EVALUATION OF BIOGEN AND YEASTURE PROBIOTICS ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF NILE TILAPIA

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ABSTRACT

Growing concern for the high consumption of antibiotics in aquaculture has initiated a search for alternative methods of disease control by improving resistance against infectious disease which can be achieved by using of probiotics. The objective of the present study was to evaluate the influence of some feed additives (Yeasture and Biogen) on the immune response of O.niloticus. The experimental fish were divided into five groups, the first group was served as control fed on probiotic – free diet, the second and third groups were fed on diet supplemented with Yeasture (1 and 3 g/kg feed) and the fourth and fifth groups were fed on diet supplemented with Biogen (1 and 3 g/kg feed). After 70 days of feeding, the results indicated that, the fish groups which received diet supplemented with probiotics revealed highly significant growth performance and significant increase in immune response as detected in challenge test as probiotic fed fish groups showed high resistance to the challenged pathogenic microorganisms.

Key words: probiotics, ***Oreochromis niloticus***, growth performance, liver enzymes and challenge test.

INTRODUCTION

The immune system of aquatic organisms, such as fish, is continuously affected by periodic or unexpected changes of their environment. Adverse environmental situations may acutely or chronically affect the health of fish, altering some of their biochemical parameters and suppressing their innate and adaptive immune responses ***(Giron-perez et al., 2007)***. Antibiotics have been used for long time as growth promoting agents but adversely affected growth and food conversion as well as multiple of drug resistant bacteria and antibiotic residues in fish meat ***(Wary and Davies, 2000)***. Therefore, using of natural feed additives to substitute antibiotic had become great interest ***(Kumar et al., 2003)***. Probiotics are defined as microbial dietary addetives that beneficially affect the host physiology by modulation immunity system, as well as improving nutritional and microbial balance in the intestinal tract ***(Villamil et al., 2002)***. Using of some kind of probiotics in aquaculture water regulated the microflora of aquaculture water, controlled pathogenic microorganisms, enhanced decomposition of the undesirable organic substances in aquaculture water, and improved ecological environment of aquaculture. In addition, the use of probiotics can increase the population of food organisms, improve the nutrition level of aquaculture animals and improve immunity of cultured animals to pathogenic microorganisms ***(Patterson and Burkholder 2003)***.

Further, they improved feed conversion ratio and feed utilization, Probiotics produce of compounds that inhibit pathogens and competition for attachment sites. Many researches showed improvement in the immune response of fishes treated with probiotices ***(Watson et al., 2008)***.

This study was planned to evaluate the effect of probiotics on the growth performance, blood parameters and immune response of cultured ***O.niloticus.*** concerning the effect of both commercial products Yeasture and Biogen on the health status of ***O.niloticus***.

MATERIAL AND METHODS

Experimental fish:

A total of 150 apparently healthy fish were collected from three private fish farms at Tolompate 7, Kafer El-Sheikh Governorate. They seems to be likely healthy and had a uniform size and with average weight 20±0.6 gram. The experimental fish were acclimatized to the aquaria conditions in indoor tanks for 2 weeks. Aquarium (80 x 32 x 40 cm) containing about 100 liters of dechlorinated water and water temperature was adjusted at 25±2.5 °C as well as continuous oxygen supply.

Experimental diets formulation and feeding system design:

In this experiment tilapia fingerlings were divided into 5 groups each group subdivided into3 subgroup that distributed in 3 aquaria 10 fish per each aquarium , the first group was the control group supplied with a probiotic free diet the second (Y1) and Third (Y2) fed on Yeasture (1 and 3 g/kg diet) the fourth (B1) and the fifth (B2) fed on Biogen (1 and 3 g/kg diet),. The feeding duration longed for a period of 70 days. Every seven days, the fish in each aquarium were weighed and the amount of feed from the diet which was formulated according to ***NRC (1993)*** requirements for ***O.niloticus*** was corrected according to the new fish biomass as 3 % of live body weight.

Table (1): Physical and chemical composition of the experimental diets:

Physical composition	(%)	Chemical analyses%	(%)
Fish meal (local)	15	Dry matter (DM)	88.9
Soybean meal (44%)	50	Crude protein (CP)	32.1
Yellow corn	16.5	Ether extract (EE)	3.85
Wheat bran	12	Crude fiber (CF)	8.1
Wheate straw	5	Ash	6.1
Vitamin and mineral mixture*	0.5		
Fish oil	1	Nitrogen free extract (NFE)	49.84

Vitamins and minerals premix at 0.5% of the diet supplies the following per kg of the diet: 75000 IU Vit.A; 9000 IU Vit. D3 ; 150 mg Vit. E ; 30 mg Vit. K3 ; 26.7 mg Vit. B1; 30 mg Vit. B2; 24.7 mg Vit. B 6 ; 75 mg Vit.B12; 225 mg Nicotinic acid ; 69 mg Pantothenic acid ; 7.5 mg Folic acid; 150 mg vit. C; 150 mg Biotien; 500 mg Choline chlorid 300 mg DL-methionine; 93 mg Fe; 11.25 mg Cu; 210 mg Zn; 204 mg Mn; 5 mg Se and Co 5 mg (Local market).

Probiotic used in the study:-

1- Yeasture (***Saccharomyces cerevisae, Lactobacillus acidophilus*** and ***Streptococcus faecium).***

Dosage: high and low levels 3 and 1g/kg feed level

2- Biogen: ***(Allicin*** and ***Bacillus subtilis*** and high-unit hydrolytic enzymes).

Dosage: high and low levels 3 and 1g/kg feed level

Calculations of feed utilization parameters:-

Average daily gain ***(Castell and Tiews, 1980)***

$ADG = (W1-W0)/T$

Where, W0 and W1 were the initial and final body weight per gram, and T is the number of days in the feeding experimental period.

Total weight gain (TG) ***(Castell and Tiews, 1980)***

$TG\ (g) = Wt_1-Wt_0$

Where wt1 is the final body weight (g) and wt0 is the initial body weight (g)

Survival rate (SR%)

S.R= (No. of fish at end / No. of fish at the start) ×100

Feed conversion ratio *(Tacon, 1987)*

FCR= Feed intake (g)/ weight gain (g)

Where, the weight gain is (the biomass of fish at the start + the biomass of the dead fish- the biomass of the fish at the end)

Protein efficiency ratio *(Davies and Morries, 1997)*

PER (%) = weigh gain (g) / protein intake (g)

Chemical analysis of feed and fish body:

The proximate analysis of feed sample moisture, crude protein (**CP**), ether extract (**EE**), crude fiber (**CF**), total ash content and fish body at the end of experiment were determined according to ***A.O.A.C. (1990)***.

Blood sample collection and immune response assay:

Blood film was prepared according to the method described by ***Lucky (1977)***. Differential leukocytic count was calculated according to ***Schalm (1986)***. Mean Corpuscular Volume **PCV** and hemoglobin concentration **Hb** were calculated according to the formula mentioned by ***Dacie and lewis (1975).*** Red blood cell (**RBCs**) and White blood cell (**WBCs**) counts were counted by haemocytometer according to ***Stoskopf (1993).*** In addition to **M.C.V.** Mean Corpuscular Volume, **M.C.H.** Mean Corpuscular Volume hemoglobin and **M.C.H.C.** Mean Corpuscular Volume hemoglobin concentration were calculated according to the formula mentioned by ***Dacie and lewis (1975)***.

M.C.V. = PCV x 10 / RBCs as m/mm^3.

M.C.H. =HB contentgm/100ml x 10/ RBCs as m/mm^3.

M.C.H.C. =HB contentgm/100ml x100 / PCV.

The concentration of total protein (**TP**) **Weichsellbaum (1946)** and albumin (**Alb**) ***(Doumas et al., 1971)*** were measured by colorimetric methods, While, globulin concentrations (**Glo**) were determined by subtracting the concentration of total protein from albumin concentration. The activity of the liver enzymes, Aspartate Amino Transaminase (**AST**) and Alanine Amino Transaminase (**ALT**) was determined according to ***(Reitman and Frankel, 1957)***.

Challenge test:

After 70 days of feeding, a total number of 50 fish (10 fish from each treatment) were injected I/P with the pathogenic ***A. hydrophila*** (0.3 ml of 10^8cells/ml) according to ***(schaperclaus et. al., 1992)***, the injected fishes were kept under observation for 14 day to record the mortality rate.

$$\textbf{Mortality rate \%} = \frac{\text{No. of death in specific period x 100}}{\text{Total population during that period}}$$

Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range ***Duncan (1955)*** was used to determine differences among water resources treatments mean at significance level of 0.05. All statistics were run on the computer using the SAS program ***(SAS, 1998)***.

RESULTS & DISCUSSION

Fish culture is increased to compensate the shortage of animal protein allover the world. Fish under intensive culture conditions will be badly affected by different microbial pathogens that have been treated with antibiotics were intensively used producing great problems of bacterial drug resistance on one hand and the public health hazards as residues in fish meat ***(Robertson et al., 2000)***. The use of natural immunostimulants in fish culture for the prevention of diseases in a promising new development and to solve the problems of massive antibiotic use.

Table (2): feed utilization of Nile tilapia (***O. niloticus***) fed diet containing probiotics.

Treatment	IW	FW	TG	DWG	FCR	FI	PER
C	20.8± 0.8a	41± 3.1 a	20.4± 2.03a	0.29± 0.01a	3± 0.1c	66.45± 2.9c	1.03± 0.1a
Y1	20.5± 1.1a	44.6 ± 2.3b	24.1± 1.3b	0.34± 0.01b	2.2± 0.4b	52.4± 3.2b	1.41± 0.12b
Y2	20.9± 2.4 a	49.3± 4.2c	27.4± 1.04c	0.39± 0.02c	1.73± 0.02a	47.48± 4.3a	1.8± 0.2c
B1	20.5± 0.9a	44.06± 1.6b	23.5± 1.32b	0.34± 0.01b	2.4± 0.8b	56.6± 1.9b	1.32± 0.74b
B2	20.9± 1.3a	47.3± 3.7c	26.36± 1.4 c	0.37± 0.01c	1.93± 0.04a	51.03± 5.2b	1.6± 0.1c

Group with different letter within the same column are significantly different at P< 0.05 . IW= Intial Weight, FW= Final Weight, TG= Total weight Gain, DWG= Daily Weight Gain, FCR= Food Conversion Ratio, FI= Feed Intake and PER Protein Efficiency Ratio.

The best results ***O.niloticus*** fingerlings of growth performance were obtained in the diet supplementation with probiotic Yeasture and Biogen compared with control group table (2). This means that Nile tilapia fingerlings grow well when either probiotic had been used as growth promoter in fish feeding such improvement may be due to the creation of balanced microbial population in the intestinal tract of fish ***(Fuller, 1997)***. The best results of total weight gain, FCR and PER % had been recorded for fish group fed the diet supplemented with high levels of probiotics Yeasture and Biogen (3g/ kg diet) without significant differences followed by fish group fed diet supplemented with the high levels of probiotics Yeasture and Biogen (1g/ kg diet). While, the lowest values were recorded for fish groups fed the free additives diet table (2). The explanation of good results of FCR recorded in groups feed on diet containing probiotics as adding Lactobacillus supplements is their effect in suppressing pathogenic coli forms in the stomach and intestinal epithelium ***(Prescott and Baggot, 1993)***.

Table (3): Carcass chemical composition of Nile tilapia (***O. niloticus***) fed diet containing probiotics.

Treatment	DM	CP	EE	Ash
Control (C)	25.9±2.01[a]	52.65±3.2 [a]	30.0±0.90 [c]	11.3±2.3[b]
Yeasture1 (Y1)	26.35±1.3 [a]	54.8±3.70 [a]	27.8±2.40 [b]	10.1±1.5 [a]
Yeasture2 (Y2)	26.7±1.50 [a]	63.9±1.56 [c]	25.4±2.40 [a]	8.5±1.4 [a]
Biogen 1 (B1)	26.4±2.10[a]	57.5±1.70 [b]	28.53±3.2 [b]	10.1±1.8 [a]
Biogen 2 (B2)	26.6±1.10 [a]	61.1±4.2 [c]	26.5±0.50 [a]	8.9±0.7 [a]

Group with different letter within the same column are significantly different at P< 0.05 .

The chemical composition of Nile tilapia (***O. niloticus***) fingerlings at the end of feeding experiments indicated that carcass dry matter (DM) content and the crude protein values (CP) were higher in fish groups fed the diet supplemented with the yeasture and biogen. Ether extract (EE) content of fish carcass and ash content were lowered in fish groups fed significant differences diet supplemented with high level of Yeasture and Biogen followed with by lower level table (3). Similar trend of changes in ***O. niloticus*** fed on diet supplemented with some probiotics was observed by ***Magouz et al. (2002)*** and ***Saad et al., (2009)*** that suggested probiotics plays a role in enhancing feed intake with a subsequent enhancement of fish body composition and the better feed intake in probiotics supplemented diets may be due to the improvement in fish appetite and in turn in improvement of growth. Generally, body composition of fish in all groups is within the range reported by ***Abdelhamed et al. (2000).***

Table (4): Hematogram of ***O.niloticus*** groups post-treatment with probiotics.

Treatment	RBC X 10^6	WBC X 10^3	H %	M %	E %	L %	Hb	PCV	MCV	MCH	MCHC
C	1.17± 0.1a	60.5± 2.3 a	41± 1.7c	2± 0.1a	3 ±0.2b	54 ±3.7a	5.3 ±1.2a	15.5 ±2.1a	130.3± 11.2b	43.23± 3.7c	34.1± 1.6b
Y1	1.54± 0.1b	71.8± 2.1c	29.5 ±3.4a	3.5± 0.3c	2.5 ±0.4b	64.2± 5.4b	6.02± 1.3b	20.05± 2.1b	129.6± 12.7b	38.9± 3.1b	30.04± 2.6a
Y2	1.6± 0.3c	72.9± 3.8c	29.5± 1.2a	3.5± 0.4c	2± 0.04a	64.5± 5.1b	6.15± 1.3b	22± 1.7c	133.27± 11.3b	37.19± 3.4b	28± 2.1a
B1	1.42± 0.04b	66± 5.3b	33.5± 1.8b	2.5± 0.07b	1.5± 0.1a	60.5± 3.5b	5.3± 0.7a	16.8± 1.5a	128.3± 7.1b	37.6± 21b	31.8± 1.7a
B2	1.51± 0.7b	67.6± 4.3b	31± 4.1b	2.5± 0.1b	2.5± 0.1b	63.5± 6.3b	5.4± 1.3a	18.4± 1.7b	118.6± 16.5a	35.1± 0.9a	29.6± 3.7a

Group with different letter within the same column are significantly different at P< 0.05 .

H= Heterophil, M= Monocyte and E= Esinophil and L= Lymphocyte.

The results indicated a positive effect represented by significant increase in RBCs count, PCV%, Hb Conc., WBCs and differential leukocytic count (Table, 4). These could be attributed to the fact that, the probiotics used increased the blood parameters valued as a result of hemopiotic stimulation. These results supported the results of **Rajesh *et al.* (2006)**. Also agreed with results obtained by **Marzouk *et al.*, (2008)** who stated that ***O. niloticus*** fed on diets supplemented with probiotic (tow commercial probiotics Diamond and Meglo) showed significant increase in RBCs count, PCV %, Hb Conc., WBCs and differential leukocyte count. Concerning the non-specific immune situation in ***O.niloticus*** fish groups received diets supplemented with probiotics. It was clear that high non-specific immunity was developed as manifested by increased number of lymphocytes and monocytes in the differential leucocytic count. These results confirmed by that obtained by **Abo state (2005)** who reported that ***O.niloticus*** fish fed on diet suuplemented with probiotic (Premalac and Biogen) had greater mean total leukocyte count than the control group, particulary when added to fish diet at 2 g/ kg diet.

Table (5): Protein profile and activities of serum enzymes (ALT & AST) in ***O.niloticus*** groups post-treatement with probiotics.

Treatment	TP (g/100ml)	Alb (g/100ml)	Glo (g/100ml)	Alb/ Glo	ALT (U/l)	AST (U/l)
C	3.15± 0.3a	2.1± 0.6b	1.05± 0.2a	2±0.2c	16.5± 1.2b	20± 2.1b
Y1	3.9± 0.4b	1.07± 0.1a	2.32± 0.3b	0.46± 0.02a	10.5±1.3a	12.5± 0.1 a
Y2	3.8± 0.27 b	1.7± 0.7 b	2.1± 0.2b	0.81± 0.02b	13.5± 0.9a	16± 1.6b
B1	3.7± 0.04b	1.6± 0.09b	2.15± 0.3b	0.74± 0.1b	14.05± 1.7a	16± 2.1b
B2	3.6± 1.02 b	1.5± 0.3 b	2.12± 0.07 b	0.71± 0.06b	14.6± 1.3a	16.9± 1.4b

Group with different letter within the same column are significantly different at P< 0.05 .

TP= Total Protein, Alb= Albumin, Glo= Globuline, Alb/Glo= Albumin Globuline ratio, Alt= Alanine amino transaminase and AST= Aspartate transaminase.

The results indicate a significant increase in total protein and decrease A/G ratio which could be attributed to the immuno-modulatory effect of Yeasture and Biogen on the liver cells which activate the anabolic capacity of the hepatocytes to produce blood proteins particularly globulin and this was also supported by the results of hepatic enzymes analysis which decreased in ***O.niloticus*** kept on protibiotics in comparison to control group indicating a normal, positive and beneficial effect of both probiotics on the maintenance of the integrity of hepatocytes ***Nayak et al. (2004)*** and ***Safinaz (2006)***. Also all values of pervious blood parameters were within the normal range reported by ***Shalaby, (2004)*** and ***El-Dakar (2004)*** in Nile tilapia.

Table (6): Mortality rate of ***O.niloticus*** fed with diet containing probiotics challenged with ***A. hydrophila***.

Treatment	Total no.	Dead no.	Sur %	Mor %
C	8	8	0	100
Y1	8	2	75	25
Y2	8	1	87.5	12.5
B1	8	1	87.5	12.5
B2	8	3	62.5	37.5

Total no. = Total number of fish, **Dead no.** =Dead number, **Sur %**= Survival rate and **Mor %**= Mortality rate.

Concerning the challenge of the ***O.niloticus*** fish groups with specific fish pathogen ***Aeromonas hydrophilia*** (Table, 6). The results indicated an appearance of characteristics clinical signs and post mortem lesions in ***O.niloticus*** control group lead to a total mortality percentage reach 100% post challenge.

On the other hand the ***O.nilotiocus*** in the groups 2 and 3 kept on diet supplemented with Yeasture did not show and mortality within 5 days post challenge and survival rate was 100% while ***O.niloticus*** of group 4 and 5 kept on diet contain ***Biogen*** showed 37.5% mortality after 2 weeks post infection (Table, 6) These results confirmed the immune stimulatory effect of Yeasture and Biogen also their inhibitory effect to ***Aeromonas hydrophilia***. Also there was variation in the mortality rate in ***O. niloticus***. These results agreed with that obtained by ***Marzouk et al., (2008)*** who stated that ***O. niloticus*** fed on diets supplemented with the two commercial probiotics Diamond and Meglo showed low mortality rate (0-14.3 %)comparing with control group (28.6 %)

In conclusion, the results of this study revealed that Yeasture and Biogen frequently used feed addetives improve Carcass quality and utilization efficiency of feed. Also the diets supplemented with Yeasture and Biogen improved the non-specific immune response which reflected on the increasing WBCS count and total protein and globulin.

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تقييم بعض محفزات النمو (البيوجين و اليستيور) علي النموالاستجابةالمناعية لأسماك البلطي النيلي

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نظراً للتوسع في الانتاج السمكي وشيوع الامراض بها اتجة المختصون لاستخدام بدائل للمضادات الحيوية في العلاج وكمنشط للنمو والمناعة لذا تهدف الدراسة لتقييم نوعين من إضافات الأعلاف (البيوجين – اليستيور) علي الأداء الإنتاجي والحالة المناعية في اسماك البلطي النيلي. تم إضافة النوعين في مستويين (1– 3 جرام/ كيلوجرام علف). تم التغذية علي النوعين لمدة 70 يوم ثم تم تحليل جسم و دم الأسماك لتقدير مدي استجابة الأسماك للإضافات والتي أظهرت تحسن معنوي في معدل النمو اليومي وكفاءة التحويل للعلف للمجموعات التي تم تغذيتها على نوع الإضافات البروبيونك وتم أيضاً عملُ إصابة تجريبية ببكتريا الايروموناس هيدروفيلا لتقيم الحالة المناعية للأسماك وأثبتت الأسماك التي تم تغذيتها علي الإضافات مقاومة اعلي من المجموعة الضابطة للإصابة البكتيرية. من نتائج الدراسة أتضح أن إضافة اليستيور أو البيوجين في العلائق بمستويات مختلفة تصل إلى 3جرام / كيلو علف تعمل على تحسين الحالة الصحية والنمو والاستفادة من الغذاء لأصبعيات البلطي النيلي.