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# 'TUDY ON THE EFFECTS OF SOME PROBIOTICS ON 'HE PERFORMANCE,CLINICOPATHOLOGICAL AND HISTOPATHOLOGICAL CHNGES OF OREOCHROMIS NILOTICUS

ABD ELAZIZ , M.*, ALI, M.** SAFAA YASSIN ***
RAAFAT, A. *** AND SAFINAZ RASHAD****
Dept. of Fish Diseases and Management Faculty of Vet. Med.Cairo Uni.
* Dept. of Pathology, Faculty of Vet. Med. Cairo Uni.
**Dept. of Clinical Pathology, Faculty of Vet. Med. Cairo Uni.
*** Research Student Clinical Pathology Dept. Faculty of Vet. Med. Cairo Uni.

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## INTROUCTION

In the recent years culturing of fish was encouraged and developed allover the world in order to cover the shortage in animal protein, specially when put in our consideration that the obtaining of fish from natural resources becomes more expensive and harder as a result of increase the cost of fishing as well as depletion of many stocks in natural water due to pollution (Abd EL-Aziz, 2002). Tilapia is one of the popular fish in Egypt. The success in the culture of tilapia is attributed to its ability to tolerate wide organic, animal and agriculture wastes (Mohamed, 2002). For nearly 50 years animal producers have incorporated growth promoters in feeds to enhance growth and feed efficiency rate in pigs, chickens, turkeys, beef, cattle, fish and other meet-producing animals (Bayoumi, 2004).The growth performance can be increased by many methods including the use of chemicals as steroids (steroids sex hormones, synthetic steroids and non steroids ) proteins or polypeptides which includes growth hormones, growth hormones releasing factor and thyrotropin releasing hormones and the antimicrobial feed additives as probiotics and antibiotics (Brander et al., 1991).

Protein is the most expensive feed nutrient in all live stock feeds, particularly fish. The use of probiotics which have been used as growth promoters to reduce the cost of the fish diet and to replace the widely used antibiotics and synthetic chemical feed supplements may have a potential

in aquaculture (Abo State, 2005).

Probiotics are live microbial feed supplements, which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989 & Rosales et al 2006 ). They are used in aquaculture as a mean of disease control, supplementing or even, in some instances replacing the use of antimicrobial compounds (Khalil et al.,2001). A wide range of microalgae, yeasts, Gram-positive *(Bacillus, Carnobacterium, Enterococcus, Lactbacillus and Streptococcus) and Gram-negative bacteria (Photorhodobacterium, Pseudomonas and Vibrio* ) have been evaluated to be used. However, the mode of action of the probiotics is not deeply investigated. The possibilities of their actions include competitive exclusion and/or by stimulation of the non specific host immunity (Abo State 2005) . Probiotics may stimulate also appetite and improve nutrition (Irianto and Austin, 2002b & Salminsen et al., 2005).

**The main objects of this study is to evaluate the use of some commercial products in Oreochromis niloticus through:**

1.Determination of the effect of such products on the growth rate of *O.niloticus.*

2.Determination of the effect of these products on some hematological parameters.

3.Evaluation of the effect of these products on the biochemical constituents of fish blood.

4-histopathological examination of samples from liver, kidney, gills and spleen were taken from both probiotics fed and a control *O. niloticus.*

## MATERIAL AND METHODS

**1-Fish** A total of 50 apparently healthy Oreochromis niloticus with an average body weight of 75 gm were obtained from a private fish farm at El Hamool, Kafer El Sheikh governorate were used in this study.

**2- Requirements of aquaculture units:**

**a- Aquarium:** Fish were kept in glass aquaria measuring 100 x 50 x 30 cm. Aquaria were supplied with an air pump for aeration.

**b- Water:** Tap water was stored for 2 days in plastic tanks for dechlorination and used after that for filling the aquaria and replacing the changed water. Water pH was measured by using an electric digital pH-meter and water temperature were recorded daily by using glass thermometer.

**3- probiotics** Two different commercial probiotics which namely biogen and moreyeast were used and mixed with the fish diets:

**a- Biogen:** was supplied from China Way-Taiwan Company which composed of allicin (which is one of the garlic byproduct not less than 0.247 micromole/gm), *Bacillus subtilis* Natto (not less than $6x10^7$/gm) and high unit hydrolytic enzyme (not less than 3690 unit/gm).

**b- Moreyeast:** supplied from Norchem Industries, USA. Moreyeast is a highly effective

yeast culture which composed of live yeast cells (*Saccharomyces cerevisiae*) mixed with yellow corn, gluten, barly, molasses and other palatable products.

**Fish diet:** Fish were fed a basal diet containing proteins (25%), fat (2.2%), fiber (4.33%) as well as minerals and vitamins in the form of dry pellets.

**Experimental design:** Fifty *Oreochromis niloticus* fish were included in this study. Fish were transferred to the glass aquaria for 15 days before start of the experiment for acclimatization. At this period the fish were fed daily on the control diet (without probiotics) at a rate of 3% of their average body weight. Water was changed every week to maintain good water quality and the glass aquaria were cleaned every morning prior to feeding by syphoning the wastes which had accumulated on the bottom. The pH value was measured weekly by electric digital pH-meter. In addition, water temperature was daily measured and adjusted at an average of 22-28°C throughout the experimental period. Fish then were divided into three groups :

oup (1): consisted of 20 fish which fed diet supplemented by biogen at a dose of 2 kg/ton diet.

oup (2): consisted of 20 fish which fed diet supplemented by moreyeast at a dose of 5 kg/ton diet.

Group (3): control group which consisted of 10 fish fed diet without probiotics.

Diets were fed to each group of fish during the experimental period (8 weeks) in the form of dried pellets. Feeding rate of all experimental diets was 3% of the total biomass of fishes per day. The amount of feed was divided into two equal portions and distributed in the aquaria two times daily at 9 a.m. and 5 p.m. Every two weeks the fishes in each aquarium were weighted and the amount of feed was corrected according to the new fish biomass.

Blood samples from each group were collected every two weeks for hematological and biochemical examinations. Blood samples were collected from the caudal vertebral vessels according to Feldman et al. (2000) by a needle and syringe moisted with 3.8% sodium citrate solution. Each sample was divided into two portions, the first was used for hematological studies and the other portion was centrifuged at 3000 r.p.m. for 10 minutes for plasma separation for biochemical studies. Tissue samples (liver, kidney, gills and spleen) were collected for histopathological examinations.

**1- Growth performance parameters**

The performance parameters including body

weight gain (W.G.), specific growth rate (SGR%), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as follows:

**Body weight gain (W.G.):**Total weight was determined to the nearest gram according to (Annet, 1985). Specific growth rate (SGR%):Specific growth rate (SGR%) was calculated as the percentage increase in weight per fish per day as suggested by Pouomonge and Mbonglang (1993). Feed conversion ratio (FCR):Was determined according to De silva and Anderson (1995). Protein efficiency ratio (PER):Was determined according to De silva and Anderson (1995).

**2- Hematological studies:**

Hemoglobin concentration (gm/dl):Hemoglobin concentration was determined using the cyanomethemoglobin according to Stoskopf (1993). Packed cell volume (PCV%):The microhematocrit method described by Dacie and Lewis (1991) was used for estimation of the PCV%. Erythrocyte and leukocyte count:A manual method for counting erythrocytes and total leukocytes as described by Stoskopf (1993) using a hemocytometer counting chamber and Natt-Herrik solution, was carried out. Differential leukocytic count:Was carried out according to (Thrall, 2004).

**3- Biochemical parameters:**

Alanine aminotransferase activity (ALT): Colorimetric determination of ALT activity was performed according to Reitman and Frankel (1957). Aspartate aminotransferase activity (AST): Activity of AST was assayed colorimetrically according to Reitman and Frankel (1957). Glucose:Glucose was estimated colorimetrically at wave length 500 nm according to the technique described by Trinder (1959). Blood urea nitrogen (BUN):Colorimetric determination of blood urea nitrogen (BUN) was carried out according to the berthelot reaction described by Fawcett and Scott (1960). Creatinine:Kinetic determination of creatinine was performed at wave length 495 nm according to the method of Larsen (1972). Cholesterol:Colorimetric determination of cholesterol was performed according to Trinder (1969). Triglycerides:Measurement of triglycerides levels was carried out according to Wahlefeld (1974). Total proteins:Assay of total proteins was carried by a test kit according to biuret method described by Weichselbaum (1946). Albumin:was carried out according to Dumas and Biggs (1972). Globulin:Globulin was calculated by mathematical substraction of albumin value from total proteins. Albumin:Globulin (A:G) Ratio:Albumin:Globulin ratio was calculated from data of albumin and globulin.

**4- Histopathological examination:**

Samples for histopathological studies were obtained from liver, kidney, gills and spleen. The specimens were fixed in 10% neutral formalin, embded in paraffin, sectioned a micron thickness

d stained with hematoxyline and eosin accord- g to the method described by Drury et al. 976).

**Statistical analysis:**

ata of hemogram and plasma biochemistry were atistically analyzed for the mean and standard eviation of the mean. Significance of the results as determined by conducting a one way analysis f variance (F-test) and the least significant differ- nce between pair groups according to the method f Snedecor and Cochran (1973) .

## RESULTS

**1- Results of growth performance parameters:**

The mean values of growth performance parameters of control *Oreochromis niloticus* fishes and those fed on the two probiotics at the end of the experimental period are illustrated in table (1). At the end of the experimental period both biogen and moreyeast administrated groups revealed a significant increase in the body weight gain (W.G.), specific growth rate (SGR) and protein efficiency ratio (PER) compared to control. Furthermore, the feed conversion ratio (FCR) was decreased significantly (improved) in both fish groups received probiotic comparing to control.

**2- Results of the haematological parameters:**

**Table (1): Growth performance parameters of control *Oreochromis niloticus* fishes and those fed on the two probiotics at the end of the experimental period.**

| Group | W.G. (gm) | SGR (%) | FCR | PER |
|---|---|---|---|---|
| Control | 20.80 ± 4.16 | 0.32 ± 0.19 | 6.51 ± 1.38 | 54.20 ± 4.80 |
| Group (1) | $^{a}$46.50 ± 1.50* | $^{a}$1.05 ± 0.05* | $^{a}$3.02 ± 0.12* | $^{a}$131.65 ± 4.25* |
| Group (2) | $^{ba}$44.67 ± 9.71* | $^{ba}$1.00 ± 0.09* | $^{ba}$2.90 ± 0.20* | $^{ba}$123.80 ± 26.98* |
| LSD | 12.31 | 0.24 | 1.62 | 31.98 |

Group(1) =*Oreochromis niloticus* received biogen.
Group(2) =*Oreochromis niloticus* received moreyeast.
Values represent means ± standard deviations.
* Significantly different from control P< 0.05.
Means with different alphabetical letters are significantly different P< 0.05.

**a- Hemogram:**

The mean values of erythrogram and leukogram of different groups of *Oreochromis niloticus* fishes are illustrated in tables (2&3). The results showed significant increase in the values of RBCs, PCV and Hb throughout the experimental period except group 1 (received biogen) at the 6th week of experiment comparing to control group. Fish group received moreyeast (Group 2) had the pronounced effect than group 1 except at the 8th week of feeding..

Assessment of leukogram revealed significant leukocytosis allover the experimental period in different groups except during the 2nd week in group 2 (received moreyeast) and at the 6th week in both probiotic recieved groups comparing to control group. Fish group received biogen had pronounced effect comparing to moreyeast administrated group. Regarding differential leukocytic picture, heterophilia was noticed only at the 2nd week in both probiotic supplemented groups and at the 4th week in group 2 (received moreyeast) which then showed heteropenia at the 8th week comparing to control group. Exceptionally, biogen increased the heterophils count comparing to moreyeast at the 8th week. On the other hand, lymphocytosis was observed in both probiotic received groups at the 4th and 8th week while group 2 (received moreyeast) showed lymphopenia at the 2nd week.

**Table (2): Erythrogram of control *Oreochromis niloticus* fishes and those fed on the two probiotics during different time intervals.**

| Weeks | Group | RBCs ($x10^6$/µl) | PCV (%) | Hb (g/dl) |
|---|---|---|---|---|
| 2 | Control | 1.23 ± 0.29 | 15.50 ± 1.00 | 4.20 ± 0.42 |
| | Group (1) | $^{a}$1.51 ± 0.23* | $^{a}$18.00 ± 1.83* | $^{a}$5.40 ± 2.09* |
| | Group (2) | $^{b}$1.69 ± 0.10* | $^{ba}$19.50 ± 1.00* | $^{ba}$5.96 ± 0.87* |
| 4 | Control | 1.23 ± 0.33 | 15.25 ± 4.19 | 4.20 ± 0.98 |
| | Group (1) | $^{a}$1.47 ± 0.05* | $^{a}$17.50 ± 4.20* | $^{a}$5.43 ± 0.97* |
| | Group (2) | $^{b}$1.66 ± 0.21* | $^{ba}$17.50 ± 2.89* | $^{ba}$5.18 ± 1.25* |
| 6 | Control | 1.61 ± 0.20 | 13.00 ± 1.83 | 3.98 ± 0.52 |
| | Group (1) | $^{a}$1.66 ± 0.24 | $^{a}$14.00 ± 0.00 | $^{a}$4.35 ± 0.79 |
| | Group (2) | $^{b}$1.82 ± 0.19* | $^{ba}$15.00 ± 0.82* | $^{b}$5.90 ± 0.32* |
| 8 | Control | 1.13 ± 0.17 | 10.50 ± 0.58 | 3.08 ± 0.17 |
| | Group (1) | $^{a}$1.57 ± 0.20* | $^{a}$14.75 ± 1.71* | $^{a}$4.68 ± 0.64* |
| | Group (2) | $^{b}$1.38 ± 0.21* | $^{b}$13.00 ± 1.83* | $^{ba}$4.40 ± 0.78* |
| LSD | | 0.16 | 1.6 | 0.68 |

Group(1) = *Oreochromis niloticus* received biogen.
Group(2) =*Oreochromis niloticus* received moreyeast.
Values represent means ± standard deviations.
* Significantly different from control P< 0.05.
Means with different alphabetical letters are significantly different P< 0.05.

Table (3): Leukogram of control *Oreochromis niloticus* and those fed on the two probiotics during different time intervals.

| Weeks | Group | TLC ($x10^3/\mu l$) | Heter. ($x10^3/\mu l$) | Lymph. ($x10^3/\mu l$) |
|---|---|---|---|---|
| 2 | Control | 58.33 ± 7.02 | 3.91 ± 1.14 | 55.35 ± 8.16 |
| | Group (1) | $^{a}$74.33 ± 5.51* | $^{a}$12.62 ± 2.46* | $^{a}$61.52 ± 11.84 |
| | Group (2) | $^{b}$55.33 ± 4.93 | $^{ba}$13.92 ± 5.02* | $^{b}$42.71 ± 7.65* |
| 4 | Control | 46.33 ± 4.73 | 3.10 ± 0.60 | 41.84 ± 4.35 |
| | Group (1) | $^{a}$72.67 ± 11.59* | $^{a}$4.46 ± 1.19 | $^{a}$58.14 ± 4.80* |
| | Group (2) | $^{b}$61.00 ± 14.18* | $^{ba}$5.37 ± 0.83* | $^{ba}$56.89 ± 12.86* |
| 6 | Control | 61.33 ± 12.06 | 5.68 ± 1.41 | 53.33 ± 9.61 |
| | Group (1) | $^{a}$67.67 ± 15.31 | $^{a}$5.73 ± 0.97 | $^{a}$58.46 ± 9.78 |
| | Group (2) | $^{b}$57.67 ± 5.13 | $^{ba}$6.47 ± 1.22 | $^{b}$47.69 ± 7.05 |
| 8 | Control | 52.00 ± 12.49 | 5.84 ± 0.69 | 46.49 ± 4.02 |
| | Group (1) | $^{a}$83.33 ± 1.53* | $^{a}$4.93 ± 0.68 | $^{a}$78.89 ± 1.84* |
| | Group (2) | $^{b}$65.00 ± 6.00* | $^{b}$2.67 ± 0.81* | $^{b}$62.58 ± 5.13* |
| LSD | | 7.93 | 1.56 | 6.69 |

Group(1) =*Oreochromis niloticus* received biogen.
Group(2) =*Oreochromis niloticus* received moreyeast.
Values represent means ± standard deviations.
* Significantly different from control P< 0.05.
Means with different alphabetical letters are significantly different P< 0.05.

**b- Biochemical parameters:**

**Protein profile:**

The mean values of protein profile in different groups are illustrated in table (4). The results showed, non significant changes allover the experimental period except at the 2nd week significant hypoproteinemia due to hypoalbuminemia was noticed in both fish groups received probiotic compared to control group, while at the 8th week group 2 (received moreyeast) showed hyperproteinemia due to hyperglobulinemia. On the other hand, A:G ratio was decreased significantly in both probiotics supplemented groups at the 8th week of experiment. Moreover, fish group received moreyeast showed significant elevation in the total protein and albumin concentration comparing to group 1 (received biogen) at the 8th week of the experiment.

Table (4): Protein profile of control *Oreochromis niloticus* and those fed on the two probiotics during different time intervals.

| Weeks | Group | T.P. (g/dl) | Alb. (g/dl) | Globu. (g/dl) | A:G ratio |
|---|---|---|---|---|---|
| 2 | Control | 3.87 ± 0.07 | 2.07 ± 0.12 | 1.79 ± 0.18 | 1.17 ± 0.18 |
| | Group (1) | $^{a}$3.07 ± 0.08* | $^{a}$1.68 ± 0.11* | $^{a}$1.39 ± 0.18 | $^{a}$1.23 ± 0.24 |
| | Group (2) | $^{ba}$2.91 ± 0.35* | $^{ba}$1.56 ± 0.20* | $^{ba}$1.35 ± 0.42 | $^{ba}$1.23 ± 0.41 |
| 4 | Control | 3.15 ± 1.19 | 1.14 ± 0.13 | 2.02 ± 1.19 | 0.68 ± 0.31 |
| | Group (1) | $^{a}$3.21 ± 0.78 | $^{a}$1.31 ± 0.10 | $^{a}$1.90 ± 0.71 | $^{a}$0.74 ± 0.21 |
| | Group (2) | $^{ba}$3.50 ± 0.61 | $^{ba}$1.16 ± 0.16 | $^{ba}$2.34 ± 0.47 | $^{ba}$0.50 ± 0.07 |
| 6 | Control | 3.97 ± 0.50 | 1.10 ± 0.66 | 2.87 ± 0.99 | 0.49 ± 0.48 |
| | Group (1) | $^{a}$3.94 ± 0.53 | $^{a}$0.96 ± 0.52 | $^{a}$2.98 ± 0.33 | $^{a}$0.33 ± 0.18 |
| | Group (2) | $^{ba}$3.94 ± 0.53 | $^{ba}$0.97 ± 0.24 | $^{ba}$2.98 ± 0.68 | $^{ba}$0.35 ± 0.15 |
| 8 | Control | 2.53 ± 0.44 | 1.65 ± 0.19 | 0.88 ± 0.44 | 2.33 ± 1.35 |
| | Group (1) | $^{a}$2.73 ± 0.32 | $^{a}$1.44 ± 0.08 | $^{a}$1.29 ± 0.25 | $^{a}$1.13 ± 0.17* |
| | Group (2) | $^{b}$3.59 ± 0.14* | $^{b}$1.87 ± 0.56 | $^{ba}$1.72 ± 0.42* | $^{ba}$1.18 ± 0.57* |
| LSD | | 0.46 | 0.27 | 0.51 | 0.41 |

Group(1) =*Oreochromis niloticus* received biogen.
Group(2) =*Oreochromis niloticus* received moreyeast.
Values represent means ± standard deviations.
* Significantly different from control P< 0.05.
Means with different alphabetical letters are significantly different P< 0.05.

**Liver enzymes:**

The mean values of plasma alanine aminotransferase (ALT) and aspartate aminotrasferase (AST) in different groups are illustrated in tables (5). The present results revealed that both groups recieved probiotics showed significant decrease in the ALT activity throughout the experimental period except during the 2nd week in both groups which recorded elevation of ALT activity compared to control group. Fish group received moreyeast showed significantly elevated ALT activity comparing to group 1 (received biogen) just at the 2nd week while it decreased it at the 8th week.

Regarding the AST activity, significant decrease were observed allover the experimental period (8 week) except during the 2nd and 4th week in group 2 (received moreyeast) which showed significant elevation comparing to control group. Comparing to group 1 (received biogen), moreyeast supplemented group showed significant increase in the AST activity at the 4th week while it

was significantly decreased at the 8th week.

**Lipids:**

The mean values of some plasma lipids of different groups are illustrated in tables (5). The present findings indicated that oreochroms niloticus fed on supplemented diets with both probiotics showed significant hypercholesterolemia throughout the experimental period except group 1 at the 2nd week while group 2 (received moreyeast) revealed hypocholesterolemia. Comparing to group 1 the cholesterol concentration were lower in group 2 at the 2nd and 4th week while it increased at the 6th and 8th week.

On the other hand, both biogen and moreyeast supplemented groups showed significant increase in the concentration of triglycerides during the eight weeks of experiment except at the 2nd week in group 2 which registered significant decrease in triglycerides concentration compared to control. Also both groups recorded no changes at the 6th week.

**Table (5): Activities of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and levels of some plasma lipids of control *Oreochromis niloticus* and those fed on the two probiotics during different time intervals.**

| Weeks | Group | ALT (U/L) | AST (U/L) | Cholest. (mg/dl) | T.G. (mg/dl) |
|---|---|---|---|---|---|
| 2 | Control | 12.67 ± 2.05 | 21.50 ± 3.78 | 109.41 ± 15.36 | 113.07 ± 20.19 |
| | Group (1) | $^{a}$18.67 ± 4.18* | $^{a}$24.50 ± 2.90 | $^{a}$109.88 ± 19.24 | $^{a}$95.47 ± 20.68 |
| | Group (2) | $^{b}$25.66 ± 2.70* | $^{ba}$27.94 ± 3.50* | $^{b}$92.88 ± 12.79* | $^{ba}$86.24 ± 11.72* |
| 4 | Control | 20.95 ± 1.75 | 10.67 ± 1.88 | 107.87 ± 16.45 | 79.43 ± 3.41 |
| | Group (1) | $^{a}$11.06 ± 2.40* | $^{a}$13.33 ± 1.89 | $^{a}$128.74 ± 17.84* | $^{a}$208.29 ± 26.87* |
| | Group (2) | $^{ba}$13.00 ± 0.00* | $^{b}$24.67 ± 0.47* | $^{b}$113.77 ± 17.63 | $^{b}$140.03 ± 13.22* |
| 6 | Control | 24.44 ± 3.50 | 25.63 ± 2.06 | 100.00 ± 2.91 | 115.58 ± 25.37 |
| | Group (1) | $^{a}$17.00 ± 3.55* | $^{a}$19.50 ± 4.12* | $^{a}$135.39 ± 15.51* | $^{a}$120.12 ± 5.57 |
| | Group (2) | $^{ba}$14.00 ± 1.75* | $^{ba}$17.67 ± 2.36* | $^{b}$158.56 ± 19.70* | $^{ba}$124.65 ± 14.91 |
| 8 | Control | 19.50 ± 6.13 | 27.93 ± 3.50 | 101.58 ± 5.33 | 114.10 ± 5.88 |
| | Group (1) | $^{a}$21.00 ± 4.32 | $^{a}$19.33 ± 2.36* | $^{a}$131.55 ± 10.77* | $^{a}$185.90 ± 11.75* |
| | Group (2) | $^{b}$11.00 ± 1.41* | $^{b}$10.40 ± 1.64* | $^{b}$177.76 ± 29.56* | $^{b}$223.07 ± 7.70* |
| LSD | | 4.33 | 4.43 | 11.90 | 25.12 |

Group(1) = *Oreochromis niloticus* received biogen.
Group(2) = *Oreochromis niloticus* received moreyeast.
Values represent means ± standard deviations.
* Significantly different from control P< 0.05.
Means with different alphabetical letters are significantly different P< 0.05.

**Renal function parameters:**

The mean values of plasma blood urea nitrogen (BUN) and creatinine of different groups are illustrated in table (6). The results showed significant decrease in the blood urea nitrogen concentration allover the experimental period in fishes received both probiotics compared to control except at the 8th week in group 1. Regarding creatinine concentration marked elevation was noticed at the 6th week in group 1 (received biogen) and at the 8th week in both groups comparing to control group.

**Glucose:**

The mean values of plasma glucose of different groups are illustrated in table (6). Changes in the plasma glucose concentration appeared only at the 4th and 6th week in group 2 (received moreyeast) and at the 8th week in group 1 (received biogen) in form of significant hypoglycemia as compared to control group and with each other.

**Table (6): Some kidney function parameters and plasma glucose of control *Oreochromis niloticus* fed on the two probiotics during different time intervals.**

| Weeks | Group | BUN (mg/dl) | Creatin. (mg/dl) | Glucose (mg/dl) |
|---|---|---|---|---|
| 2 | Control | 4.41 ± 0.60 | 0.25 ± 0.10 | 50.71 ± 11.76 |
| | Group (1) | $^{a}$2.60 ± 0.52* | $^{a}$0.20 ± 0.08 | $^{a}$48.81 ± 9.41 |
| | Group (2) | $^{b}$3.16 ± 1.11* | $^{b}$0.13 ± 0.02* | $^{ba}$48.66 ± 6.16 |
| 4 | Control | 4.42 ± 0.74 | 0.13 ± 0.01 | 32.26 ± 3.14 |
| | Group (1) | $^{a}$2.68 ± 0.18* | $^{a}$0.19 ± 0.04 | $^{a}$31.45 ± 2.66 |
| | Group (2) | $^{ba}$2.83 ± 0.84* | $^{ba}$0.16 ± 0.02 | $^{b}$26.55 ± 4.56* |
| 6 | Control | 4.95 ± 0.93 | 0.13 ± 0.03 | 44.86 ± 3.67 |
| | Group (1) | $^{a}$4.00 ± 0.47* | $^{a}$0.27 ± 0.09* | $^{a}$46.01 ± 7.93 |
| | Group (2) | $^{b}$2.28 ± 1.17* | $^{b}$0.07 ± 0.04 | $^{b}$31.89 ± 2.58* |
| 8 | Control | 4.67 ± 1.38 | 0.29 ± 0.08 | 40.01 ± 6.56 |
| | Group (1) | $^{a}$4.47 ± 1.34 | *0.55 ± 0.24* | $^{a}$22.12 ± 4.14* |
| | Group (2) | $^{b}$2.99 ± 0.08* | $^{ba}$0.56 ± 0.06* | $^{b}$41.20 ± 10.22 |
| LSD | | 0.36 | 0.065 | 4.82 |

Grou,)(1) = *Oreochromis niloticus* received biogen.
Group(2) = *Oreochromis niloticus* received moreyeast.
Values represent means ± standard deviations.
* Significantly different from control P< 0.05.
Means with different alphabetical letters are significantly different P< 0.05.

**. Histopathological results:**

he histopathological examination of different ish Groups revealed that both probiotics received roups showed slight congestion of the branchial lood vessels (Fig. 1). The splenic tissue showed narked activation of melano-macrophage centers nd aggregation of melanophores around blood essels were noticed (Fig.2). Vacuolar degeneration of renal tubules were also observed (Fig.3). Exceptionally, in the moreyeast received group, lamellar oedema was noticed in one case of this group (Fig. 4).The histopathological examination of the negative control group (administrated diet without probiotic) revealed apparently normal structure of such organs.

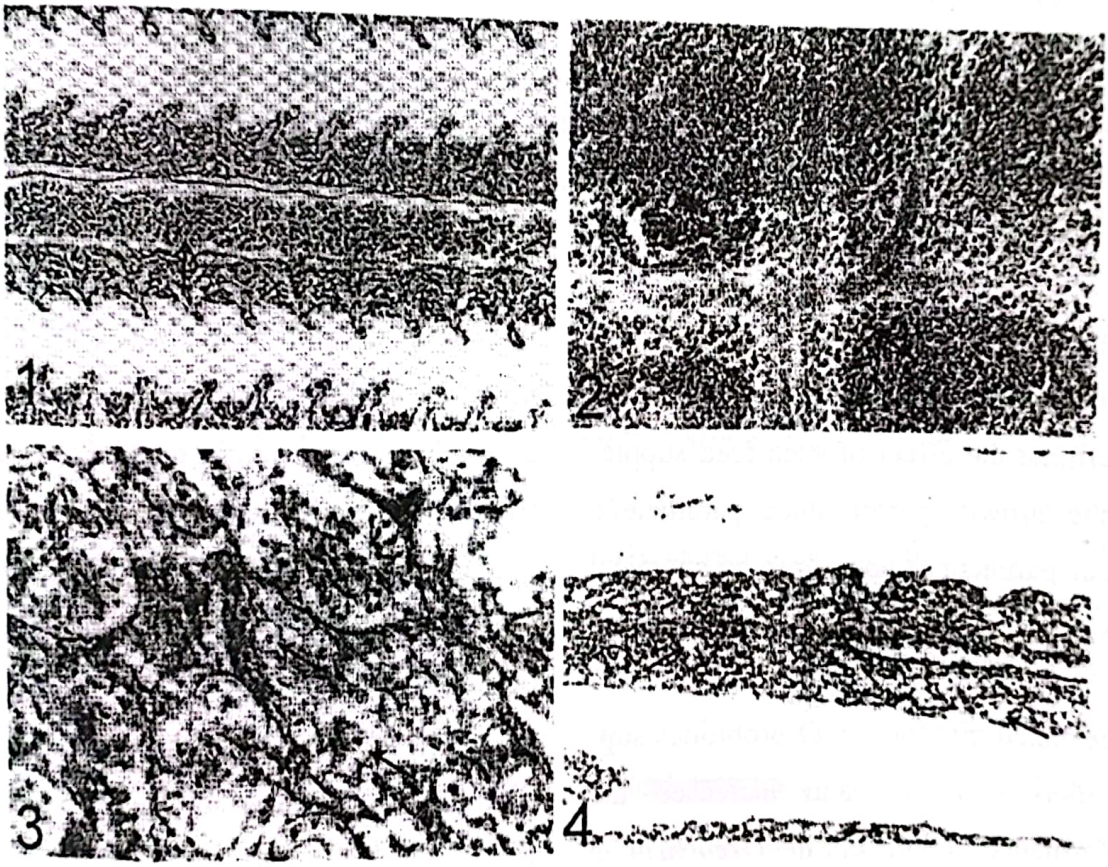


Fig. (1): Gills of Nile tilapia (Oreochromis niloticus) showing lamellar congestion was a common picture in the 2 probiotic recievd groups (arrow).(H&E stain x200)
Fig. (2): Spleen of Nile tilapia (Oreochromis niloticus) in the probiotics received groups showing melanophores around blood vessels (arrow).(H&E stain x 200)
Fig. (3): Kidney of Nile tilapia (Oreochromis niloticus) in the probiotic received groups showing vacuolar degeneration in renal tubules (arrow). (H&E stain x400)
Fig. (4): Gills of Nile tilapia (Oreochromis niloticus) received moreyeast showing lamellar oedema (arrow). (H&E stain x200)

## DISCUSSION

Anti-microbial feed additives were commonly used as growth promoters which involves probiotics and antibiotics (Brander et al., 1991). The worldwide increase in bacterial resistance to antibiotics (Van der waaij and Nord, 2000) has stimulated investigations to the use of probiotics which are single or mixed cultures of live microbes that benefically affect health by improving the properties of the friendly bacteria residing in the intestinal tract (Zilberter, 2001), denaturate the potentially indigestible components in the diet, produced vitamins and stimulate the immune system (Hoshino et al., 1997).

In the present study, attempts were made to evaluate the use of some probiotics as growth promoters and determine the effect of such feed supplements on the growth performance parameters, hematological parameters and some biochemical constituents of blood.

This study revealed that the use of probiotics supplemented diets enhanced and increased the growth performance parameters in *Oreochromis niloticus*. The findings of the present work agree with those of Ramadan et al. (1991) who found that probiotic had a significant growth promoting effect in tilapia fish either by arising natural metabolities as active substances for nutritional performance promotion or by its positive effect on intestinal flora and improving digestion.

The beneficial effect of biogen supplemented diets on different growth performance parameters such as weight gain (W.G.), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) may be attributed to its particular good flavor and appetizing function that considered as a palatability enhancer for increasing food consumption by fish and consequently increase the growth rate. On the other hand, various kind of biogen contents may explain the biogen growth stimulating effect such as biogen enzymes (hydrolytic, amylolytic, lipolytic, proteolytic and cell separating enzymes) which increase in the digestibility and absorbability of food by activation of the intestinal villi and promote the secretion of digestive fluids (Bayoumi, 2004). Moreover, allicin which is one of the garlic byproduct stimulate the secretion of anabolic hormones specially the growth hormone via activation of various endocrine glands which reflected on the enhancement of growth parameters (Lun et al., 1994). Also, biogen share in the improvement of water quality through degradation of waste organic matter in water leading to better growth rate and this was in close agreement with the finding of Badawi and Abd El-Aziz (2004) who recorded that probiotics improved water quality by decreasing the level of ammonia which reflected on the better body weight gain of Nile tilapia.

Furthermore, the competitive exclusion process of *Bacillus subtilis* bacteria which replace the pathogenic microorganism leads to intestinal microbial balance and improves the digestibility, absorbability and utilization of dietary proteins resulted in the decrease of FCR and increases the PER. All these factors improve the health state of fishes and provide nutrients for increasing growth rate (Khalid et al., 1995). On the other hand, it is interesting to state that moreyeast supplemented diets enhanced the growth performance parameters and this could be attributed to the growth promotion mechanism of yeast (*Saccharomyces cerevisiae*) which adher to the intestine and modify the gastrointestinal microbial balance by prevention of colonization of the pathogenic bacteria leading to maintaining a normal gut microflora, that reflected on the enhancement of the physiological function of fishes by increasing its body resistance. Moreover, yeast intestinal adherence stimulates the secretion of amylase which denaturate the indigestible compounds in the diet leading to improving the nutrition and providing the nutrients which cannot used by pathogens (Nunes, 1994).

Significant increase in the RBCs count, PCV percent and hemoglobin concentration have been encountered after administration of probiotics supplemented diets, these findings simulates that described by Ramadan et al. (1989) who found that probioticum "s" was significantly elevated the RBCs count, PCV percent and hemoglobin concentration than flavomycin when administrated in chickens. The increase in the concentration of hemoglobin, PCV percent and RBCs count could be attributed to the high content of essential amino acids, vitamins and minerals in the probiotic, this confirms that this substances considered as erythrocyte maturing factors as well as they share in the heme and globin synthesis (Lilly and Stillwell, 1965). Regarding to this point, Sarma et al. (2003) attributed this increase of the RBCs count, PCV percent and hemoglobin concentration to the hepato-stimulatory and hepato-protective effects of probiotics and according to this more RBCs and hemoglobin are formed under the control of erythropoietic factors released by hepatic cells.

Evaluation of leukogram of fishes administrated probiotics revealed significant increase of the total leukocytic count, lymphocytes and heterophils. Such results were in agreement with those previously recorded by Ramadan et al. (1989), Ramadan et al. (1991), Muscettola et al. (1994), Irianto and Austin (2002a) and Choudhury et al. (2005). This could be attributed to the immunomodulatory and immunostimulatory effects of probiotics through stimulation of bone marrow to produce more leukocytes. Moreover, minerals and vitamins which are found in probiotics may enhance immune system (Ballas et al., 1996). On the other hand, lymphocytes are the bulky leukocyte in the peripheral blood of most normal fish that play a major role in the humoral and cell mediated im-

munity of fish, therefore, lymphocytosis is suggestive of immunogenic stimulation (Thrall, 2004).

Fishes administrated probiotics revealed hyperproteinemia, hyperglobulinemia and lower A:G ratio in the moreyeast received group at the 8th week of experiment compared to control group. This could be attributed to the moreyeast particular good flavor which enhance the palatability and increase the food consumption leading to the significant elevation of the plasma total proteins. This agree with the result of Johnston et al. (1989) who found that plasma protein level in both mature and immature Atlantic salmon (*Salmo salar*) was increased with increasing food consumption. Furthermore, the significant increase of globulins concentration reflect the immunopotentiating effect of moreyeast (Khalil et al., 2001) and explain the lower A:G ratio.

The significant hyperproteinemia may be attributed to the different contents of both probiotics as biogen enzymes and *Bacillus subtilis* as well as the special yeast species (*Saccharamyces cerevisiae*), vitamins, minerals and amino acids of moreyeast which increase the nutritional value of the diet, palatability, stimulate the appetite and activate the intestinal villi leading to increase the digestability and absorbability of nutrients in the gastrointestinal tract of fish. This supports the finding of Goel et al. (1977) who found that increasing the plasma total proteins level indicates the improvement in the nutritional value of the diet. The same was noticed by Choudhury et al. (2005). This result reflect the positive effect of both biogen and moreyeast on the intestinal flora, thereby improving the digestion, availability of natural feed supply of nutrients and utilization of energy which enhance the fish immunity (Ramadan et al., 1991). Moreover, biogen have a direct effect on the enhancement of the B cells of lymphatic system to proliferate (Angelo et al., 1998). Furthermore, the increased globulins level may be attributed to the mode of action of yeast (*Saccharamyces cerevisiae* ) which include bacterial exclusion, neutralization of toxins and immunostimulation (Newman, 1994). Since the gamma fraction makes the largest portion of globulin, it can be infer that the dietary probiotics supplementation enhance the antibody response in Nile tilapia.

Assessment of plasma biochemistry revealed significant decrease in the ALT and AST activities in probiotic administrated fish compared to control groups. This could be attributed to the positive and beneficial effect of both probiotics on the maintenance of the integrity of hepatocytes and their role in the improvement of the histology of liver and preventing any toxicity for the hepatic cells (Segner et al., 1989). This supports the finding of Nakano et al. (1995) who showed that probiotic supplemented diet have the potential for

improving the liver function (decrease the ALT and AST activities) and increase the defensive potential level against oxidative stress in fishes.

Marked increase of the cholesterol and triglycerides concentrations which were observed in probiotic administrated fish compared to control negative and control positive groups confirmed the idea that probiotics have a hepato-protective and hepato-stimulatory effect (Sarma et al., 2003) specially when we know that the liver is a major site of cholesterol and triglycerides synthesis. So, it is interesting to state that probiotics stimulate and enhance the hepatic tissue of fishes.

Regarding the kidney function parameters, marked elevation of the creatinine concentration was observed at the 6$^{th}$ week in group 1 (received biogen) and at the 8$^{th}$ week in both biogen and moreyeast received groups comparing to control group. The elevation of creatinine concentration could be attributed to the vacuolar degeneration of renal tubules which was noticed in both probiotics received groups. The gills appear to predominate over the kidneys as the major organ of urea excretion in most fish converse to the creatinine which mainly excreted through the kidneys (Stoskopf, 1993). Therefore, increases in the plasma BUN concentration may be more indicative of branchial epithelial disease while the increase of the plasma creatinine reflects renal disease. The plasma glucose level revealed significant hypoglycemia only at the 4th and 6th week in group 2 (received moreyeat) and at the 8$^{th}$ week in group 1 (received biogen) as compared to control group. The reduced blood glucose level in both probiotics administrated groups indicated that probiotics might play a role in ameliorating the physiological effects of stress in fish through improving the gastrointestinal microbial balance, degradation and minimization of the waste organic matter found in water, prevention of colonization of pathogenic bacteria and providing nutrients which reflected on the enhancement of the physiological function of fish and reduce stress factors, so, the increased blood glucose level is a good indicator of stress in fish as described by Hattingh (1975).

The biogen and moreyeast administerd groups showed congesion of branchial blood vessels and marked activation of the melano-macrophage centers. In this concern, the results may explain the potential and the immunoenhancment effect of both probiotics which may attributed to addition of natural bacteria which modify the bacterial composition of the water and lead to removal or decrease in the population density of pathogens and improve the water quality through the more rapid degradation of waste organic matter. Also, probiotics secretes and produce pathogen inhibitory substance which inhibits the microbial toxins, stimulats IgA and the defense mechanism of fish against microorganism by the activation of the melano-macrophage center which is a component

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# دراسة عن تأثير استخدام بعض محفزات النمو على كفاءة أسماك البلطي النيلي وكذلك احداث تغيرات باثولوجية وباثولوجية إكلينيكية

محمد عبد العزيز أحمد*محمود على محمود** صفاء يس سيد أحمد***علاء رأفت أحمد - صافيناز رشاد على عبد العزيز****

*قسم أمراض الأسماك ورعايتها -كلية الطب البيطرى - جامعة القاهرة

**قسم الباثولوجى كلية الطب البيطرى - جامعة القاهرة

***قسم الباثولوجى الإكلينيكية - كلية الطب البيطرى - جامعة القاهرة

****طالبة بحث بقسم الباثولوجيا الإكلينيكية كلية الطب البيطرى - جامعة القاهرة

أجريت هذه الدراسة للإستدلال على تأثير إثنين من إضافات الأعلاف المسماه بالبروبيوتيك (بيوجين و موريست) على معدلات النمو، مكونات الدم الخلوية و الكيميائية، و قد تمت التجربة على خمسين سمكة من أسماك البلطي النيلي (أوربيوكرومس نيلوتيكس) تم تقسيمها إلي مجموعات ثلاث كما يلى المجموعة الأولي تحتوي على ٢٠ سمكة تم تغذيتها على علف مضاف إليه البيوجين بجرعة ٢ كجم/ طن علف ، المجموعة الثانية تحتوي علي ٢٠ سمكة تم تغذيتها على علف مضاف إليه الموريست بجرعة ٥ كجم/ طن علف. المجموعة الثالثة المجموعة الضابطة حيث تحتوي علي ١٠ أسماك تم تغذيتها على علف خالي من اية إضافات.

تم إطعام هذة الأعلاف لكل مجموعة من الأسماك علي مدي فترة التجربة (٨ أسابيع) في صورة حبيبات جافة. أضيفت هذة الأعلاف بمعدل تغذية ٣٪ لكل يوم من الوزن الكلي للأسماك في كل المجموعات التجريبية. وقد أشارت النتائج إلى أن إستخدام كلا النوعين من إضافات الأعلاف المعروفة بالبروبيوتيك أدى إلى تحسن الصحة العامة في المجموعات المختلفة من الأسماك المغذاة على هذه الأعلاف مقارنة بالمجموعات الضابطة. حيث تجسد ذلك في إرتفاع معدلات النمو المختلفة و مكونات الدم (كرات الدم الحمراء، خلايا الدم المكدسة، تركيز الهيموجلوبين، العد النوعي و الكمي لكرات الدم البيضاء). بالإضافة إلى التغيرات الكيميائية للبلازما التى إشتملت على إرتفاع في مستوى البروتينات الكلية، الجلوبيولينات، الدهون و الكربوهيدرات بينما قل تركيز البومين و الجلوكوز ما عدا في المجموعات المعدية تجريبيا. كذلك لوحظ إنخفاض في إنزيمات الكبد المختلفة.كما لوحظت تغيرات باثولوجية في الخياشيم والطحال والكلى في المجموعتين الأولى والثانية مقارنة بالمجموعة الضابطة.