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Evacuation of Fish meal using Soy protein and Carbohydrate sources in diets of *Tilapia mossambicus* and its physiological alterations

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ABSTRACT:

Achieving factual sustainability in fish farming needs the addition of most of the fish meal exploited as feed stuffs. The current experiment described two feeding trials, that resulted in the complete replacement of fish meal in the fingerling of *Tilapia mossambicus*. The initial trial was accompanied with three stages of fish meal replacement (50, 75 and 100% of dietary protein) viz., one level of soy protein and two levels of Lactat ® Probiotic (0 (or) 0.3% of the diet). Since probiotic has been reported to promote gut health, it was incorporated inorder to examine the growth enhancement and whether it would ease high levels of fish meal replacement in *T. mossambicus*. Lipids were provided by Cod liver oil. The better weight gain was observed in the treatments 50/50$^+$ of fish meal replacement and 0/100$^+$ of fish meal replacement. The optimum Specific Growth Rate, Food Conversion Ratio and Survival were also observed in 50/50$^+$ fish meal replacement and 0/100$^+$ fish meal replacement. The higher serum Acetyl Choline, Leucocytes, and Erythrocyte were observed in 50/50$^+$ and 0/100 $^+$ than the other diets. The Lysozyme activity was higher in 0/100$^+$ and 50/50$^+$ than the other diets.

In the Second feeding trial, fish meal was replaced by various carbohydrate sources on the growth performance and hepatic carbohydrate metabolic enzyme activities of the fingerlings of *T. mossambicus.* Five experimental diets were formulated to contain glucose, sucrose, maltose, dextrin, corn starch and control were maintained separately. The results indicated that the better weight gain, SGR, FCR and survival were also better in starch, dextrin and sucrose diet fed fish. There were significant differences in the total plasma, glucose and triglyceride concentration in fish fed with different carbohydrate sources. Plasma total protein, red blood cell, leucocytes and hemoglobin were significantly affected by various carbohydrate sources. The activities of glucose 6-Phosphate dehydrogenase, (G6PD), 6- Phospho fructokinase (PFK) and fructose 1, 6 – bisphosphatase (FBase) were significantly affected by these carbohydrate sources. While this two feeding trail indicated that the 50/50$^+$ and 0/100$^+$ of soy flour replacement with fish meal showed the optimum growth performance and in carbohydrate sources the corn starch, dextrose, and sucrose showed the better growth for tilapia fingerlings.

Keywords:

Fish meal replacement, probiotic, *Tilapia mossambicus*, growth

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INTRODUCTION

Tilapia (*Tilapia mossambicus*) has a good potential for aquaculture in many tropical and subtropical regions (Fitzsimmons, 2000). Tilapia is the third greatest cultivated fish group in the biosphere, after carps and salmonids (FAO, 2004). Tilapia culture in farming activities is well known, with an normal yearly growth rate of 13.4% during 1970–2002. They are broadly cultured in about 100 countries in the tropical and subtropical counties. As a consequence, the production of cultivated tilapia has increased from 383,654 mt in 1990 to 1,505,804 mt in 2002, in lieu of about 6% of total farmed finfish in 2002 (FAO, 2004). Nourishment is the most expensive component in the challenging aquaculture industry, where it denotes over 50% of operational costs. Furthermore, protein itself signifies about 50% of feed cost in comprehensive culture. Therefore, the choice of proper measure and eminence of nutritional protein is a required tool for effective tilapia culture practices. The chief risk facing tilapia nutritionists in emerging countries is the progress of profitable, cost effective tilapia feedstuffs using locally accessible, cheap and eccentric resources (El-Sayed, 2004). Feed Cost increases and it has became a vital financial factor in the commercial production of Tilapia.

The alternative use of more economical, plant or animal protein ingredients are needed for the formulation of cost-effective fish diets. The most expensive factor in the prepared diets are protein and it should be more enough for the cultured animal (Abdel – Tawwab *et al*., 2007). A wide account of studies has pointed on evaluating the potential to reduce addition of fish meal in fish diets over the past four decades. (Cho *et al*., 1994; Wilson and Poe 1985; Fowler 1991). Endurable replacements for fish meal protein are most often from the plant origin, mainly the grains, pulses and oil seeds (Lunger *et al*., 2006; Gatlin *et al*., 2007). Soy bean meal has been one of the most studied options of fishmeal (Gatlin *et al*., 2007). Soybean meal has low crude protein levels, particularly when compared to fish meal. Hence for replacement of fish meal with other sources need high protein level. (Barrows *et al*., 2007).

Carbohydrate is the extra low-slung source of diet energy but is not well encouraged by many creatures (Philips *et al*., 1948). Carbohydrate utilization varies among fish species. The hybrid tilapia (*Oreochromis niloticus* X.O. aureus) grows better when they are fed with starch diet. Generally herbivorous and omnivorous fish have a higher capacity to utilize the dietary carbohydrate than carnivorous fish (Wilson 1994). This study establishes the use of soybean meal and carbohydrate sources in aqua feeds that can be accounted commercially feasible in terms of cost – effectiveness and also to study the growth performance, physiological alterations and enzyme activities in *T. mossambicus* fingerlings.

MATERIALS AND METHODS

Experimental Design: Healthy fingerlings of *T. mossambicus* were obtained from the river Thambiraparani, Tirunelveli (8.73000 N,7.770000 E). Fish were adapted in covered tanks for two weeks by nursing a diet comprising 30% of protein. After that they were separated and distributed in 18 plastic troughs of 100L. Each trough was well aerated and the temperature was 28 ±1^{o}C. Part of the water with fish excreta was siphoned out every day and replaced by pure aerated water.

Graph 1. Feeding Trial 2: Physiological Measurements of *O. mossambicus* fed with experimental diets

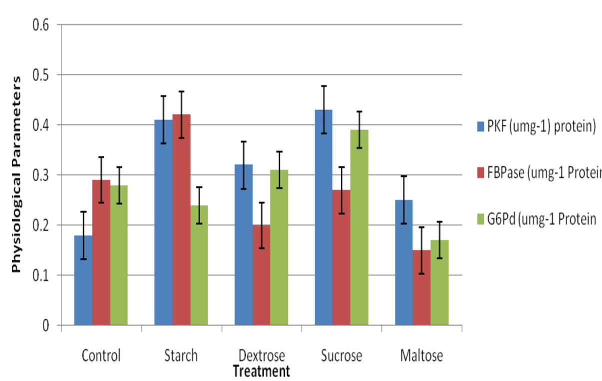

Table 1. Composition of experimental diets for feeding trial 1

Ingredients	Control	50/50	50/50+	25/75	25/75+	0/100	0/100+
Anchovy meal	63.8	31.9	31.9	16.0	16.0	0.0	0.0
Soy protein	0.0	32.9	32.9	49.4	49.4	65.9	65.9
Dextrose[d]	13.0	13.0	13.0	13.0	13.0	13.0	13.0
fish oil	4.7	7.2	7.2	8.4	8.4	9.6	9.6
Mineral premix [a]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin premix [b]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Amino acid mix	0.0	1.0	1.0	1.0	1.0	1.0	1.0
Lactact ® [c]	0.0	0.0	0.3	0.0	0.3	0.0	0.3
Wheat flour	9.5	6.0	4.7	4.2	2.9	2.5	1.2

[a]Mineral premix to supply the following elements (mg kg-1 diet): zinc (as sulphate) 72, iron (as sulphate), 36, manganese (as sulphate) 12,copper (as sulphate) 24, cobalt (as chloride) 0.6 ,iodine (as iodate) 1.2,chromum (trivalent, as chloride) 0.8, selenium (as selenate) 0.2, and molybdenum (as molybdate) 0.2.

[b]Vitamin premix was added to supply the following elements (mg/kg^{-1} diet): B12,0.1; nicotinic acid, 80.0:riboflavin,50; pantothenic acid ,180; menadione,40 folic acid, 6.0; biotin,0.6; thiamin hydrochloride, 15 ;pyridoxine, 60; thiamine ,40; inositol, 400; astaxanthin,60; choline chloride,20.0; vitamin C,250, and (IU) vitamin A,6000; vitamin D3,2000; vitamin E,6000 IU.

[c]Poseidon Biotech, Chennai, India

Feeding trial 1

Experimental feeds for the first feeding trial were produced as summarized in Table. 1 Seven diets were formulated and fed in triplicate, thus leading to the stocking of 21 tanks. All diets were formulated to provide 40% crude protein. The first feeding trial was designed as a 3 x 2 factorial with fish meal replacement level by soy bean meal as one factor (50,75 and 100% of dietary protein) and Lactact ® inclusion as the other factor (with and without). The diets were maintained isolipidic by increasing the levels of fish (cod liver) oil as fish meal levels decreased. All diets except the control were supplemented with an amino acid mix (Glysine, lysine and proline: 20/30/50 w/w/w, respectively). The remaining diets containing probiotic were formulated by adding Lactact ® (Poseidon Biotech, Chennai) a commercial product, at a level of 0.3% of the diet.

Feeding Trial 2

In the second feeding trial, a total of five iso nitrogenous and iso lipidic diets were prepared to contain 10, 20, 30 and 40% of carbohydrate content in Table 2, (15 tanks stocked). Experimental diets in this trial were formulated more similar to commercial formulations utilized for fingerling *T. mossambicus* under production conditions. The second feeding trial was designed as a fish meal replacement level by carbohydrate sources.

Experimental Diets and Control

All the dry ingredient powders were completely mixed and then lipid (fish oil and cholesterol) and water were added and mixed thoroughly. The 2.0 mm diameter pellets were wet extruded, air dried and packed in air tight container.

Experimental Animal

Fingerlings of *T. mossambicus* were obtained from Manju fish farm of Tirunelveli district. Before the experiment, the fish were acclimated and fed with commercial diet (35% crude protein and 6.2 crude lipids) for two weeks. At the beginning of the experiment, fish initial weight about (Trial 1: 3.7 g and Trial 2: 4.2g) were weighed and sorted into Trail 1: 21 tanks and Trial 2:15 tanks. Three replicate groups of fish were used for testing each diet. Fish were fed 4% of its body weight for thrice a day. The culture tanks were cleaned weekly, feeding trail 1 and 2 lasted for eight weeks. During the experimental period, temperature ranged from 28- 33^{o} C, pH from 7.0 – 7.1 and dissolved oxygen was not < 5.0 mg L-1was maintained. Every fortnight of the feeding trial, fish in each tank were weighed. At the end of the

Table 2. Composition of experimental diets for feeding trial 2

Ingredients	Control	Dextrose	Sucrose	Starch	Maltose
Fish meal	60	-	-	-	-
Wheat flour	35	35	35	35	35
Soyflour	0	45	45	45	45
Carbohydrate source[d]	0	15	15	15	15
Fish oil	0	1	1	1	1
Cholesterol[e]	0	1	1	1	1
Vitamin premix [a]	2	1	1	1	1
Mineral premix [b]	2	1	1	1	1
Monosodium phosphate[c]	1	1	1	1	1

[a]Mineral premix to supply the following elements (mg kg-1 diet): zinc (as sulphate)72,iron(as sulphate),36, manganese (as sulphate) 12,copper (as sulphate)24,cobalt (as chloride) 0.6 ,iodine (as iodate) 1.2,chromum (trivalent, as chloride)0.8, selenium(as selenite) 0.2, and molybdenum (as molybdate)0.2

[b]Vitamin premix was added to supply the following elements (mg/kg^{-1} diet): B12,0.1; nicotinic acid, 80.0: riboflavin,50; pantothenic acid ,180; menadione,40 folic acid, 6.0; biotin,0.6; thiamin hydrochloride, 15 ; pyridoxine, 60; thiamin ,40; inositol, 400; astaxanthin,60; choline chloride,20.0; vitamin C,250, and (IU) vitamin A,6000; vitamin D3,2000; vitamin E,6000 IU.

[c] Bentoli Agri Nutrition, U.S.A.

[d] Sigma-Aldrich, U.S.A.

8th week, the experimental fish were sampled for blood analysis. Blood was collected from the Caudal Veins of three fish from each tank and used to determine blood characteristics according to the method described by kikuchi *et al*.,(1994). Liver was frozen in freezer for conducting hepatic enzyme activities. Crude protein and lipid were analyzed in the dorsal muscle according to the standard methods (Association of Official Analytical Chemists 1995). The activities of fructose – 1,6 bisphosphatase (FBP ase), glucose -6- phosphate dehydrogenase (G6PD) and 6-Phospho Fructo Kinase (PFK) were analyzed as per the methodology followed by Meton *et al*., 2003; Zhang *et al.,* 2009. Complete enzyme activities were communicated in per mg of total protein (Specific activity). The total protein content in the crude extract was resoluted at 30^{o} C using bovine serum albumin as a typical based on the technique of Bradford (1976). One unit of enzyme activity was defined as the amount of NADH or NADPH generated by protein per/mg minute at 30^{o} C.

The parameters were calculated as follows:

$$\text{Specific growth Rate (SGR)} = \frac{\text{Ln Final weight-Ln Initial Weight}}{\text{Experimental duration}}$$

$$\text{Food Conversion ratio} = \frac{\text{Food Consumed (g, DW)}}{\text{Weight gain (g)}}$$

All data were subjected to one-way ANOVA. When there were significant differences, the group means were further compared using Duncan's multiple range tests. All Statistical analyses were performed using SPSS 15.0.

RESULTS

Feeding Trial 1

Weight gain, Specific growth rate, Food conversion ratio and Survival

Weight gain, specific growth rate, food conversion ratio and survival were all significantly affected by the fish meal replacement level in the experimented diets (Table 3). At the end of the first feeding trial the fingerlings of *T. mossambicus* fed diets showed the best Weight gain, FCR and SGR in the 50/50+ fish meal replacement when compared to the fish fed diets containing 100% fish meal replacement. Survival was also significantly higher in the fish fed with

Table 3. Feeding Trial 1: Growth performance of *O. mossambicus* fed with experimental diets

Dietary Treatment	I.W (g)	F.W (g)	W.G (g)	SGR (%/day)	FCR	Survival (%)
Control	3.24±0.07[a]	11.25±0.11[a]	8.01±0.21[a]	2.77±0.22[a]	2.33±0.02[a]	82%
50/50	3.39±0.08[a]	13.46±0.22[b]	10.07±0.22[b]	3.04±0.33[a]	1.73±0.05[a]	89%
50/50+	3.67±0.09[a]	16.28±0.93[c]	11.19±0.16[b]	3.30±0.24[a]	1.26±0.02[a]	100%
25/75	3.43±0.08[a]	13.33±0.29[c]	9.9±0.15[b]	3.02±0.36[a]	1.89±0.05[a]	89%
25/75+	3.37±0.02[a]	13.56±0.52[c]	10.19±0.56[b]	3.08±0.37[a]	1.56±0.03[a]	92%
0/100	3.36±0.03[a]	12.83±0.73[c]	9.46±0.33[b]	2.97±0.25[a]	2.01±0.05[a]	86%
0/100+	3.3±0.03[a]	13.96±0.72[c]	10.73±0.52[b]	3.24±0.38[a]	1.33±0.03[a]	98%

The mean values having different superscripts in the same row are significantly different at p<0.05 % level and ± indicates the standard deviation

50/50+ fish meal replacement diets. Addition of Lactact ® had some significant effects on weight gain, FCR, SGR and survival in this trial.

Immunological parameters

Fish meal replacement also had significant impacts on serum ACH, Leucocytes, Erythrocytes and Lysozyme activity. (Table 4) Fish fed on the 50% fish meal replacement which is significantly larger than the 50/50+ when compared to the other diets. Inclusion of Lactact® probiotic had significant effect on Serum Activity, Leucocytes Erythrocytes and Lysozyme activity.

Feeding Trial 2

Weight gain, FCR, SGR and Survival

In the second feeding trial, diet showed significant differences in the fish fed with the carbohydrate sources and control. Fish fed with starch, dextrose, sucrose were achieved significantly greater weight gain. *T. mossambicus* fed with other diets had the less weight gain compared to starch, dextrose and sucrose.

Specific growth rates were also significantly higher in the fish fed with starch, dextrose and sucrose compared to maltose and control diets. The least food conversion ratio was observed in starch and dextrose diets. Survival values were impacted significantly by diet following the trend similar to weight gain, SGR and FCR. The best survival was observed in starch and dextrose diets.

Physiological Measurements

6-Phosphofructokinase, fructose 1,6 bisphosphatase and Glucose 6-phosphate dehydrogenase of *T. mossambicus* were significantly affected by the dietary carbohydrate sources (Meton *et al.*, 2003; Zhang *et al.,* 2009) (Graph -1). Fish fed with sucrose had relatively higher PFK and G6PD. Fish fed with starch showed relatively higher FBPase activity compared to the other diets.

DISCUSSION

In the present study, the best growth performance, hepatic metabolic enzyme activities and physiological parameters were observed in fish meal replaced diet with soy bean meal and in fish fed with the starch and dextrose diet. Weight gain and the specific growth rate of fingerlings were positively related to soy bean meal and carbohydrate sources. Similar results were obtained on yellow tail (Furuichi *et al*., 1986) and (Salze *et al*., 2010) *Rachycentron canadum*. In the first feeding trial, *T. mossambicus* reacted well to fish meal replacement in 50/50+ and 0/0+ diet. These diets fed fishes showed better SGR, Weight gain, FCR and survival. Fish fed with control diet consisting of anchovy meal showed lower values than the experimental diets. The control diet was only with anchovy meal without soy flour, fish oil and amino acid mix. These other ingredients supplementation in experimental diets and replaced fish meal with soy flour caused a better performance in this study.

Table 4. Feeding Trial 1: Immunological Parameters of *O. mossambicus* fed with experimental diets

Treatments	Serum ACH	Leucocytes	Erythrocytes	Lysozyme activity
Control	38±3.02[a]	1.33±0.03[a]	1.52±0.2[a]	237±20[a]
50/50	63±5.3[a]	3.4±0.2[b]	1.93±0.1[b]	533±20[c]
50/50+	69±.7[a]	3.8±3.2[b]	1.97±0.2[b]	563±21[c]
25/75	42±4.1[a]	1.5±0.2[b]	1.73±0.1[b]	256±19[c]
25/75+	45±45[a]	1.7±0.9[b]	1.78±0.1[b]	273±20[c]
0/100	62±6.1[a]	2.3±0.1[b]	2.03±0.91[b]	551±23[c]
0/100+	65±6.3[a]	2.8±0.2[b]	2.13±0.93[b]	532±32[c]

The mean values having different superscripts in the same row are significantly different at p<0.05 % level and ± indicates the standard deviation

Use of probiotics is a new method to control potential pathogenic microorganisms (Gomez-Gil *et al*., 2000). Probiotic bacteria have an antagonistic activity against the pathogens in the intestinal tract and enhance the immune system (Gatesoupe, 1997). Probiotics not only improve the intestinal tract, but also improve the water quality by modifying the microbial community (Verschuere *et al*., 2000). The use of probiotic stimulates *Oreochromis niloticus* immunity by stimulating the physiological and biochemical parameters (Khattab *et al*., 2004). There was a significant increase were observed from Lactact® the dietary probiotic supplementation in the present experiment. When replacing 100% fish meal in the first feeding trial, a positive trend was observed in the fish fed with 50/50+. This diet fed fishes showed 100% survival and best SGR and followed by 0/100+ diet fed fishes showed 98% survival and better SGR. This clearly showed that it is due to the presence of fish meal, soy flour and probiotic in 50/50+ and second category is due to the major component as soy flour and probiotic. Our observations were supported by Manju *et al*., 2011 the incorporation of Efinol ® FG (mixed probiotics) in fish diet showed better growth performance in *Channa striatus*.

In the Second feeding trial, the best growth performance and feed utilization were observed in the fish fed with starch and dextrose. Specific growth rate, food conversion ratio and weight gain of *T. mossambicus* were marked effective by the carbohydrate sources. Similar results were observed in hybrid tilapia (Shiau and Chuang 1995) and yellow fin sea bream (Wu *et al*., 2006).

The main aspect of this study was the total elimination of fish meal by using soy flour and carbohydrate sources. In the second feeding trial, the concept has attained a desired level of performance by carbohydrate sources and soy flour. The best survival rate was observed in the starch and dextrose fed fishes. The same trend was also observed in Juvenile cobia fed with carbohydrate sources (Cui *et al*., 2010)

In the First feeding trial the immunological parameters such as serum complement activity, leucocytes, erythrocytes and lysozyme activity were observed in the fishes fed with experimental diets.

Lysozyme is one of the vital part in the immune defense system in both vertebrates and invertebrates and it can be fragmented peptidoglycan in bacterial cell walls particularly of the gram positive species and cause lysis of the cells (Chipman and Sharon, 1969). In our present study the feeding trial 1 consist of probiotic Lactact ® in the feed also enhances the lysozyme activity of *T. mossambicus* in fish. This report was supported by Panigrahi *et al*. (2004) who demonstrated that *Lactobacillus rhamnosus* JCM1136 could change the lysozyme activity in fish.

Our present study showed an increase in erythrocyte count in fish fed on replacement of fish meal and added with probiotic. This report was supported by

Table 5. Feeding Trial 2: Growth performance of *O. mossambicus* fed with experimental diets

Dietary Treatment	I.W (g)	F.W (g)	W.G (g)	SGR (%/day)	FCR	Survival (%)
Control	2.36±0.02[a]	4.21±0.13[a]	11.85±0.88[a]	4.00±0.13[a]	2.9±0.11[a]	95%
Starch	2.38±0.02[a]	15.36±0.22[b]	12.98±0.37[b]	4.15±0.13[a]	1.9±0.21[a]	100%
Dextrose	2.4±0.03[a]	15.00±0.21[b]	12.6±0.73[b]	4.0±0.12[a]	2.1±0.10[a]	100%
Sucrose	2.41±0.04[a]	14.7±0.15[b]	12.3±0.98[b]	4.02±0.12[a]	2.5±0.09[a]	98%
Maltose	2.38±0.03[a]	12.3±0.10[b]	9.92±0.33[b]	3.64±0.17[a]	3.5±0.11[a]	85%

The mean values having different superscripts in the same row are significantly different at $p<0.05$ % level and ± indicates the standard deviation

Irianto and Austin (2002), who recorded an increase in erythrocyte count in fish, fed on probiotic bacteria than control group.

The complement pathway activity was also higher in the fish fed with replacement diet incorporated with probiotic in our study. These results were correlated with Nikoskelainen *et al*., 2003 who showed elevated levels of complement activity observed in rainbow trout fed with *L. rhamnosus*. According to Leucocytes it also showed a significant increase in the number of fish fed with Lactact® and this report was supported by Villamil *et al*., 2002 in turbot larvae.

In the second feeding trial some key carbohydrate metabolic enzymes including PFK of the glycolysis pathway, FBPase and G6Pd were measured and found to be higher in starch fed fishes than the other diet fed fishes. Our results were in harmony with sturgeon juvenile by Hung *et al.,* 1989.

G6PD was higher in the fishes fed with sucrose diets than the other diets fed fishes. In contrast to these results, Enes *et al*., 2006 observed higher G6PD in Glucose fed diets. The activities of glucose 6-Phosphate dehydrogenase, (G6PD), 6- Phospho Fructokinase (PFK) and Fructose 1, 6 – Bisphosphatase (FBase) were significantly affected by these carbohydrate sources.

In conclusion, data from the present study the *T. mossambicus* adopted in two feeding trials has been successfully achieved in aqua feeds. The replacement of fish meal with soy flour and additionally with probiotic showed a better performance than other diets. This type of work is to reduce the feed cost and also to enhance the intestinal microflora of *T. mossambicus*. Because world wide *T. mossambicus* farms are there and it helps the fish farmers in increasing the yield of the fish weight by increasing their economic value. The next one using Carbohydrate sources with partial replacement of fish meal has also showed a better performance in *O. mossambicus.* The use of commodity traded plant based protein source as an optional to fish meal represents the economical endurability in the global aquaculture industry.

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