Studies on growth performance and body composition of Nile tilapia *(Oreochromis niloticus)* and common carp *(Cyprinus carpio)* as affected by dietary supplemented biogen®

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ABSTRACT

Three months feeding experiments were carried out in concrete ponds, filled with fresh water, in order to evaluate the probiotic (Biogen) potential, and assess its effect on growth and net returns of both *O. niloticus* in average initial body weight $13.8^{\pm 1.02}$ g. and body length $13.5^{\pm 0.9}$ cm and *Cyprinus carpio* at mean initial body weight $15.3^{\pm 0.05}$g and body length $13.5^{\pm 0.05}$ cm Fish were fed basal diet that containd 30% crude protein, and Biogen® at four levels (0, 2,3 and 4g./ kg. diet). *O. niloticus* and *C. carprio* were divided into (4) treatment groups . T_1 was the control fed on basal diet without adding Biogen® , T_2 , T_3 and T_4 fed on basal diet and Biogen® at levels 2, 3 and 4 g. /kg diet.

The results show that: (FBW), (WG), (ADG) (S.G.R) and nutrient utilization (P.P.V), protein efficiency rate (PER) values were significantly ($p<0.0l$), higher in the treatments receiving probiotic than those offered the control diet in both *O. niloticus* and *C. carprio*. Concerning the influence of different dietary Biogen® levels on the chemical proximate analysis of whole carcass ash was significantly ($p<0.0l$) higher for fish offered the control diet, while protein has a significant difference ($p<0.0l$) for fish offered basal diet contained Biogen® , than those offered the control diet, but results showed no clear trend. Differences were observed in carcass lipid and gross-energy content with significantly ($p<0.01$) higher values recorded in fish offered the control diets compared to fish fed a diet containing 3. g /kg. diet Biogen®.

The production and subsequent cost - benefit analysis clearly indicated that the diets containing 3.g / kg. diet recorded the highest return. In comparison between *O. niloticus* and *C. carpio*, the results showed that the former recorded the highest significant values than *C. carpio* in all growth parameters and the net return. At the end of this research, results indicated that fish offered diets containing Biogen® exhibited greater growth than control. The observed improvement in fish growth, survival and net return by using probiotic bacteria can open a new scope for intensive use in aquaculture.

Key words: *Oreochromis niloticus*, *Cyprinus carpio*, Biogen® , growth performance, net return, body composition .

INTRODUCTION

The use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms was increasingly reviewed as an after-native to antibiotic treatments. The use of probiotic in human and animal nutrition is well documented (Molder *et al.,* 1997; Rinkinen *et al.,* 2003). Recently , they have begun to be applied in aquaculture. Bachere (2003) and Watson *et al.* (2008) described the principal mechanism of action and criteria for selection of probiotics in aquaculture with some considerations similarly to humans and terrestrial animals. It can be assumed in aquaculture that the intestinal macrobiotic does not exist as entity by itself but there is a constant interaction with the environment and the host function. Probiotic Biogen® consists of *Bacillus subtilis* and *Bacillus licheniformis*. The advantage of these spore-forming bacteria is that they are able to survive the pellitization process after transits through stomach, then they germinate in the intestine and use a large amount of sugars (carbohydrates) for their growth and production at a range of relivant digestive enzymes (amylase, protease and lipase). The beneficial effects of probiotics include higher growth and feed efficiency, prevention of intestinal disorders and pre digestion of anti nutritional factors present in the ingredients.

Probiotics in Aqua feed should be tested for their efficacy as had already been demonstrated in aquaculture and used in vitro models of specific bacteria as antagonists of pathogens. Vine *et al.* (2004) measured the survival of probiotics in the fish gut, Andild, *et al.* (1998) studied the evaluation of beneficial effect of probiotics on health management, diseases resistance and Immune response of fish (Gatline & Li , 2004). Other studies have focused on growth prombting of fish by probioticl supplements. Gatesoupe (2002), reported that dietary supplement of probiotic and Robiotics® has significantly enhanced the growth and diseases resistance of hybrid striped bass.

The aim of present study was to evaluate the Biogen® potential on *O. niloticus* and *C. carpio* growth performance and final net return.

MATERIALS AND METHODS

1) Experimental site and fish culture technique:

The present study was done in EI-Kanater EI-Khayria fish research station, that belongs to National institute of Oceanography and Fisheries, Qalubyia, Egypt. *O. niloticus* with average Initial weight $13.8^{\pm 1.02}$ g , and length $13.5^{\pm 0.09}$ cm and *C. carpio* with average weight 15.3 g and length 13.5 cm, were obtained from EI-Abbasa fish station, Sharkya Govcrnorate. The experiment was carried out in concrete ponds, each pond area was $16m^3$ (4x4x1 m), filled by fresh water from Darawa irrigation canal, the fish were adapted for two weeks to the new conditions, before the start of the experiment and fed with a commercial diet twice a day. The duration period was 12 weeks. The turnover rate of water was $0.4m^3$ day / pond. Fish were held under natural light. At the beginning of the experiment fish were divided to four groups; each group contained 96 fish in

polyculture system. During the trial, water temperature was recorded daily, dissolved Oxygen (D.O) was measured by using an YSI model 56 Oxygen meter, (Yellow Spring OH, U.S.A.) pH was determmed by using a pH meter (Orion pH meter, U.S.A.) Ammonia and alkalinity were measured at weekly intervals according to APHA (1985).

All dietary treatments were tested in duplicate groups, where each pond was considered as experimentel unit during the experiment, and all fish were fed diets at a rate of 5% from the body weight for six days a week, and offered to fish three times daily.

2) Experimental diets:

Four isonitrogenous (30%) crude protein and lsocalories (4690) Kcal/kg diet were formulated for the experiment. Table (1) illustrates chemical composition and proximate analysis for the used diet. The control diet had no Biogen®, and the three other tested diets included Biogen®, at 2 , 3 and 4 g. /kg diet levels. Biogen® was obtained from the EI-Zahar veterinary trading company (exclusive agent) of the manufacturer, China way corporation, Taiwan). The Biogen®, is reported to contain Allicin (not less than 0.247 mol/g, *Bacillus subtilis* not less than ($6xl0^7$ g^{-L}) and High unit hydrolic Enzymes (not less than 3690.0µ./g). The diet was prepared by blending dry ingredients into a homogenous mixture and then passing the mixed feed through a laboratory pelleit at the, California pellet Mill. Co.(San, Franc , CA,U.S.A).

Table (1) Chemical composition and proximate analysis of diet contained different levels of Biogen®:

Treatments Ingredients	**Control 0.0 g. Biogen®**	**2g Biogen Kg^{-1} diet**	**3g Biogen kg^{-1} diet**	**4 g Biogeo kg^{-1} diet**
Fish meal	15.0	15	15.0	15.0
Soya bean meal	32.4	32.4	32.4	32.4
Yellow corn	20.0	20.0	20.0	20.0
Wheat Bram	26.0	26.0	26.0	26.0
Vitamin	2.0	2.0	2.0	2.0
Oil	2.0	2.0	2.0	2.0
Biogen	-	2	3	4
Allicin (ml mol)	-	0.494	0.741	0.988
Bacillus subtilis (No)	-	$12x\ 10^7$	$15x10^7$	$24x10^7$
Hydrolic inzymes ug^{-1}	-	7380	11070	14760

PROXIMATE COMPOSITION:

% crude protein	30
% ether extract	73
% crude fiber	6.4 for all the treatment diets.
Ash %	9.3
Nitrogen free extract	46.6
Growth energv kcal/Kg diet	4960

3) Sampling and chemical analysis:

At the beginning of the experiment, ten fish from the common population were collected to serve as initial carcass sample.

At the end of experiment, 15 fish from every treatment groups were sampled at random and anaesthetized with T-amyl alcohol. Fish were pooled, autoclaved ground into homogeneous sturry, oven- dried, reground and stored at 20ºc until. analyzed for dry matter (DM) and ash according to AOAC (1995). Crude protein (%N x 6.25) by Kjeldahle method using a Kjettech autoanalyzer (Model 1030 Tecator Hoganas Sweden), and total lipid according to the methods of Bilgh and Dyer (1959). Gross energy content of carcass samples were measured by using an automated bomalarimeter (Model1272). Par Instruments Inc. Molin, IL, U.S.A.).

Growth performance parameter: mean fish weight of each treatment (pond) was calculated by dividing total fish to determine (FBW) final body weight, (ADG) average daily gain, specific growth rate (SGR), fed conversion ratio (FCR), Protein productive value (P.P.V) and protein efficiency rate (PER) were calculated by using the following .

W.G = final body weight - Initial body weight (g).

SGR = (Ln FBW- Ln IBW) / period x 100.

FCR = feed intake (g) / weight gain (g).

PER = weight gain (g) / protein intake (g) x 100.

PPV = Retained protein (g) / protein intake gx100.

Net production kg /pond= TB MH- TBMS.

Where:

TBMH = Total fish biomass at harvesting (kg) / pond.

TBMS = Total fish biomass at start (kg) / pond.

Total income = TBMH x price.

Net retun (LE) pond = total income (LE) pond / total cost.

Profit % = net return (LE) pond /total cost (LE) pond x 100.

Statistical analysis :

The data were analized by analysis of variance (ANOVA) using the SAS ANOVA procedure (SAS 1988), Duncan's multiple range test (Duncan, 1955).

RESULTS

Data given in Table (2) show that *O. niloticus* was affected by adding Biogen® at different levels, (0, 2,3and 4.0 g. /kg /diet). Growth performance including final body weight, weight gain, average daily gain and specific growth rate had signilicant differences ($p<0.01$) between fish groups offered diet contained Biogen® and the control fish group offered basal diet without Biogen®. There was significantly higher values (92.7, 78.5, 0.87 and respectively) obtained from the fish group offered diet contained 3.0 g /kg diet compared to fish fed the control diet (without adding Biogen®) which recorded

the lowest values (58.5 , 44.7 , 0.49 and 1.2 respectively). From Table (2) survival percent had a higher value (100%) fish fed the highest Biogen® level in contrast to the control fish groups (90%).

In Table (2), the nutrient utilization for *O. niloticus* was affected by adding Biogen® in different levels (0, 2 , 3 and 4.0 g. /kg diet), includes protein efficiency ratio and protein productive value that had a significant difference ($p<0.0l$) for fish groups offered diet contained 3g./kg. (4.5, 36.3) in contrast to fish groups fed diets without Biogen® (3.3,30.8). The growth rate had the maximum value of (380.6%) , for fish group fed diet contained Biogen® at leve1 3 g./kg. diet. compared to the control diet (244.8%)

Cyprinus carpio has been affected by adding Biogen® at different levels (0, 2, 3 and 4g./kg diet); growth performance, (FBW), (WG), (ADG) and (S.G.R) were given in Table (3). The given data achieved significant differences for fish groups offered diet with Biogen® and the control fish groups fed diet without add. Biogen®. ($p<0.01$). Fish groups offered diet contained Biogen® at level 3.0 g. /kg diet had a significantly higher values of 90.7 ,75.4 ,0.85 and 1.47 respect, while the lowest values (55.2, 39.9, 0.44 and 0.98 respectively) were obtained from the control diet. From Table (3), survival percent showed significantly higher value (95%) for fish groups that had diet contained 3.0 g. /kg diet in contrast to the control diet (85%).

In order to evaluate the effect of using dietary supplemented Biogen to *O. niloticus* diets on fed conversion ratio (F.C.R), Table (2) showes that there were significant differences ($p<0.01$) between fish groups offered diets contained Biogen® and the control fish group. Differences were recorded with significantly higher value (1.9), recorded from fish group fed the control diet in contrast to fish groups offered diet contained Biogen® in level 3.0 g./kg diet which showed the best vlue (1.4).

Table (2) Effect of adding Biogen® to *O. niloticus* diets on growth performance, survival, FCR. PPV and PER

Treatments / **Items** **Biogeo levels g/kg diet**	**T_1** **0.0**	**T_2** **2**	**T_3** **3**	**T_4** **4**	**SE±**
- Average initial body weight (g).	13.8	13.8	13.8	13.8	1.02
- Aver. final body weight g/fish.	58.5^{c}	75.0^{b}	92.3^{a}	90.0^{ab}	3.01
- Aver. weight gain (g/fish)	44.7^{c}	59.0^{b}	78.5^{a}	76.2^{ab}	1.01
- Aver. daily gain g/fish/day.	0.49^{c}	0.65^{b}	0.87^{a}	0.84^{ab}	0.02
- Specific growth rate %	1.2^{c}	1.4^{b}	1.6^{a}	1.5^{ab}	0.01
- Survival%	90^{c}	100^{b}	100^{a}	100^{ab}	OJ) 1
- Feed Conversion%.	1.9^{c}	1.7^{b}	1.4^{a}	1.6^{ab}	0.01
- Protein Productive value.	30.8^{c}	31.9^{b} 36.2^{a}		32.1^{ab}	0.92
- Protein Efficiency Ratio.	3.30^{c}	3.70^{b}	4.5^{a}	3.9^{ab}	0.01

SE+ is the standard error calculated from residual mean in the analysis of variance.

a,b,c... etc means in the same row the different superscript are significant different ($p<0.01$)

Also, Table (3) showed that *C. carpio* has been affected by adding Biogen® at different levels. Nutreint utilization including (P.P.V.) and (P.E.R.), has significant differences (P<0.01) in fish groups offered diet contained Biogen® compared to control fish groups. Differences were observed in nutrteint utilization with significantly higher value (35.3 and 4.11 respectively.) for fish groups offered diet contained Biogen® at level 3.0 g /kg in contrast to the control fish groups, which recorded (30.7 and 3.1). There were no significant differences (p>0.01) among all treatments fish groups that offered Biogen® .

Table (3) recorded also the same trend for *C. carpio* feed conversion ratio. Differences cleared that significantly highest value (1.9) was recorded for control fish groups fed diets without fed Biogen® compared to lowest and best value of (1.4) obtained from fish group offered diet with Biogen® at level 3.0g./kg diet .

Table (3) Effect of adding Biogen® to diets on growth performance, survival, FCR, PPV and PER of *Cyprinus carpio*.

Items / Treatments Biogeo levels g/kg diet	0.0 1	2g/kg 2	3g/kg 3	4g/kg 4	MSE±
- average initial body weigh (g).	15.3	15.3	15.3	15.3	0.09
- average final body weigh (g).	55.2^{c}	70.5^{b}	90.7^{a}	90.0 ^{ab}	2.03
- average weight gain(gm/fish).	39.9^{c}	55.0^{b}	75.4 ^{a}	74.7 ^{ab}	1.50
- average daily Gain g/fish/day.	0.44 c	0.60^{b}	0.85 ^{a}	0.83 ^{ab}	0.01
- Specific growth rate%	0.98c	01..22^{b}	1.47^{a}	1 .4 7 ^{ab}	0.04
- Survival %.	85c	95^{b}	95 ^{a}	92 ^{ab}	-
- Protein productive value%.	30.7c	31.2^{b}	35.3 ^{a}	34.5 ,1^{ab}	1.05
- Protein Efficiency %.	3.1 c	3.5^{b}	4.1 ^{a}	3.9 ^{ab}	0.03
- Feed conversion ratio%.	1.9c	1.8^{b}	1.4 ^{a}	1 .80 ^{ab}	0.01

MSE± : Is the standard error calculated from residual mean in the analysis of vanance .

a,b,c... etc. meaning in the same raw with different superscripture signicantiy different (p<0.01).

In order to clear the influence of different dietary Biogen® levels on chemical composition analysis of whole carcass for *C. carpio* as shown in Table (4), no statistical differences (p>0.01) appeared in carcass, moisture content, and ash. Protein was significantly higher at fish group offered diet contained 3.0g/kg diet. Differences were more clear in carcass lipid and gross energy content. The highest values of (6.3and 1595 respectively) were obtained from the control fish group, in contrast to fish offered Biogen® at level 3.0g/kg diet, which recorded 5.9and 1320 respectively).The same observation for *O. niloticus* as indicated in

Table (4) where significantly higher values (6.5 and 1390) were recorded from fish groups offered the control diet compared to fish offered diet contained Biogen® at level 3.0g./kg diet which recorded (6.2and 1390 respectively).

Table (4) : Effect of adding Biogen® on chemical composition of whole body carcass for *O. niloticus* and *C. carpio*.

Items% / Treatments		Moisture	Crude protein	Lipid	Ash	Cross-energy kcal./kg diet
I At start:		73.3	14.01	6.07	5.01	1410
Cyprinus carpio						
II at harvest						
Conrol	T_1	73.0	13.06^{b}	6.3^{a}	7.1^{b}	1595
	T_2	72.5	13.7^{ab}	6.01^{ab}	7.8^{b}	1580
	T_3	70.9	15.5^{a}	5.9^{ab}	7.0^{a}	L320
	T_4	70.9	13.25^{b}	5.4^{b}	7.3^{b}	1560
Oreocromis niloticus						
Conrol	T_1	72.9	12.9^{b}	6.5^{a}	7.4^{b}	1390
	T_2	72.7	13.1^{ab}	6.7^{ab}	7.5^{ab}	1396
	T_3	71.9	$13,8^{a}$	6.2^{ab}	$7,9^{a}$	13950
	T_4	72.9	13.1^{b}	6.5	$7,5^{ab}$	1330
$MSE^{\pm}$		2.6	1.01	0,3	0.43	30.3

$MSE^{\pm}$: Is the standard error calculated from residual mean in the analysis or vanance.

a,b, c...etc. meaning in the same raw with different superscript signicantly different ($p<0.01$).

Table (5) shows fish production and economic efficiency inclusion: Net production (kg /pond), total income (LE /pond), net return (LE/ pond) and the profit percent for *O. niloticus* as affected by adding Biogen® with different levels (0 , 2 , 3 and 4.0g./ kg diet) . There were significant differences ($p<0.01$) between fish groups offered diet containing Biogen and the control fish group fed diets without Biogen®. The maximum values (3.6 , 36.0 , 20.0 and 125 respect) were obtained from fish groups offered diet contained Biogen® at level 3.0 g. / kg diet, while the lowest value (1.6 , 16.0 , 4.4 and 36.6 respect.) were recorded from the control fish groups in the case of *C. carpio* fish , net production , total income, net-return and profit percent as given in Table (5) recorded significant differences ($p<0.01$) between fish groups offered diets contained Biogen® and the control fish groups.

Differences were clearly observed in fish economic efficiency with significantly higher values (3.6 , 36.0 , 18.0 and 100.0 respect.), in contrast to the control fish groups which recorded the lowest values (1.58 , 15.8 , 2.8 and

15.5 respect.).

Table (5) Fish production and economic efficiency to the experimental diets for *C. carpio* and *O. niloticus*

Items \ Treatments	T_1	T_2	T_3	T_4
I ***cyprinus carpio***				
Fish number / pond	40	46	47	47
Total biomass:				
At starting	0.734	0.734	0.734	0.734
At harvesting	2.22	3.229	4.36	4.1
Net production kg/pond	1.58	2.4	3.6	3.3
Total income LE/pond	15.8	24	36	33
Total cost LE/pond	13	15	18	18
Net return LE/pond	2	9	18	15
PrQfit %	15.4	60	100	83.3
II ***Oreochromis niloticus***				
Fish no. / pond	40	46	47	46
Total biomass:				
At start	0.662	0.662	0.662	0.662
At harvest	2.32	3.45	4.33	4.14
Net production kg/pond.	1.64	2.74	3.6	3.2
Total income LE/ pond	16.4	27	36	32
Total cost LE/ pond	12	13	16	16
Net return LE/ pond	4.4	14.9	20	16
Profit %	36.6	114	125	100

DISCUSSION

In the present study, all probiotic supplemented diets resulted in higher growth in *O. niloticus* than the control diets, indicating that the addition of probiotic enhance the growth performance and feed utilization, Similar result were observed by Abd EI-Hakim *et al*., (2005), who revealed that *O. niloticus* Growth performance (FBW, TWG, ADG and SGR), was improved by supplementing the fry diet with Biogen®. The best values on growth parameters were obtained from the diet contained 3.0 g/kg diet in *O. niloticus*. Also these results are in accordance with the finding of Jena *et at*. (1996), who indicates that Catla and Rohu fry fed on diet with probiotic had significant differences in growth at different stages compared with those fed the control diet. The improvement in growth and feed conversion might be attributed to the presence of any of the compounds in Biogen® or to an interaction among these compounds.

The growth performance of *C. carpio* as affected by adding Biogen® in the level 3.0 g/ kg diet recorded the maximum value than those offered the control diet. These results are in agreement with Noh *et at*. (1994) who studied the effect of supplementing *C. carpio* with different additives, including antibiotic, Yeast and bacteria (*S. faecium*). They also observed a better growth response with probiotic supplemented diets. Bogut *et at*. (1998), showed similar results on *C. carpio* , with supplementing diets with (*S. faecium*). The mechanism by which probiotic improved growth efficiency indicated that the

probiotic activity inhibit the colonization of potential pathogens in the digestive tract, (Hoshino *et al.* 1997), and by stimulating host immunity (MacFarlane 1997) Another possible exptanation for increasing growth performance of *O. niloticus* is an improvement in nutrient digestibility, which in turn could explain the better growth and feed efficiency seen with the supplemented diet. Mendez and Madrid (2003) recorded the same trend, where the addition of 0.1 % probiotic in tilapia in diets improved animal growth and apparent protein digestibility.

The best feed conversion (FCR) value observed with probiotic supplemented diets, addition of probiotic improved feed utilization. Similar observation has been reported for probiotic used in diets for *O. niloticus* fingerlings by EI-Haroun *et al.* (2006). In practical terms, this means that probiotic use can decrease the amount of feed necessary for fish growth, which could result in production costs reduction. In the Present study, all treatment groups were fed at level 3.0 g/kg diet, Biogen® showed a higher weight gain associated with lower feed intake compared to fish fed control diets. These results may be due to the positive effect of probiotic to enhance feed conversion ratio.

Results of growth and survival showed that the best recorded value from fish offered with dietary supplemented diet contained 3.0 g./ kg. diet than those offered the control diet which recorded the lowest value. These results are in agreement with Shelb *et al.*(2006), who found that young Nile tilapia showed enhancement in growth and survival rate.

In fish receiving Bioplus probiotic, *Bacilrus subtilis*, the protein productive value and protein utilization efficitency rate results indicated that supplementing diets with probiotic significantly improved dietary protein and energy utilization. Ringo & Gatesoupe (1998) showed a similar improvement in the biological value of diet supplemented with probiotic from nutritional point of view and in agreement with data of Shelby *et al.*(2006). The present result showed that the use of Biogen® as a feed additive for Nile tilapia and *C. carpio* is recommended to stimulate productive growth performing and nutrient utilization.

Carcass composition of *O. niloticus* and *C. carpio* had significant differences in moisture level and crude protein proportion among treatments. They do not follow the same trend and thus showed variations that are probably due to inherent variation with a positive correlation between Biogen® intake and lipid content in the carcass. Similar results were achieved by Abdel-Monem *et al*. (2002) and Shelby, *et al.* (2003) who found no effect of Biogen® supplementation to Tilapia feed on any carcass proximate composition parameters.

From the data of total production, economic efficiency, the present study revealed that the best and highest net return and a high profit bercent were recorded from the fish fed supplemented Biogen® , than those offered the control

diet.

These results indicated that dietary Biogen® supplemetation. Could decrease the feed costs for the offered diets that contain Biogen® and raise the profit percent with adding Biogen® to the basal diet, while its addition did not confer any additional cost. Similar results were obtained by EI-Dakar *et at*. (2007), who used the same brand of NEM feed supplemented in the feed of tilapia fingerlings.

CONCLUSION

At the end of this research it was concluded that dietary supplemented Biogen® can improve both *O. niloticus* and *C. carpio* growth, that translates to financial benefits for farmers by decreasing feed cost per unit growth intake from fish, while could be beneficial for the reared fish.

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