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Evaluation of (*Bio-Nutra 200*) as A Commercial Probiotic Product in Nile Tilapia (*Oreochromis niloticus*) Diets.

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ABSTRACT

The study was conducted over a 150 days period, in order to evaluate the potential benefit of super biobuds as a probiotic agent in Nile tilapia (*Oreochromis niloticus*) feeds. Hapa hatchery, each measuring (2x4x1m) suspended in an earthen pond (10.000m²) was used. There were four treatments, each consisting of two replicates, each of 5 fish / m² (mean individual initial weight 8 g). A total number of 320 Nile tilapia were randomly distributed into four treatment groups, and were fed daily at a rate of 3% of fish live body weight. Fish were fed a balanced diet of 28 % protein along the period of the experiment. The treatments were: **1**- Diet1 (control group 15% fish meal without probiotc). **2**- Diet 2 (10% fish meal + 0.1% *Bio-Nutra*200). **3**- Diet 3 (5% fish meal + 0.2% *Bio-Nutra 200*). **4**- Diet 4 (0.3% *Bio-Nutra 200* without fish meal in fish diet). The results indicate that, the fish groups received probiotic supplemental diets revealed significant improvement in growth parameters (body weight gain, feed conversion ratio, and protein efficiency ratio), since the diets contained *Bio-Nutra200* at levels (0.1% and 0.2%) showed highest values of growth parameters and protein utilization. Carcass composition of the experimental fish was relatively affected by different dietary treatments. Internal organs indices showed no significant differences among all treatments but the tested parameters of blood hematology and biochemistry of the experimented groups indicated significant differences among the fish groups which were superior for those received diet supplemented with probiotic (*Bio-Nutra200*). Cost benefit analysis showed that low profit index and high incidence cost were obtained by the control diet. This study suggested that *Bio-Nutra200* at levels of 0.1% and 0.2% can be used in feed of Nile tilapia without negative effect on growth parameters, feed utilization, and blood parameters.

Key Words: Nile tilapia, Probiotic, *Bio-Nutra200*, Growth parameters, Feed utilization, Blood parameters, Cost benefit.

INTRODUCTION

Tilapia is one of the most important groups of fish for aquaculture with annual production exceeds two million metric tons. They have been cultured for quite a long time ago. However, their aquaculture production has been developed remarkably during the last few decades

due to the increased level of intensification and cultured area (Tri nuh, 2008). In the past, fish meal was used as a main protein source in tilapia feed. Due to the escalating price and unstable supply of this ingredient, many studies have been conducted to replace fish meal by the less expensive plant or animal protein sources and some probiotics.The global aquaculture industry currently accounts for over 45% of all seafood consumed. This figure has been projected to increase 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. Some of the most utilized growth-promoting feed additives include hormones, antibiotics, ionospheres and some salts (Klaenhammer and Kullen, 1999). Probiotics are also used as feed 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 probiotic 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 (Carnevali *et al.*, 2006). Thus, the use of probiotic in aquaculture has received some attention (Diab *et al*., 2007; El-Dakar *et al*., 2007 and Abdelhamid *et al.*, 2009). Some common bacterial strains are used as probiotic products such as *Lactobacillus acidophilus, L. bulgaricus, L.plantariu, Streptococcus lactis* and *Saccharomyces cerevisiae* (FAO, 2004). Piraret *et al*., (2006) found that number of mortality was significantly lower in probiotic supplemented fish than in control fish. Thus, the present study was conducted to determine the effect of using graded levels of probiotic (*BIO-NUTRA 200*) on growth performance, feed utilization, carcass composition, internal organs indices, blood parameters, and economic evaluation of feed costs of Nile tilapia *(O.niloticus)* fingerlings.

MATERIALS AND METHODS

The present work was conducted in a fish farm in Kafr El-Sheikh governorate during season 2008 in order to evaluate a commercial probiotic product in Nile tilapia (*O. niloticus*) diet on growth performance,

feed utilization, body composition, internal organs indices , blood parameters, and economical efficiency, under Egyptian conditions.

1- Fish culture system:

A total number of 320 Nile tilapia fish (*Oreochromis niloticus*) with average body weight of 8 gram, were obtained from Metobase area, in Kafr El-Sheikh Governorate, Egypt. Each size of fishes was randomly distributed among 8 hapa hatchery, each measuring (2 × 4 × 1 m) as width, length, and depth, respectively, suspended in an earthen pond 10.000m², at a stoking density of 5 fish/ m². Thirteen fish were frozen at-20ċ for chemical analysis at initial. The experimental pond was supplied with freshwater from El-kefah Canal in Metobese area. The water exchange rate was 15% of total pond area / day. The experimental period was 150 days from 1 July to 30 November 2008. Water pH was measured using pH meter (model 68 Engineered System and Designs). Water dissolved oxygen and temperature were measured daily at 2.00 O'clock p.m. by oxygen meter (WPA 20 Scientific Instrument).

2- Experimental diets:

Fingerlings were fed the experimental diets containing 28% CP and 453.66 Kcal/100g at a feeding rate of 3% of fresh biomass in each hapa (six days per week). Fish were fed two times daily at (8 am and 2 pm) with feed amounts adjusted at approximately 14-day intervals in response to weight gain. A basal diet was formulated from the commercial ingredients (fish meal, soybean meal, yellow corn, wheat bran, Vit.&Min. mixture and oil). The dry ingredients were grounded through a feed grinder to small particle size (0.5mm). The ingredients were weighed and mixed by a dough mixer for 20 minutes till homogeneity of the ingredients. The estimated amount of oil was gradually added (few drops gradually) and the mixing operation was continued for 20 minutes. After homogenous mixture was obtained, forty ml water per hundred g diet was slowly added to the mixture according to Shimeino *et al*., (1993). The diets were cooked on a water bath for 20 minutes. Thereafter, the different doses (0.1, 0.2, and 0.3% of *Bio-Nutra 200*) were added to the ingredients prior to pelletizing.

Probiotic (*Bio-Nutra200*) Contained: (**a**) - Digestive enzymes, such as amylase, proteases, cellulose, and lipase. (**b**)- *Aspergillus oryzae*, Bio-Nutra200 adds to this biological tool chest, already providing active dry yeast and four digestive enzymes at guaranteed level of activity, a source of *Aspergillus oryzae* fermentation soluble. This particular *Aspergillus* mold has been so cultured to produce digestion direction, while the

metabolites contribute undefined factor improving microbial digestion. **(c)**- *Bacillus subtilus*: The biological benefits to greater digestive powers for livestock and poultry are completed in Bio-Nutra200 with the inclusion of the total fermentation produce of the versatile spore former *B.subtilus*. Significant number of these bacterial spores plus its growth media is meticulously dried to retain optimum enzymatic powers. The biological catalysts produced by this organism, alpha-amylase, protease and gumase resent in *Bio-Nutra200* act to speed digestion, absorption and utilization of complex carbohydrates and protein in animals so fed. **(d)**- Active dry yeast & culture: *Bio-Nutra 200* with its live yeast cells is an important tool helping animals. Requited to grow rapidly and reproduce consistently, to meet production goals under intense management. Nutritionists measure and report the results of animal experimentation in absolute number; so should yeast culture products have accurate and defined counts help properly carry out their beneficial activities in the digestive tracts of diverse economic animals. *Bio-Nutra200* is manufactured by (Ameco – Bios&company, California, Georgia, Indiana, U.S.A.).

The diets were pelleted through fodder machine and the pellets were dried under room temperature for 24 h before use. The required amount of the diet was prepared every two weeks and stored in a refrigerator. The chemical analysis of feed ingredients used in the experimental diets is presented in Table (1). These diets were designated as diet (1) to (4), respectively they were isonitrogenous (28.24 to 28.60%) and isocaloric (435.47 to 453.66 Kcal/100g). Composition of the mixed diets is presented in Table (2).

Table (1): ***The proximate analysis of tested ingredients (% on DM basis).***

Ingredients	DM	CP	EE	CF	Ash	NFE%*	GE Kcal/kg**
Fish meal	92.1	72.0	8.7	-	12.6	6.70	512.798
Soybean meal	92.4	44.0	1.2	5.8	6.5	42.5	431.978
Yellow corn	89.2	8.8	3.8	2.6	1.8	83.0	425.452
Wheat bran	88.2	11.9	3.0	11.0	5.0	69.1	378.270
Bio-nutra200	91.5	21	3	6	3.3	66.7	419.390

**NFE= 100-(CP+EE+CF+Ash).*

*** Gross energy was calculated by multiplication the factor 4.1, 5.6 and 9.44 kcal GE/Kg DM carbohydrate, protein and fat, respectively (Jobling,1983).*

Table (2): ***Ingredients and composition of the experimental diets.***

Ingredients	Diet 1 Control	Diet 2 (0.1% Bio-Nutra200)	Diet3 (0.2% Bio-Nutra200)	Diet4 (0.3% Bio-Nutra200)
Fish meal (72% CP)	15	10	5	-
Yellow corn	40.5	36.9	31.8	28.7
Soybean meal (44% CP)	30	38.5	48.5	56.5
Wheat bran	10	10	10	10
Sunflower Oil	4	4	4	4
Vit.&Min. [1]	0.5	0.5	0.5	0.5
(*BIO-NUTRA 200*)	-	0.1	0.2	0.3
Total	100	100	100	100
Chemical analysis (% on DM basis):				
Dry matter	89	88.50	88.90	88.70
Crude protein	28.54	28.26	28.60	28.24
Ether extract	8.5	7.8	7.5	7.9
CF	4	5.6	5.8	5.4
Ash	6.4	7.1	7.8	7.5
NFE	52.56	51.24	50.30	50.96
Calculated Values:				
Gross energy (GE)(Kcal/100g)2	453. 66	440. 14	435.47	439.85
Digestible energy(DE)(Kcal/100g)3	324.32	313.98	311.10	314.22
P/E ratio4	62.91	64.20	65.67	64.20
Protein energy5 (%).	28.54	28.26	28.60	28.24

1- Vitamin and mineral mixture (product of HEPOMIX) each 2.5 kg contain: 12.000.000 IU Vit.A; 2.000.000 IU Vit. D3; 10 g Vit. E; 2g Vit. K3; 1g Vit. B1; 5g Vit. B2; 1.5 g Vit. B 6 ; 10g Vit.B12; 30 g Nicotinic acid ; 10 g Pantothenic acid ; 1g Folic acid; 50g Biotin; 250g Choline chloride 50% ; 30g Iron; 10g Copper; 50g Zinc; 60g Manganese; 1g Iodine; 0.1g Selenium and Cobalt 0.1g.

2- (Gross energy) (Kcal/100g), based on 5.6Kcal/g protein, 9.44 Kcal/g lipid, 4.1 Kcal/g carbohydrate, according to (Jobling, 1983).

3- (Digestible energy) (Kcal/100g), based on 5.0 Kcal/g protein 9.0Kcal/g lipid, 2.0 Kcal/g carbohydrate. According to (Wee and shu.1989).

4- (P/E) (protein to energy ratio) = mg crude protein / Kcal of gross energy.

5- (Protein energy) = (energy in protein/gross energy) x100

3-Growth parameters

Average total gain (ATG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %) and survival rate (SR %) were calculated according to the following equations:

a- ATG (g/fish) = [Average final weight (g) – Average initial weight (g)] as reported by (Annet.,1985).

b- ADG (g/fish/day) = [ATG (g)/experimental period (d)].

c- SGR (%/day) = [Ln final body weight–Ln initial body weight] x 100/experimental period (d) according to Pouomonge and Mbongland (1993).

d- FCR= Feed Intake (g)/Live weight gain as reported by De Selva and Anderson (1995).

e-PER = Live weight gain (g)/ protein intake (g) as reported by De Selva and Anderson (1995).

f- PPV (%) =100[final fish body protein (g) – initial fish body protein (g)]/crude protein intake (g).

g- SR = 100[total No. of fish at the end of the experimental period /total No. of fish at the start of the experiment.

4 – Proximate analysis

Dry matter, crude protein, ether extract, (crude fiber) and ash contents of the tested ingredients and whole body of fish at the beginning and at the end of the experiment were performed according to A.O.A.C. (1990).

5-Organs indices

All fish were killed and soon abdominal cavity was opened to remove liver, kidneys, gonads, and spleen which were weighed individually. Hepato (HSI), kidney (KSI), gonado (GSI), and spleeno somatic indices (SSI) were calculated as follow:

HSI =Liver weight x 100/Gutted fish weight (Jangaard *et al*; 1967).

KSI = Kidneys weight x 100 / fish weight (Alabaster and Lioyd, 1982).

GSI = Gonads weight x 100 / fish weight (Tseng and Chan, 1982).

SSI = Spleen weight x 100 / fish weight (Abdelhamid *et al.,* 2004).

6- Clinic pathological examination

A- Hematogram

a- Hemoglobin concentration (Hb)

Blood sample from the different groups were collected from the caudal peduncle. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by

using commercial kit (Diamond Diagnostic, Egypt).

b- Packed cell volume (PCV %)

PCV was estimated by the microhaematocrite method described by Decie and Lewis (1991).

c- Erythrocyte and leukocyte count

A manual method for counting using a hemocytometer counting chamber and Natt-Herrik solution was carried out according to Stoskopf (1993).

B- Biochemical parameters

a- Alanine aminotransferase activity (ALT) and Aspartate aminotransferase activity (AST): Colorimetric determination of ALT and AST activity was performed according to Reitman and Frankel (1957) using commercial kit.

b- Total protein: Assay of total proteins was carried out by a test kit according to method described by Weichselbaum (1946).

c- Serum albumin from all experimental groups was estimated method according to Dumas and Biggs (1972) using commercial kit.

d- Globulin: was calculated by mathematical subtraction of albumin value from total protein.

e- Albumin/Globulin (A/G) ratio: Albumin: Globulin ratio was calculated from data of albumin and globulin concentration.

7- Statistical analysis

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F- test was significant, least significant difference was calculated according (Duncan *(*1955).

RESULTS AND DISCUSSION

Chemical composition of the experimental diets

Chemical composition and calculated energy of deferent diets are presented in Table (2). The chemical analysis revealed that no differences were observed among all diets in DM, CP, and ash, while there were some differences observed among different diets for EE and CF, these differences may be due to the ingredients themselves. The CP content was between 28.24 to 28.60% on DM basis. Such level was within the range suggested by Jauncey and Ross (1982) and NRC (1993). The calculated energy was similar in the tested diets, where the GE values ranged from 435.47 to 453.66 Kcal/100g; it was higher than that suggested by NRC (1993) in the practical diets for tilapia. However, it was nearly similar to that used by Hassanen *et al.* (1995) and Abd-El-Maksoud *et al*. (1998).

Quality parameters of rearing water

All tested water quality criteria were suitable for rearing Nile tilapia fingerlings as cited by Abdel-hakim *et al*. (2002) and Abdelhamid (2009). Since water temperature ranged between (27 and 28Ċ), pH values (7.5 and 9), and dissolved oxygen ranged between (7 and 8.5 mg/l). Also, Abdelhamid *et al*. (2002) suggested that these values are suitable for rearing Nile tilapia. In the same trend, Abdelhamid *et al*. (2004) found that all the tested water quality (temperature, pH value, conductivity mg/l and dissolved oxygen mg/l) criteria were suitable for rearing Nile tilapia fish.

Growth performance and survival rate

At the end of the experimental period, both groups T2 and T3 received probiotic supplemented diets revealed significant increase in the total weight gain (TWG), average daily gain (ADG), and specific growth rate (SGR). There were significant ($P \leq 0.05$) differences among various groups of fish concerning final body weight, daily gain, specific growth rate, and survival rate of the experimented fish, being the best values in favor of T2 and T3, which seem even more better than the control and T4. These results are demonstrated in Table (3).

The best growth parameters observed with probiotic – supplemented diets suggested that, the addition of probiotic improved feed utilization in practical terms, this means that probiotic used can decrease the amount of feed necessary for animal growth which could result in production cost reduction. Similar results have been reported by Lara-Flores *et al*. (2003).

The pervious results of growth parameters indicated a positive acceptable effect of the used probiotic (*Bio-Nutra200*). The obtained results could be attributed to the ability of *Bacillus subtilus* to adhere to the intestinal mucosa of *O.niloticus* producing a wide range of relevant digestive enzymes (amylase, lipase, and protease) which have the ability to denaturate the indigestible components in the diets, the ability to detoxify the potentially harmful components of feed and ability to produce a lot of essential vitamin B complex members, particularly biotin and vitamin B12, the matter of which resulted in high food utilization (and an increase in digestibility of different dietary components). These results supported those of Piraret *et al*. (2006) and Marzouk *et al*. (2008), they used Bacillus *subtilus* in the food and found that these probiotic bacteria increase the food absorption by enhancing the protease level and consequently gave a better growth. Also, EI-Haroun *et al*. (2006) in this study with Biogen as

Table (3): ***Means**± standard errors of the growth performance of the experimented tilapia fish as affected by the dietary treatments for 150 days.***

Treatment	Initial weight	Final weight	T.W.G (g/fish)	A.D.G (g/fish/day)	S.G.R. (%/day)	S.R.%
T1	8.20±0.100a	124.56±0.90b	116.36±0.81b	0.78±0.01b	1.82±0.01b	95.50±050b
T2	8.48±0.15a	143.17±1.17a	139.69±1.02a	0.93±0.01a	1.88±0.01a	99.00±0.00a
T3	8.36±0.00a	138.40±2.60a	130.04±2.60a	0.87±0.02a	1.87±0.01a	96.00±0.00b
T4	8.26±0.00a	109.20±4.10b	100.94±4.10b	0.67±0.03b	1.72±0.01b	94.00±1.00b

**Means (in the same column) superscripted with different letters significantly (P≤0.05) differ. T1= Diet1 (Control group), T2= (Diet2 + 0.1% Bio-Nutra200), T3= (Diet3 + 0.2%Bio-Nutra200), and T4= (Diet4 + 0.3%Bio-Nutra200).*

food additive containing *Bacillus subtilus* came to the conclusion that, this organism germinates in the intestine of fish, using a large numbers of sugar (carbohydrates) and produces a wide range of digestive enzymes (amylase, lipase, and protease) which have a beneficial effects including higher growth rate.

Also, Scholz *et al.* (1999) reported that the probiotic improved the growth and survival of sea bass fry. They attributed this action to adherence of *S. cerevisiae* cells to the gut and the secretion of amylase enzymes which shared in the increased digestibility of the diet. On the other hand, the increased growth performance of *O. niloticus* treated with commercial products (*Bio-Nutra200*) yeast containing living *Aspergillus oryzae* and *Bacillus subtillus* could be attributed to the inhibition of some intestinal bacterial flora and increasing the non-specific immunity of the treated *O.niloticus*.

Feed and Protein Utilization

All criteria studied and presented in Table (4) showed again that T2 and T3 were the best (P≤0.05) treatments (even than the control, and T4) concerning FI, FCR, PER, and PPV in Nile tilapia then followed by T1. There were significant (P≤0.05) differences among treatments for FCR, PPV, and PER. While, there were no significant differences between T2 and T3 in data of FCR, PER, and PPV. Again, T4 was the worst one, compared to other treatments.

The (FCR) of *O.niloticus* kept on a basal diet (control) was higher than T2 and T3 groups receiving the diets supplemented with probiotic which in turn represented a positive aspect of probiotic supplemented diets. The best FCR, PER, and PPV values were observed with probiotic-supplemented diets suggested that, the

Table (4): ***Feed intake and conversion as well as protein utilization in the experimented Nile tilapia (x*± SE) as affected by the dietary treatments during the 150 days experimental period.***

Treatment	FCR	PER	PPV%
T1	2.35±0.03ab	1.49±0.14ab	34.71±3.67b
T2	1.83±0.04c	1.93±0.03a	48.27±1.25a
T3	2.04±0.09bc	1.71±0.07ab	45.08±1.77a
T4	2.82±0.16a	1.25±0.05b	35.40±1.96b

a, b and c means in the same column bearing different letters differ significantly at 0.05 level. T1= Diet1 (Control group), T2= (Diet2 + 0.1% Bio-Nutra200), T3= (Diet3 + 0.2%Bio-Nutra200), and T4=(Diet4 + 0.3%Bio-Nutra200).

addition of probiotics improved feed utilization, in practical terms this means that probiotic used can decrease the amount of feed necessary for animal growth which could result in production cost reduction. Similar results have been reported by Lara-Floras *et al*. (2003). PER, and PPV results indicated that supplementing diets with probiotic significantly improved protein utilization in tilapia. This may be contributed to optimizing protein (which is the most expensive feed nutrient) use for growth. The improvement in the biological value of the supplemented diets in these treatments with high population and low dietary protein demonstrated that the probiotics supplements performed more efficiently in stress situation. This agreed with the results obtained by Scholz *et al.* (1999) and Marzouk *et al*. (2008).

Internal organs indices

Data of the internal organs indices of the tested Nile tilapia are given in Table (5). Hepato somatic index (HSI), Kidney somatic index (KSI), gonado somatic index (GSI), and spleen somatic index (SSI) were highly in T1 (basal diet), but not significantly (P>0.05) affected by the

Table (5): ***Means*± standard errors of the internal organs indices of tilapia fish at the end of the 5 months period as affected by the experimental diets.***

Treatment	HSI%	KSI%	GSI%	SSI%
T1	3.70±0.12	0.06±0.001	0.73±0.24	0.46±0.06
T2	2.97±0.14	0.04±0.007	0.63±0.28	0.29±0.03
T3	2.53±0.70	0.03±0.006	0.64±0.29	0.31±0.05
T4	2.39±0.01	0.05±0.001	0.65±0.24	0.32±0.07

T1= Diet1 (Control group), T2= (Diet2 + 0.1% Bio-Nutra200), T3= (Diet3 + 0.2%Bio-Nutra200), and T4= (Diet4 + 0.3%Bio-Nutra200).

Table (6): ***Means* ± standard errors of proximate analysis (% on the dry matter basis) of experimental fish fed on graded levels of (Bio-Nutra200).***

Treatment	DM	CP	EE	Ash	GE Kcal/100g
T1	27.80±0.60	61.25±0.01a	19.65±0.01b	17.65±0.45ab	534.77±2.19
T2	27.20±0.90	61.31±0.05a	19.45±0.05a	18.25±0.05a	533.82±0.50
T3	26.10±0.30	60.78±0.03b	19.37±0.02ab	17.23±0.02ab	536.40±0.06
T4	25.62±0.51	60.25±0.05c	18.16±0.05c	16.66±0.46b	531.50±2.03

a,b and c means in the same column bearing the same letter do not differ significantly at 0.05 level.
T1= Diet1 (Control group), T2= (Diet2 + 0.1% Bio-Nutra200), T3= (Diet3 + 0.2%Bio-Nutra200), and T4= (Diet4 + 0.3%Bio-Nutra200).

dietary treatments (T1, T2, T3, and T4). These results agree with the findings of Lara-Floras *et al.* (2003) found that when up to 33% of dietary fish meal was replaced with probiotic in diets fed to tilapia better growth and feed utilization results were obtained compared to control diet.

Body composition

Values of dry matter (DM), crude protein (CP), ether extract (EE) and ash of the fish body are summarized in Table (6). The results of carcass composition of Nile tilapia showed that the difference were not significant (P>0.05) in dry matter and GE content. But crude protein, ether extract and ash percentages differed significantly among fish groups. These results agree with the findings of Ringo and Gatesoupe (1998) and Scholz *et al.* (1999), but EL-Haroun *et al.* (2006) found that no differences were observed for moisture, ash and protein content among (T1 and T2 and T3).

Clinicopathological findings

Biochemical Parameters

The results of protein profile and liver enzymes showed significant increases in total protein and globulin (G) and significant decrease in albumin (A), A/G ratio and liver enzymes (ALT) significant between T1 and other treatments, but (AST) no differed significantly among fish groups. These results are illustrated in Table (7). The

Table (7): Protein profile and activities of serum enzymes (ALT & AST) in O. niloticus groups post treatment with probiotic (*Bio-Nutra200*).

Treatment	Total protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	A/G ratio	ALT (µ/l)	AST (µ/l)
T1	2.95±0.01b	0.60±0.20a	2.35±0.15b	0.25±0.10	25.90±0.30a	19.80±0.20
T2	4.00±0.20a	0.54±0.26a	3.46±0.06a	0.17±0.10	16.55±0.55b	19.15±1.75
T3	3.52±0.11a	0.46±0.01b	3.06±0.16a	0.15±0.02	16.40±0.25b	19.10±0.60
T4	3.00±0.01b	0.45±0.10b	2.55±0.05ab	0.17±0.05	16.80±1.30b	19.18±0.03

a,b means in the same column bearing different letters differ significantly at 0.05 level.

Table (8): ***Hematogram of O.niloticus groups post-treatment with probiotic.***

Treatment	RBCs (x106/mm³)	Hb (g/100ml)	PCV (%)	WSCs (x10³/mm³)
T1	1.45±0.05	8.85±0.95	25.78±1.52	45.57±0.25
T2	1.79±0.03	9.93±1.10	32.74±2.51	45.59±0.26
T3	1.76±0.05	9.90±1.80	30.75±2.79	45.83±0.22
T4	1.30±0.01	8.15±0.95	26.28±3.03	37.77±0.15

results could be attributed to the immuno-modulatory effect of *Bio-Nutra200* on the liver cell which activate the anabolic capacity of the hepatocytes to produce blood protein particularly globulin Jessus *et al*. (2002), and this was also supported by the results of hepatic enzymes activity which decreased in *O.niloticus* kept on probiotic in comparison to the control group. These results were supported by the findings of Nayak *et al*. (2004) and Yan-Bo. (2008).

Hematogram

The results of hematogram revealed no significant increase in RBCs count, Hb value, PCV%, and WBCs in the tow groups (T2 and T3) treated with probiotic (*Bio-Nutra200*). These results are presented in Table (8). These could be attributed to the fact that, the probiotic used increases the blood parameter values as a result of hemopiotic stimulation. In this connection Sarma et al. (2003) and Rajesh *et al.* (2006), found that when up to 33% of dietary fish meal was replaced with probiotic in diets fed to tilapia better RBCs, PCV%, and Hb results were obtained compared to control diet.

Economic efficiency

The economic parameters of the tested diets are presenters in Table (9). The calculation depends on the average price (LE) of dietary ingredients of the local market at year (2008) vit.&min. 12100/kg, fish meal 10000/ton, yellow corn 1250/ton, soybean meal 2000/ton, wheat bran 900/ton, oil 4000/ton and probiotic (*Bio-Nutra200*) 60/kg. The calculated figures showed lower cost of one ton of all diets containing probiotic However; the control diet recorded the highest price, being 2916.2 LE/ton. The diets containing (0.1%, 0.2%, and 0.3% *Bio-Nutra200*) showed low cost of fish diet comparing with the control diet. Yet, diets No 1, and 4 showed high cost/kg gain but diet No.2 and 3 gave the lowest feed cost/kg gain, being 4.70 and 4.57 LE. These results support the results of Eid and Khalid (2008).

Table (9): ***Data of the economical efficiency due to feeding fish on graded levels of Probiotic.***

Treatment	Feed intake g/fish	Cost(LE) of one ton diet	Decrease in feed cost(LE)	Total gain g/fish	Feed cost/kg gain (LE*)
T1	274.00	2916.2	00.0	116.36	6.86
T2	255.75	2571.2	345	139.69	4.70
T3	265.84	2237.5	678.7	130.04	4.57
T4	284.80	1888.7	1027.5	100.94	5.32

**Feed cost/kg gain (LE) =feed intake x cost (LE) of one ton feed/1000xtotal gain.*

CONCLUSION

From the pervious results, it could be concluded the positive influence of adding *Bio-Nutra200* to the diets of Nile tilapia on growth performance, showed positive active effects. From feed utilization data and the economical point of view, the diets supplemented with 0.1% and 0.2% *Bio-Nutra200* were the best treatment.

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تقييم البايوناترا ٢٠٠ كبروبيوتك تجارى في علائق اسماك البلطي النيلى.

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تم إجراء هذا البحث لمدة ١٥٠ يوما في هابات للتفريخ و التي تم إنشائها في حوض سمكي ترابي بغرض دراسة وتقييم أحد أنواع البر وبيوتك, ودراسة مدى تأثيرها على إنتاجية الأسماك المستزرعة و على بعض الصفات الكيماوية لجسم الأسماك وأيضا مدى تأثيرها على مكونات دم الأسماك, و دراسة مدى التقليل في نسبة مسحوق الأسماك في علائق الأسماك نظرا لأنة يعتبر المكون الأغلى ثمنا في علائق الأسماك، وبغرض الحصول على علائق منخفضة التكاليف وذات جودة عالية ومن هنا نتمكن من خفض الأسعار المرتفعة للعلائق بإضافة هذا المكون في علائق الأسماك. حيث تم توزيع ٣٢٠ من اسماك البلطي النيلى وبالتساوي على ٨ هابات تفريخ وبعمل ٤ معاملات وكل معاملة في مكررتين وقد تغذت الأسماك على عليقة متوازنة بها ٢٨% بروتين, وتم تغذية الأسماك بمعدل ٣% من الوزن ألحى للأسماك وكانت التغذية مرتين يوميا (٨ صباحا و ٢ ظهرا) وتعدل هذه النسبة بناء على التغيير في وزن الأسماك المستزرعة. وكانت نسبة الإضافة لمسحوق السمك و البروبايوتك في العلائق المصنعة كانت كالاتى:

١- ١٥ ٪ مسحوق سمك بدون إضافة البروبايوتك (مجموعة الكنترول)
٢- ١٠ ٪ مسحوق سمك + ١ ,٠ ٪ من البروبايوتك (المجموعة الثانية)
٣- ٥٪ مسحوق سمك + ٢ ,٠ ٪ من البروبايوتك (المجموعة الثالثة).
٤ - ٣ ,٠ ٪ من البروبايوتك مع عدم إضافة لمسحوق السمك بالعليقة (المجموعة الرابعة).

وبعد خمسة شهور(وهى مدة التجربة) تم التوصل إلى مجموعة من النتائج الهامة وهى أن اسماك المجموعة التي تغذت على علائق تحتوى على مادة البايونترا ٢٠٠ بتركيزات (١ ,٠ ٪ , ٢ ,٠ ٪) قد أعطت أحسن معدل للنمو ومعدل النمو اليومي، والنمو النسبى للاسماك، و بالإضافة لأحسن معدلات للتحويل الغذائي ومعدلات الاستفادة من البروتين بالمقارنة بأسماك الكنترول, والأسماك التي تغذت على علائق تحتوى على نسبة ٣ ,٠ ٪ من البروبايوتك مع عدم إضافة لمسحوق السمك بالعليقة)، وأيضا حدثت اختلافات معنوية في التركيب الكيماوي لجسم الأسماك باختلاف المعاملات, كما اثرت إضافة هذه المادة في علائق الأسماك تأثيرات ايجابية في قياسات الدم المختلفة للأسماك. ومن خلال التحليل الاقتصادي وجد أنه حدث انخفاض في تكلفة انتاج طن العليقة المصنعة، وبالتالي حدث انخفاض في تكلفة إنتاج الكيلو الحى للأسماك. لذلك يوصى باستخدام هذه المادة من أجل تخفيض تكلفة إنتاج طن العليقة، وبالتالي حدوث تحسين اقتصادي لإنتاجية الأسماك المستزرعة.