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Effect of dietary probiotic mix (SPILAC) on growth performance and nutritive physiology of Nile tilapia, *Oreochromis niloticus* (Linn.) under laboratory conditions

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Abstract
The present study was carried out to evaluate the influence of dietary supplementation of probiotic mix (*Lactobacillus sporogenes* and minerals, a commercially available product) on growth performance, and nutritive physiology on the fingerlings of *Oreochromis niloticus.* Along with water quality parameters, excretory patterns of total ammonia (NH_4-N) and reactive phosphate (o-PO4) production were also monitored. The fish with mean body weight 1.22±0.04g were distributed randomly into five treatment groups which were fed on a diet containing 'probiotic mix' in four concentrations (0.25, 0.5, 0.75 and 1.0 g 100 g^{-1} of diet). The control group was fed on a diet without supplementation of probiotic mix for the same duration. All the diets were isocaloric and isonitrogenous. All fish were fed daily @ 5% of the body weight per day in two equal instalments. The feeding rate was kept at 5% body weight day^{-1} for the whole rearing period of 70 days, and the amount of feed was adjusted every tenth day following a bulk weighing of each group of fish. All diets contained about 40% of crude protein. Significantly ($P<0.05$) high growth performance (percent gain in BW, SGR and length), were observed in the group fed diet containing probiotic mix at a concentration of 0.75 g 100 g^{-1} of diet. Similarly, accumulation of carcass protein, apparent protein digestibility, nutrient retention (PER, GPR, GER and APD) and digestive enzyme activity were also significantly ($P<0.05$) higher at this dietary inclusions of probiotic mix (0.75 g 100 g^{-1}). Excretion of metabolites remained lower, while the values of VSI and HSI remained higher in this treatment. Muscle glycogen and liver glycogen remained significantly ($P<0.05$) lower, while the values of muscle protein were significantly ($P<0.05$) higher in fish fed diet-3 containing probiotic mix at a concentration of 0.75g, 100g^{-1} of diet. These results suggest that supplementation of diets with appropriate concentration of probiotics could be used effectively for economic gains in aquaculture.

Keywords: Growth performance, Intestinal enzymes, probiotic, Nile tilapia, Spilac

1. Introduction
Probiotics are cultures of special microorganisms, which have been used as feed additives that improve the health of terrestrial and aquatic livestock. These when administered in adequate amount beneficially affect the host by improving its microbial balance [1]. The probiotics may be added to feed as live microorganisms to create a balanced indigenous microfloral community in the gastrointestinal tract. Most probiotics are supplied as live supplements in food, which must have the ability to survive passage through the intestinal tract [2]. The benefit to the host may arise as a nutritional effect, whereby the bacteria are able to breakdown toxic or otherwise non-nutritious components of the diet, which the host can then digest [3]. Alternatively, the probiotic may prevent potential pathogens from colonizing the gut by production of antimicrobial compounds, or by out competing them for nutrients or mucosal space [3].

In the search of new options, several studies have been carried out to test new compounds, from which the aquaculture industry has developed the concept of "functional additives". Among the various additives employed in fish nutrition, additions of microorganisms to diets, named probiotics, has been shown to improve the energy expenditure derived from other sources such as carbohydrates and increase the incorporations of protein for growth; increase the immunity and disease resistance of host organism**.** The application of probiotics in aquaculture has now been widely used as a means of controlling disease, enhancing immune

response, providing nutritional and enzymatic contributions to the digestion of the host, and improving water quality as they are considered to be as environmentally friendly [4]. The use of probiotics, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment [5].

Aquaculture is a fast-growing and rapidly expanding multibillion dollar industry. The availability of feed for aquaculture is a significant challenge in intensifying aquaculture industry, as feed accounts for up to 70% of operating costs for most aquaculture species [6]. Feed quality and feeding methods, therefore, need to be thoroughly considered in order to improve growth performance and feed efficiency of the cultured animals. Several previous reports have suggested that probiotic supplementation can reduce disease outbreaks by enhancing the immune system of fish and shrimp [7-9], and can decrease culture costs by improving the growth and feed efficiency of fish [10, 11]. Although scientists have demonstrated beneficial effects of using probiotics as fish feed additives, information on the interaction among probiotic species, digestive enzymes and probiotics in fish diets is very scarce. Some information on the effect of probiotics for Indian major carps and other indigenous and exotic fishes of major economic importance is available [12, 13].

The present study was designed to evaluate the effect of dietary probiotics (commercially available as SPILAC) containing *Lactobacillus* sp. as one of the component at various inclusion levels on growth performance, digestibility, activity of digestive enzymes, excretion of metabolites in *Oreochromis niloticus*.

2. Materials and Methods

2.1 Source of Probiotics

The 'probiotic mix' for inclusion in the fish diet was obtained commercially from Gubyro Chemicals Mumbai with trade name SPILAC. Each kg of this probiotic mix contained - *Lactobacillus sporogenes*-33,200 cfu; Liver extract-5320 mg; Yeast extract (*Saccharomyces cerevisiae*) 1250mg. Alpha amylase-5000mg. SPIRULINA-5320mg. Ascorbic acid coated-500 mg. Xanthophyll-9000 µg. Chlorophyll-9500 µg. GLA-38000 µg. Protein-100 gm.

2.2 Diet preparation

Four experimental diets containing varying concentrations of probiotic mix (0.25, 0.5, 0.75 and 1.0%) were formulated using processed full fat soybean as the protein source. A diet without supplementation of probiotic mix was also formulated which served as the control diet. Dietary ingredients and proximate composition of the formulated diets are given in Table 1.

Table 1: Ingredient content (%) and proximate analysis (% dry weight basis) of five experimental diets with different levels of probiotics (g $100g^{-1}$ of diet)

Ingredient	Diets				
	D0 (control)	D1	D2	D3	D4
Groundnut oil cake	65.00	65.00	65.00	65.00	65.00
Rice bran	3.20	2.95	2.70	2.45	2.20
Wheat flour	3.20	3.20	3.20	3.20	3.20
Processed soybean[a]	26.60	26.60	26.60	26.60	26.60
Chromic oxide (Cr_2O_3)	1.00	1.00	1.00	1.00	1.00
Calcium	0.50	0.50	0.50	0.50	0.50
Phosphorus	0.50	0.50	0.50	0.50	0.50
Probiotic mix[b]	-	0.25	0.50	0.75	1.00
Proximate analysis % (analysed values) Parameters					
Dry matter	83.18±0.01	93.17±0.01	93.17±0.01	93.18±0.02	93.17±0.02
Crude protein	39.70±0.28	39.70±0.16	39.81±0.17	39.59±0.22	39.87±0.32
Crude fat	8.13±0.26	8.38±0.35	8.06±0.33	7.94±0.44	7.88±0.35
Crude fibre	5.81±0.21	6.00±0.19	5.75±0.13	5.75±0.19	5.75±0.19
Ash	6.38±0.13	6.44±0.15	6.56±0.18	6.69±0.19	6.56±0.24
Nitrogen free extract (NFE)	33.17±0.37	32.65±0.40	32.99±0.37	33.22±0.38	33.12±0.55
Phosphorus	0.60±0.02	0.59±0.02	0.64±0.02	0.67±0.02	0.63±0.02
Gross energy (kJ g^{-1})	18.29±0.10	18.30±0.09	18.26±0.10	18.19±0.13	18.22±0.11

[a] Soybean was hydrothermally processed in an autoclave at 121 ºC (15lbs for 15 minute) to eliminate antinutritional factors (ANFs) (Garg *et al.,* 2002) [b] Probiotics (SPILAC) : Each kg contains-*Lactobacillus sporogenes*-33,200 cfu. Liver extract-5320mg. Yeast extract (*Saccharomyces cerevisiae*) 1250mg. Alpha amylase-5000mg. SPIRULINA-5320mg. Ascorbic acid coated-500 mg. Xanthophyll-9000 g. Chlorophyll-9500 g. GLA-38000 g. Protein-100 gm.

2.3 Experimental design

Nile tilapia, *Oreochromis niloticus* fry were collected from the fish farm attached to the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar. Experiment was conducted in glass aquaria (60×30×30 cm) with aeration facilities in the laboratory where the temperature was kept as 25±1 ºC and a lighting schedule of LD 12:12. The salinity of the water in the aquaria was kept at 10.0 ppt.

2.4 Studies on growth and dietary performances

After an initial 10-day acclimation period, fish fry (mean body weight: 1.22±0.04g) were randomly distributed among the aquaria, with 20 fish per aquarium. Each diet treatment was tested in replicate of four (four aquaria per diet). All fish were fed twice daily, at 08:00h and at 14:00 h. The feeding rate was kept at 5% body weight day^{-1} for the whole rearing period of 70 days, and the amount of feed was adjusted every tenth day following a bulk weighing of each group of fish. The fish were exposed to their respective diet for 4h during each ration, thereafter; the uneaten feed was siphoned out, stored and dried separately for calculating the feed conversion ratio (FCR). The faecal matter voided by the fish in each aquarium was also collected by siphoning, dried in a hot air oven (60 ºC) and subsequently analysed for digestibility estimations. The water in the aquaria was renewed daily with water that had been stored and adjusted to the laboratory temperature (25 ºC). At

the termination of the experiment, fish from all the treatments were weighed (length was also recorded) individually to the nearest gram and processed for subsequent analyses. Eight fish were obtained from each aquarium and kept on an ice tray and the viscera of the fish were extirpated for the calculation of the viscero-somatic index (VSI). Liver was removed for calculating hepatosomatic index (HIS) and also for the estimation of liver glycogen [14]. Muscle was extirpated and used for the estimation of muscle [15]. Intestine was processed for the determination of protease [16] and amylase [17] lipase and cellulase enzyme activity [18].

2.5Analytical techniques

The feed ingredients, experimental diets, faecal matter samples and fish carcass (initial and final) were analysed following AOAC [19]. Cr_2O_3 levels in both the diets and the faecal samples were estimated spectrophotometrically following the method of Furukawa and Tuskahara [20]. Water quality parameters like temperature, Carbon dioxide, Alkalinity, Hardness and Ammonia were recorded at weekly intervals during the entire experimental duration of 70 days by using standard methods of APHA [21], pH and dissolved oxygen were monitored using an automatic analyser (Model F-set-3; Merck, Germany). At the end of the feeding schedule, water samples from each aquarium were collected at 2-h intervals over a 24-h period and used for estimating [21] the excretory patterns of total ammonia (NH_4-N) and reactive phosphate (o-PO4) production; calculations were made following [22].

Daily ammonia and orthophosphate production (DP) rates (mg day^{-1}) were estimated by summing the values obtained at two-hour intervals over a period of 24 h. The quantity of nitrogen and phosphorus excreted by the fish in the aquaria water was calculated as follows:

$$\text{Total N-NH}_4\text{/o –PO}_4 \text{ excretion (mg kg}^{-1}\text{BW 2h}^{-1}\text{)} = \frac{[(\text{N-NH}_4\text{/o -PO}_4)_{120}-(\text{N-NH}_4\text{/o -PO}_4)_0] \times a}{\text{Fish biomass/ kg}}$$

$(N\text{-}NH_4/o\text{ -}PO_4)_0$ and $(N\text{-}NH_4/o\text{ -}PO_4)_{120}$ = concentration at times 0 and 120 min (2h) post feeding

a = amount of holding water (L) in which fishes were kept.

Live weight gain (in grams), growth percentage gain, specific growth rate [% body weight (BW) per day], feed conversion ratio (FCR), gross protein retention (GPR) and gross energy retention (GER) were calculated using standard methods [23]. Apparent protein digestibility (APD) of the diets was calculated according to [24] as follows:

$$\text{Apparent Protein Digestibility} = 100 - \frac{100\% \text{ marker in diet}}{\%\text{marker in faeces}} \times \frac{\%\text{ nutrient in faeces}}{\%\text{nutrient in diet}}$$

Gross energy content of the diets and fish were calculated using the average caloric conversion factors of 0.3954, 0.1715 and 0.2364 kJ g-1 for lipid, carbohydrate and protein, respectively [25].

2.6 Statistical Analysis

ANOVA followed by Duncan's multiple range test [26] and student 't' test [27] were applied to find out the significant differences between different treatments SPSS version 11.5 for windows. Data were further subjected to orthogonal polynomials for trend analysis.

3. Results

3.1 Fish growth, digestibility and nutrient retention

No disease was encountered during the experimental period of 70 days. Survival was not affected by the inclusion levels of probiotic mix. Growth performance [(in terms of live weight gain (Fig.1), growth percent gain in BW and final length), SGR (Fig.2) and nutrient retention (PER, GPR, GER)] increased when dietary probiotics level were increased from 0.25g to 0.75g 100 g^{-1} of diet; further increase in dietary probiotics level (>0.75g 100g^{-1}) resulted in a significant ($P<0.05$) growth depression and nutrient depletion. Apparent protein digestibility (Fig. 3) was significantly ($P<0.05$) higher in fish which were fed diets containing probiotics at 0.75g 100 g^{-1} than in fish fed control diet (Control) or diets containing low or high levels of *Lactobacillus.* FCR values were also significantly ($P<0.05$) lower in fish fed diet containing *Lactobacillus* at 0.75g 100g-1 than fish fed other dietary preparations including control diet (Table 2).

Table 2: Effect of different levels of probiotics supplement on growth performance, digestibility, nutrient retention and excretion of metabolites in *Oreochromis niloticus* fry under laboratory conditions (LD 12:12 at 25±1 ºC) –70 days treatment

Parameters	Diets				
	D0 (control)	D1	D2	D3	D4
Initial weight (g)	1.22±0.02a	1.29±0.04a	1.23±0.01a	1.15±0.03a	1.20±0.02a
Initial length (cm)	4.00±0.05a	4.25±0.06a	4.14±0.07a	4.09±0.06a	4.20±0.06a
Final weight (g)	3.02±0.03e	3.53±0.02d	5.44±0.06b	6.79±0.24a	4.13±0.05c
Final length (cm)	5.77±0.07d	6.12±0.10cd	6.87±0.12b	7.72±0.12a	6.19±0.09c
Live weight gain (g)	1.80±0.02e	2.23±0.03d	4.21±0.06b	5.64±0.24a	2.93±0.06c
Growth (% gain in BW)	147.19±2.16d	174.20±7.26d	341.63±7.16b	491.21±25.59a	243.61±7.31c
Specific growth rate (SGR)	1.29±0.01e	1.44±0.04d	2.12±0.02a	2.53±0.06b	1.76±0.03c
Feed conversion ratio (FCR)	2.07±0.08a	1.99±0.05a	1.95±0.05a	1.74±0.08b	2.04±0.08a
Gross energy retention (GER)	19.97±0.96b	20.76±0.49b	21.47±0.70b	24.65±0.92a	20.49±1.07b
Gross protein retention (GPR)	24.86±1.09b	25.93±0.79b	27.33±0.85b	31.81±1.32a	25.46±1.11b
Protein efficiency ratio (PER)	1.23±0.05b	1.27±0.03b	1.30±0.03b	1.47±0.06a	1.24±0.05b
Apparent protein digestibility (APD%)	80.07±0.19d	81.28±0.26c	83.58±0.20b	85.37±0.36a	81.12±0.20c
Total ammonia excretion (mg kg^{-1} BW day^{-1})	1349.83±6.31a	1008.75±8.95b	621.46±2.11d	534.55±5.98e	799.45±7.54c
Total phosphate production (mg kg^{-1} BW day^{-1})	244.42±0.24a	176.99±11.26c	144.84±4.44d	143.28±3.12d	199.77±4.84b

All values are mean±SE of mean.

Means bearing different letters in the same row differ significantly ($P<0.05$)

D0:No probiotic, mix, D1: Probiotic mix at 0.25 g 100 g^{-1} of diet, D2: Probiotic mix at 0.5 g 100 g^{-1} of diet,

D3 Probiotic mix at0.75 1.0 g 100 g^{-1} of diet, D4: Probiotic mix at 1.0 g 100 g^{-1} of diet

A review of Figs 1, 2 and 3 indicate a positive relation between the concentrations of probiotics in the diets and fish growth. Fish fed diets containing low concentrations of probiotics had the lowest growth and the optimum dietary probiotics estimated using broken line method was approximately 0.75g 100 g^{-1}. A trend line fitted to means showed a similar level.

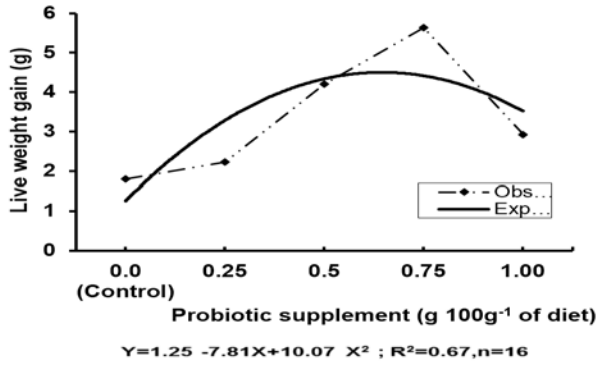


Fig 1: Relationship between dietary probiotics supplement level (0.0, 0.25, 0.50, 0.75 and 1.0 g 100g^{-1} of diet) and live weight gain in *Oreochromis niloticus* fry. Where, Y= Live weight gain (g), x = Probiotics supplement levels (g 100g^{-1} of diet), R^2 = Coefficient of determination, n = Number of observations (16).

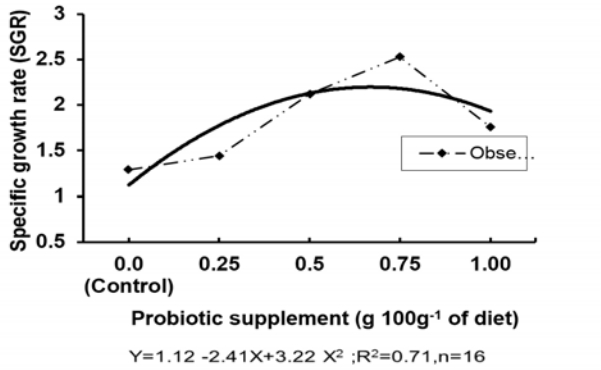


Fig 2: Relationship between dietary probiotics supplement level (0.0, 0.25, 0.50, 0.75 and 1.0 g 100g^{-1} of diet) and specific growth rate in *Oreochromis niloticus* fry. Where, Y=Specific growth rate (SGR), x = Probiotics supplement levels (g 100g^{-1} of diet), R^2 = Coefficient of determination, n = Number of observation (16).

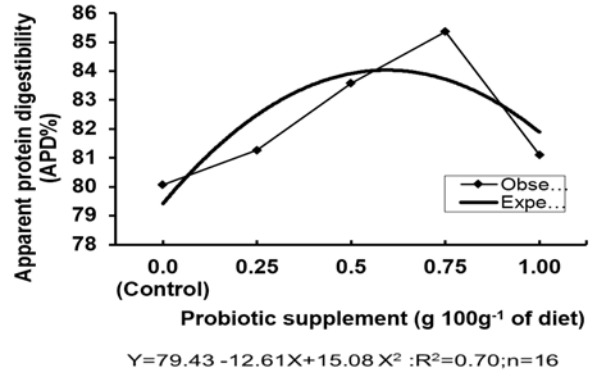


Fig 3: Relationship between dietary probiotics supplement level (0.0, 0.25, 0.50, 0.75 and 1.0 g 100g^{-1} of diet) and apparent protein digestibility in *Oreochromis niloticus* fry. Where, Y=Apparent protein digestibility (APD %), x = Probiotics supplement levels (g 100g^{-1} of diet), R^2 = Coefficient of determination, n = Number of observations (16).

3.2 Water quality and postprandial excretory levels of total ammonia (N-NH4) and reactive phosphate (o-PO4)

Water quality parameters in all the experimental aquaria remained within optimal range allowing for high growth rate of *O niloticus* (data not shown).

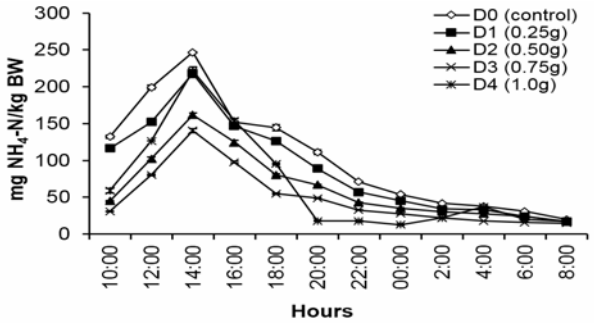
Total ammonia excretion and reactive phosphate production were significantly lower ($P<0.05$) in fish fed probiotics at 0.75g 100 g^{-1} of diet than the fish groups fed on other dietary preparations or control diet (Table 2). Irrespective of the probiotics level N-NH_4 excretion showed a peak at 6 h post-feeding, while o-PO4 production showed an initial high level at 2 h post-feeding and a peak between 14 and 16h post-feeding (Table 2 and Figs. 4A and B).

3.3 Fish carcass composition

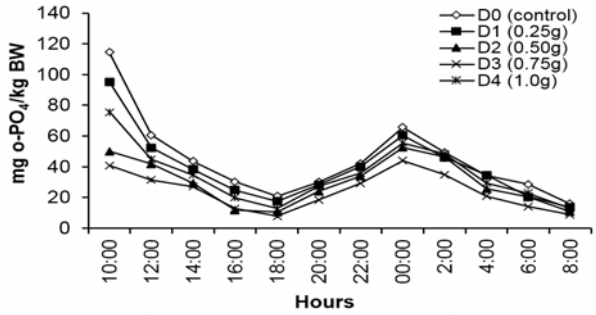
The body composition of the fish was also affected by the probiotics concentrations in diets. The accumulation of carcass protein, fat, phosphorus and gross energy were significantly ($P<0.05$) higher in groups fed diets containing probiotics at 0.5 to 0.75 g 100g^{-1} of diet. Carcass ash contents remained significantly ($P<0.05$) low at 0.75 g of probiotics level (Table 3).

3.4 Digestive enzyme activity, muscle and liver glycogen, muscle protein, VSI and HSI

In general, total and specific activity of digestive enzymes (Protease, amylase, lipase and cellulase) remained significantly ($P<0.01$) higher in fish fed diet containing *Lactobacillus* at 0.75g 100 g-1 of diet in comparison with other treatments and control. VSI and HSI values were also significantly ($P<0.05$) higher in this treatment. Muscle protein was significantly high, while, muscle and liver glycogen levels remained significantly low in fish fed at 0.75g of probiotic mix at 100g^{-1} of diet (Table 4).



(A)



(B)

Fig 4: Effect of different levels of probiotics supplement on postprandial patterns of total ammonia (A) and orthophosphate (B) excretion (mg kg^{-1} BW) in *Oreochromis niloticus* fry in holding water. All values are mean±SE of mean of eight observations

Table 3: Effect of different levels of probiotics supplement on proximate composition (% wet weight basis) in *Oreochromis niloticus* fry under laboratory conditions (LD 12:12 at 25±1 °C) -70 days experiment

Carcass composition (%)	Initial value	Diets				
		D0 (control)	D1	D2	D3	D4
Moisture	71.26±0.17	68.21±0.03a	67.85±0.12b	67.23±0.03d	66.80±0.07e	67.62±0.03c
Crude protein	15.31±0.35	18.26±0.26c	18.56±0.27c	19.79±0.37ab	20.56±0.28a	19.03±0.36bc
Crude fat	2.74±0.04	3.78±0.03d	3.82±0.03cd	3.97±0.03b	4.08±0.03a	3.89±0.03bc
Ash	2.85±0.06	3.42±0.02a	3.34±0.02a	3.21±0.02b	3.01±0.05c	3.25±0.03b
Phosphorus	0.59±0.02	0.60±0.02b	0.59±0.02b	0.64±0.02ab	0.67±0.02a	0.63±0.02ab
Gross energy (kJ g^{-1})	5.97±0.03	6.90±0.01e	7.00±0.02d	7.24±0.03b	7.42±0.04a	7.10±0.03c

All values are mean±SE of mean
Means bearing different letters in the same row differ significantly (P<0.05)
D0:No probiotic, mix, D1: Probiotic mix at 0.25 g 100 g^{-1} of diet, D2: Probiotic mix at 0.5 g 100 g^{-1} of diet,
D3 Probiotic mix at0.75 1.0 g 100 g^{-1} of diet, D4: Probiotic mix at 1.0 g 100 g^{-1} of diet

Table 4: Effect of different levels of probiotics supplement on muscle protein, muscle glycogen, liver glycogen, enzymatic activities (protease, amylolytic, cellulase and lipase) and viscero-somatic index (VSI) and hepato-somatic index (HSI) in *Oreochromis niloticus* fry under laboratory conditions (LD 12:12 at 25±1 °C) – 70 days treatment

Parameters	Diets				
	D0 (control)	D1	D2	D3	D4
Muscle glycogen (mg g^{-1})	1.33±0.02c	1.75±0.03a	1.73±0.03a	1.32±0.03c	1.45±0.02b
Liver glycogen (mg g^{-1})	2.58±0.05a	2.38±0.04b	2.23±0.03c	1.87±0.03d	2.56±0.05a
Muscle protein (mg g^{-1})	110.85±1.89c	116.99±1.29b	121.03±1.40b	133.88±2.59a	118.00±1.75b
Total protease enzyme activity (mg g^{-1} h^{-1})	3.51±0.07d	4.34±0.05c	5.44±0.06b	6.32±0.09a	5.27±0.06b
Specific protease enzyme activity[1]	1.17±0.04d	1.52±0.07c	2.35±0.06a	2.42±0.14a	1.98±0.08b
Total amylase activity (mg g^{-1} h^{-1})	0.22±0.04e	0.24±0.004d	0.30±0.005b	0.38±0.004a	0.28±0.006c
Specific amylase activity[2]	0.12±0.02b	0.13±0.21a	0.15±0.003b	0.17±0.004b	0.12±0.003b
Total lipase activity (mg g^{-1} h^{-1})	0.09±0.01d	0.17±0.01c	0.37±0.03b	0.06±0.03a	0.25±0.03c
Specific lipase activity[3]	0.05±0.009b	0.08±0.008b	0.13±0.01b	0.56±0.03a	0.11±0.01b
Total cellulase activity (mg g^{-1} h^{-1})	0.93±0.03c	1.13±0.02c	1.36±0.02b	1.60±0.05a	1.32±0.11b
Specific cellulase activity[4]	0.43±0.03d	0.51±0.04d	0.80±0.04b	0.95±0.02a	0.70±0.04c
Viscero-somatic index (VSI)	8.63±0.29d	9.00±0.20cd	10.75±0.42b	13.36±0.26a	9.92±0.46bc
Hepato-somatic index (HSI)	1.74±0.16c	1.65±0.04c	2.17±0.04b	2.81±0.13a	1.81±0.06c

[1] mg of tyrosin liberated/mg of protein/minute
[2] mg of maltase liberated/mg of protein/minute
[3] micromole fatty acid liberated/mg of protein/hour
[4] mg of glucose liberated/mg of protein/minute
All values are mean±SE of mean
Means bearing different letters in the same row differ significantly (P<0.05)
D0:No probiotic, mix, D1: Probiotic mix at 0.25 g 100 g^{-1} of diet, D2: Probiotic mix at 0.5 g 100 g^{-1} of diet,
D3 Probiotic mix at0.75 1.0 g 100 g^{-1} of diet, D4: Probiotic mix at 1.0 g 100 g^{-1} of diet

4. Discussion

4.1 Fish growth, digestibility and nutrient retention

The survival of *O. niloticus* in all the treatments was excellent. In the present, studies no obvious effect of the probiotic mix added to feeds on water quality was observed. Supplementation of probiotics in the experimental diets resulted in higher growth and feed utilization as compared to control. These findings are in agreement with freshwater species such as Nile tilapia, *Oreochromis niloticus* and carp, *Cyprinus carpio* [28]. Similar response in growth performance in *Cyprinus carpio* has also been shown by other workers [13, 29]. Similar response for marine fish species such as red drum, *Sciaenops ocellatus* [30] and Japanese flounder, *Paralichthys olivaceus,* juveniles [31] has also been reported**.** The optimum probiotics levels which resulted in high growth in *O. niloticus* in terms of live weight gain (grams), growth percentage gain, SGR and nutrient retention (PER, GPR, GER and APD) was found to be around 0.75g 100 g^{-1} of diet. FCR values decreased with each increase in the dietary probiotics contents of the diet upto 0.75 g 100g^{-1} of diet, thereafter, an increase in dietary probiotics levels resulted in an increase in FCR values. Highest carcass protein and lipid contents in *O niloticus* also coincided with higher growth. These results are similar to those observed by Bandyopadhyay and Mohapatra [12] in *Catla catla* when fed on diets containing probiotic bacterium *Bacillus circulans* PB7. A depression in growth parameters was observed at higher inclusion levels.

Although, all the feeds were isonitrogenous but the concentration of probiotic mix in dietary treatment D3 might have been helpful for proper nutrient utilization. Better body carcass composition and lesser nitrogen and phosphate excretion were also observed in dietary treatment D3 (Probiotic mix supplemented at the rate of 0.75g 100 g^{-1} of diet) which is attributed to proper probiotic concentration, whereas lesser carcass composition and greater nitrogen and phosphate excretion were observed in dietary treatment D4 (Probiotic mix at 1.0g 100 g^{-1} of diet) which could have also noticed to the overall low feed utilization level. These studies indicate a definite relationship of dietary probiotic level and the growth performance of fish species and their nutritive physiology for optimum growth of *O. niloticus.* Studies of [32] have also demonstrated a similar dose dependent response for *Catla catla, when* gut isolated probiotic *Bacillus coagulans* was incorporated in the diet. Ghosh [33] with *B. circulans* as probiotics in feed for *Labeo rohita* fingerlings and Ringpipat [34] with *Bacillus sp.* as probiotics in *Penaeus monodon* also reported similar results.

4.2 Activity of the digestive enzymes, muscle and liver glycogen, muscle protein, HSI and VSI

The nutritional value of the diet depends on the digestive capabilities of the fish, which in turn is affected by the activity of the digestive enzymes present in the digestive tract [35] The activities of the digestive enzymes (protease, amylase, lipase and cellulase) were high, in the group of fish fed on a diet having a probiotics concentration of 0.75g 100 g^{-1} of diet which may be due to better dietary protein utilization due to colonization of probiotics bacteria and its exogenous enzyme production. Most of the amino acids normally found in protein undergo transamination reaction and transaminases are localized in both cytosol and mitochondria [36] which is induced by high protein diet however, in the present studies the diets were isocaloric and even then growth rate and digestive physiology varied. Enzyme activity increased with increasing dietary probiotics levels, while at high probiotics concentration (1.0g 100 g^{-1} of diet) a decrease in their activity was observed. These results indicate that probiotics when incorporated at optimal levels to the diet stimulate the digestion through the supply of digestive enzymes and certain essential nutrients to the animals.

Probiotics are known to improve enzymatic activity in the gut by producing several enzymes not produced by the host. Similar observations were also reported by Swain [37]. These authors have further reported that yeast in the diet improves feed efficiency, organic phosphorus (phytic acid) utilization and fibre digestion. Improvement of feed utilization in fish fed diet supplemented with probiotics could also be due to improvement in intestinal microbial flora balance which in turn might have led to better nutrient digestibility, higher absorption quality, and increase in enzyme activities [38, 39] and also more degradation of higher molecular weight protein to lower molecular weight peptides and amino acids [40].

Microorganisms and their enzymes have an important role in the digestion processes they increase the total enzyme activity of the gut [41]. Similar results were also reported by [33] using *Bacillus circulans* as probiotics in *Labeo rohita* fingerlings and Rengpipat [34] using *Bacillus* sp. S11 as probiotics in *Penaeus monodon*. Renuka [13] used *Lactobacillus* bacteria as probiotics in common carp diet and had obtained similar results. They also observed higher levels of amylase, protease and lipase in *Cyprinus carpio* fed *Lactobacillus* sp. probiotics could be used effectively as a probiotics for the use in aquaculture.

The results obtained in the present study support the use of probiotics for better growth and proper nutrient utilization. The finding further suggest that the concentrations of probiotics applied in formulated diet should be optimum to increase overall physiological performance like increase in intestinal enzymes along with growth parameters and decrease in excretion of metabolites in the holding water, thus, enhancing the defence mechanisms in the fish.

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