo, M.c.; Skalli, A.; Abellan, E.; Arizcum, M. and Gardenete,G. (2006): Dietary intake of probiotics and maslinic acid in Juvenile dentex (*Dentex dentex* L.). effects on growth performance, survival and liver proteolytic activies. Aqua. Nutrition Vo. 12(4): 256-266.

Hoyos, A.G. adn Cruz, C. (1990): Mecanismos de accion propuestas de la probiotics en cedes: biotechnology en la inductria de Alimentacion Animal., 1: 73-80 (C.F. Abdel-Azeem *et al*. 2001).

Jobling, M. (1983): A short review and critique of methodology used in fish growth and nutrition studies. J. Fish Biol., 23: 685 – 703.

Lara-Flores, M.; Olvera- Novoa, M.A.; Guzman-Mendez, B.E. and Lopez-Madrid, W. (2003): Use of the bacteria *Streptococcus facium* and *Lactobacillus acidophilus,* and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia

(*OPreochromis niloticus*) Aquaculture, 216: 193-201.

Li, P. and Gatlin, D.M. (2004): Deitary brewers yeast and the prebiotic grobiotic ™ AE influence growth performance, immune responses and resistance of Striped bass **(***Morone chrysops* x *M. saxatilis*) to *Streptococcus iniae* infection. Aquaculture 231: 445-456.

Meyer, D.E. and Pera, P. (2001): Ammonia excretion rates and protein adequacy in diets for tilapia *Oreochromis* sp. Aquaculture: Book-of-Abstracts
143-JM-Parker-coliseum-Louisiana State Univ. -Baton. Rouge LA-70803-USA World-Aquaculture Society 436.

Nikoskelaine, S.;Ouwehand, A.; Salminen, S. and Bylund, G. (2001): Protection of rainbow trout (*Oncorhynchus mykiss)* from furunculosis by *Lactobacillus rhamnosus*.Aquaculture, 198: 229-236.

Noh, S.H.; Han, I.K.; Won, T.H. and Choi, Y.J. (1994): Effect of antibiotics, enzyme, yeast culture and probiotics on enzyme, yeast culture and probiotics on growth performance of Israeli carp. Korean J. Animal Sci., 36: 480-486.

NRC (National Research Council) (1993): Nutrition requirement of fish Washington, D.C.

Ogunji, J.O. and Wirth, M. (2000): Effect of dietary protein content on growth, food conversion and body composition of tilapia *Oreochromis niloticus* fingerlings fed fishmeal diet. J. Aquacult. Trop., 15: 381-389.

Ogunji, J.O. and Wirth, M. (2002). Influence of dietary protein deficiency on amino acid and fatty acid composition in tilapia, *O.niloticus*, fingerlings. Isr. J. Aquacult., Bamidgeh, 54: 64-72.

Olvera, M.A.; Lara, M.; Guzman, B.E. and Lopez, W. G. (2001): Effect of the use of probiotics on growth of tilapia *Oreochromis niloticus* reared under stress conditions. Aquacutlure-Book of abstracts 143-J.M. Parker-Coliseum-Louisiana Stat5e Univ., Baton-Rouge-LA-70803-USA.
World-Aquaculture Society 497.

Pangrahi, A.; Kiron, V.; Puangkaew, J.; Kobayashi,T.; Satoh, S. and Sugita, H. (2005): The viability of probiotic bacteria as a factor influencing the immune response in Rainbow trout *Oncorhynchus mykiss*. Aquaculture 243: 241-254.

RingØ, E. and Gatesoupe, F.J. (1998): Lactic acid bacteria in fish a review. Aquaculture, 160: 177-203.

Shelby, R.A.; Lim, C.; Yildirim, M. and Klesius, P. H. (2006): Effects of probiotic bacteria as dietary supplements on growth and disease resistance in young channel catfish. *Intalurus punctatus* (Rafinesque). J. of Applied Aquaculture Vol. 18(2): 49-60.

Statistical Analysis System, SAS, (1992): SAS/STAT user's Guide Release 6.03 edn. SAS institute, Cary, NC, 1028PP.

Tacon, A.G.J. and Rodrigus, A.M.P. (1984). Comparison of chromic oxide, crude fiber, polyethylene and acid-insoluble ash as dietary markers for the estimation of apparent digestibility coefficients in rainbow trout. Aquaculture, 43: 391-399.

2/9/2010