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Influence of supplementation of *Spirulina platensis* on nutritional, physiological and metabolic performance of GIFT Tilapia (*Oreochromis niloticus*)

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ABSTRACT

An eight weeks feeding trial was conducted to assess the influence of supplementation of *Spirulina platensis* on nutritional, physiological and metabolic performance of GIFT Tilapia (*Oreochromis niloticus*). Fingerlings (120; Initial body weight 3.4±0.01 g) were randomly assigned to four dietary treatments, viz. control (0%), T_1 (1%), T_2 (3%) and T_3 (5% *S. platensis* meal) in triplicates. The animals were fed with a ration at 5% body weight twice a day throughout the trial. The results indicated that, final body proximate composition, growth performance and nutrients digestibility coefficient were higher in T_2 group compared to other treatments and the control. At this level of inclusion, fish digestive enzymes, such as amylase, protease and lipase were significantly higher between control and treatments however, metabolic enzyme activities were not influenced by dietary treatments. The liver and intestinal histology were not affected by dietary treatments, however the intestinal tissues showed a higher intestinal villi length and muscular layer thickness than the control group. It can be concluded that, *S. plantesis* meal can be supplemented up to 3% without compromising the overall performance of GIFT tilapia.

Keywords: Digestive enzymes, GIFT tilapia, Nutrients digestibility, *Spirulina* meal

Tilapia species particularly GIFT strain plays an important role as a cheap source of protein for human consumption. The culture period is relatively short which is beneficial to the farmers and it has been demonstrated to have a capacity for utilizing unconventional feed ingredients such as microalgae during diet formulation (Wan *et al.* 2018). These unconventional feed ingredients/or alternative raw materials should increase digestion, lowering FCR, regulating microbiota in fish intestine, and enhancing immune system of the fish (Gültepe 2020).

Among non-conventional microalgae, *Spirulina platensis* has been reported to be a promising alternative supplement source of protein for tilapia species cultured. This is due to their valuable biochemical compounds such as antioxidant pigments and antimicrobial activity, high protein content, vitamin profile, essential fatty acids (gamma-linolenic acid) and high digestibility coefficient (Bin-Dohaish *et al.* 2018, Demelash 2018). Also, the algae can be incorporated at low quantity such as <20% in fish feeds to enhance appetite, growth performance and feed cost reduction (Mosha 2019). Therefore, this study aimed to assess the influence of additives of *S. platensis* and its effect on nutritional, physiological and metabolic performance of GIFT Tilapia (*Oreochromis niloticus*).

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MATERIALS AND METHODS

Experimental design and setup: The experiment consisted of four isonitrogenous (32%) and isocaloric (17 KJ/kg DM) diets formulated to replace fish meal with *S. platensis* meal at 0% (Control), 1% (T_1), 3% (T_2) and 5% (T_3) (Tables 1–3). Animals (3.4±0.01 g) were randomly stocked at a density of 10 fish per plastic trough (70 L capacity) in all experimental groups in triplicates, and fed 5% body weight in two rations for eight weeks.

Water quality parameters: Temperature (Pro 20, YSI-USA), dissolved oxygen (Pro 20, YSI-USA) and pH (Pro 20, YSI-USA) were monitored daily. Nitrite (NO_2-N) and ammonia (NH_4-N^+) were measured weekly once by titration method using spectrophotometer (Systronics, Ahmedabad, India) (APHA 2005).

Growth performance and survival: The growth parameters (final weight, average daily gain, feed conversion rate, protein efficiency rate, specific growth rate, and hepatosomatic index) and survival rate were calculated at the end of feeding trial according to Olvera-Novoa *et al.* (1990).

Proximate composition analysis of ingredients, feeds and animals: Dietary ingredients, experimental feeds and whole animals were subjected to proximate composition analysis.

Crude protein, ether extract, crude fibre, total ash, moisture and gross energy were analyzed according to AOAC (2005).

Metabolic enzyme activities: At the end of eight weeks trial, plasma protein was estimated according to Lowry *et al.* (1951). Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the blood plasma were assayed according to Reitman and Frankel (1957).

Digestive enzyme activities: Amylase, lipase and protease digestive enzymes were assayed at the end of feeding trial. Amylase activity was estimated by starch-hydrolysis method (Bernfeld 1955). Lipase and protease activity were assayed according to Cherry and Crandall (1932), and Snell and Snell (1971) respectively.

Table 1. Ingredients composition used in the experimental diets (g/kg)

Ingredient	Experimental diet			
	Control (0% SP)	T_1 (1% SP)	T_2 (3% SP)	T_3 (5% SP)
Fish meal	200.0	150.0	100.0	50.0
Spirulina meal	0	10.0	30.0	50.0
Soybean meal	220.0	220.0	280.0	340.0
Corn flour	340.0	380.0	350.0	320.0
Cassava starch	50.0	50.0	50.0	50.0
Groundnut oil cake	172.5	172.5	172.5	172.5
Methionine	2.5	2.5	2.5	2.5
Vitamin premix	5	5.0	5.0	5.0
Mineral premix	5	5.0	5.0	5.0
Chromium oxide (Cr_2O_3)	5	5.0	5.0	5.0

SP, *Spirulina* meal.

Table 2. Proximate composition of experimental feed ingredients

Ingredient	Proximate composition (%)				
	Crude protein	Ether extract	Crude fibre	Total ash	Moisture
Fish meal[a]	60.28	13.76	<1	14.83	10.62
Spirulina meal	58.37	2.35	1.99	7.12	8.80
Soybean meal[a]	47.17	1.52	5.30	7.46	5.39
Corn flour[a]	7.87	4.31	1.40	1.69	6.67
Cassava starch[a]	9	–	–	–	–
Groundnut oil cake[a]	39.21	10.11	5.35	3.77	17.65

[a]Ingredients obtained from local store, Ponneri, Tamil Nadu, India.

Nutrients digestibility: The chromium oxide for experimental diets and fecal samples were determined according to Bolin *et at.* (1952). The apparent digestibility coefficients (ADC) of dietary nutrients and gross energy were calculated by the method of Ngugi *et al.* (2017).

Histology: Liver and intestine tissues were collected and preserved in 70% formalin at the end of eight weeks feeding trial. The histological analysis of the tissues were performed in State Referral Laboratory for Aquatic Animal Health, Tamil Nadu, India, according to Yuji-Sado *et al.* (2015). The measurements of villi length and muscular layer thickness were performed using binocular microscope (Lawrence and Mayo, NLCD-120E) with a micrometer rule as described by Eyarefe *et al.* (2008).

Statistical analysis: Statistical analysis for all the parameters in the study was performed using Analysis of Variance (one-way ANOVA) followed by turkey's multiple range test at 5% ($P<0.05$) level to compare mean between the treatments. The results obtained were presented as mean±SE (standard error of mean). All calculations were performed using IBM SPSS Statistics V21 (IBM Cop. Armonk, New York, USA).

RESULTS AND DISCUSSION

The water temperature (28.60 to 30.20°C), dissolved oxygen (5.70 to 6.00 mg/L), pH (7.20 to 7.37), total ammonia (0.01 to 0.03 mg/L) and nitrite (0.001 to 0.002 ppm) were within the optimal mean ranges for tilapia species culture, and were similar to the previously reported by Plaza *et al.* (2018). This was achieved due to continuous aeration, regular water exchange (75%) and daily removal of un-eaten feeds as well as fecal matter from the experimental units.

The algae can be incorporated at low quantity (<20%) in fish feeds to enhance appetite and growth performance (Mosha 2019). In the present study, growth parameters (FW, ADG, PER and SGR) of the fishes were significantly ($P<0.05$) higher in T_2 group, while FCR value was significantly ($P<0.05$) lower in T_2 group (Table 4). This could be due to high digestibility of *S. platensis* which stimulates the intestinal flora of fish to increase the activity of digestive enzymes resulting in efficient nutrients absorption which in turn promotes growth. Also, the lower the FCR value in T_2 indicates the better and more efficient utilization of the diet which is used for growth and meet

Table 3. Proximate composition of experimental diets (g 100/g, as fed)

Parameter (%)	Experimental diet			
	Control (0% SP)	T_1 (1% SP)	T_2 (3% SP)	T_3 (5% SP)
Crude protein	32.10±0.05	31.96±0.01	31.84±0.04	31.83±0.06
Ether extract	2.92±0.01	2.87±0.01	2.85±0.01	2.90±0.02
Crude fibre	3.07±0.01	3.13±0.02	3.16±0.01	3.79±0.02
Total ash	7.87±0.02	7.58±0.02	6.67±0.01	7.21±0.01
Moisture	7.46±0.03	7.40±0.02	8.13±0.01	8.88±0.02
Gross energy (KJ/kg)	17.17±0.01	17.02±0.02	17.08±0.01	16.72±0.02

SP, *Spirulina* meal.

the nutritional requirements of tilapia fish species without compromise the intended effects. The results are in accordance to those reported in Nile tilapia, *O. niloticus* (Khalila *et al.* 2018) and Gurami, *Osphronemus gouramy* (Simanjuntak *et al.* 2018) when *S. platensis* supplemented in the diets at 3% inclusion level. In addition, the significantly (P<0.05) higher survival rate (SR) and Hepatosomatic Index (HSI) were observed in T_2 group, possibly due to accumulation of glycogen in the liver which provide energy to the fish and in hence survival (Cazenave *et al.* 2006, Raji *et al.* 2018). The finding was in collaboration with the results of Raji *et al.* (2018) in African catfish (*Clarias gariepinus*).

There were significantly (P<0.05) higher levels with respect to crude protein, ether extract and gross energy of whole fish body proximate composition among the supplemented groups, while the reverse trend was observed in crude fiber and moisture content (Table 5). The results revealed the efficient utilization and muscle's deposition rate of fish at 3% *Spirulina* inclusion level. This statement is supported by Abdel-Tawwab *et al.* (2006) who reported that, the changes in the fish body contents such as protein, can be linked to changes in the nutrients synthesis, deposition rate in muscle, and/or different growth rates. Similar results were observed in Nile tilapia (*O. niloticus*) (Bin-Dohaish *et al.* 2018) and Gurami (*Osphronemus gouramy*) (Simanjuntak *et al.* 2018) when fed with diets containing 3% *Spirulina*.

There was significantly (P<0.05) higher level with respect to plasma protein in T_2 compared to other treatments and the control (Fig. 1), possibly due to high protein conversion ratio which has been reported to be associated with stronger immune responses, effective utilization and digestibility of dietary nutrients which is reflected in the metabolic profile of fish tissues (Debnath *et al.* 2007). The finding was corroborated with results reported by Adel *et al.* (2016c) in great sturgeon, *Huso huso* when fed with diet supplemented with *Spirulina* meal. In addition, there were no difference with respect to alanine transaminase (ALT) and aspartate transaminase (AST) among the treatment groups (Fig. 1). The reason could be due to availability of the various antioxidant like tannins and

Table 4. Growth performance and survival of GIFT tilapia fed with experimental diets for eight weeks

Parameter	Experimental diet			
	Control (0% SP)	T_1 (1% SP)	T_2 (3% SP)	T_3 (5% SP)
Initial weight (IW, g/kg)	3.40±0.007^a	3.39±0.017^a	3.40±0.006^a	3.40±0.015^a
Final weight (FW, g/kg)	20.99±0.91^a	26.54±2.52^b	38.47±2.05^c	28.02±0.38^b
Average daily gain (ADG, g)	0.29±0.02^a	0.37±0.04^b	0.58±0.03^c	0.41±0.01^b
Feed conversion rate (FCR)	1.39±0.04^c	1.34±0.12^c	1.15±0.05^{ad}	1.22±0.03^{bd}
Protein efficiency rate (PER)	2.89±0.09^a	3.04±0.26^b	4.46±0.21^d	3.36±0.09^c
Specific growth rate (SGR, %)	3.03±0.07^a	3.35±0.08^b	4.04±0.09^d	3.51±0.02^c
Survival rate (SR, %)	80.33±3.33^a	90.00±4.77^d	93.33±3.67^c	83.33±3.82^b
Hepatosomatic index (HSI)	0.09±0.001^a	0.10±0.001^a	0.21±0.002^{cd}	0.19±0.001^{bd}

Values are means±standard error (n=30). Means with different superscripts letters within each row are statistically significant (P<0.05) (SP, *Spirulina* meal).

Table 5. Whole fish body proximate composition fed with experimental diets for eight weeks

Parameter	Experimental diet			
	Control (0% SP)	T_1 (1% SP)	T_2 (3% SP)	T_3 (5% SP)
Initial whole body composition (%)				
Crude protein	59.43±0.03^a	59.20±0.20^a	59.01±0.20^a	59.17±0.23^a
Ether extract	19.77±0.13^a	19.82±0.17^a	19.86±0.20^a	19.85±0.19^a
Crude fibre	0.97±0.03^a	0.90±0.06^a	0.93±0.03^a	0.90±0.06^a
Crude ash	11.08±0.22^a	11.04±0.10^a	11.07±0.22^a	11.09±0.21^a
Moisture	10.91±0.25^a	10.68±0.22^a	11.06±0.21^a	10.91±0.25^a
Gross energy (KJ/kg)	17.64±0.06^a	17.70±0.07^a	17.72±0.13^a	17.74±0.02^a
Final whole body composition (%)				
Crude protein	62.92±0.13^a	65.81±0.03^c	67.67±0.31^d	64.68±0.14^b
Ether extract	14.57±0.22^a	15.98±0.23^b	16.70±0.27^c	16.09±0.28^b
Crude fibre	1.00±0.06^d	0.70±0.05^b	0.60±0.06^a	0.77±0.03^c
Total ash	9.74±1.04^a	10.07±1.09^a	10.65±1.02^a	10.43±1.00^a
Moisture	9.84±2.99^b	3.81±0.55^a	3.73±0.31^a	4.00±0.44^a
Gross energy (KJ/kg)	20.61±0.25^a	22.31±0.11^b	23.34±0.18^d	22.87±0.11^c

Values are means±standard error (n=30). Means with different superscripts letters within each row are statistically significant (P<0.05). SP, *Spirulina* meal.

phenolic contents, which might stimulate biotransformation and detoxify oxidative stress products via catalysis of metabolites into substances that can be discharged more easily (Mithraja *et al.* 2011). Hence, maintain normal function in fish by accelerating the regenerative capacity of liver cells (Gültepe 2020). According to this study, *Spirulina* supplementation did not cause any significant liver damage in GIFT tilapia. Similar results have been reported in GIFT tilapia (Zou *et al.* 2017) and Great Sturgeon (*Huso huso*) (Yeganeh and Adel 2018) when fed with diets containing up to 10% *Spirulina* inclusion levels.

The activities of fish digestive enzymes provide insight into the possible effects of different diets on fish performance (Zou *et al.* 2017). In the present study, the mean value of amylase, lipase and protease activity was significantly (P<0.05) higher in T_2 group (Fig. 2), possibly due to an increase in algal protein assimilation as well as high impact on the lipoproteins when supplemented at an optimum level in the diets (Mustafa *et al.* 1995). The present finding was in agreement with those reported in catla, rohu and common carp (Nandeesha *et al.* 1998) when fed with *S. platensis* containing diets.

On the other hand, poor digestive activity at high algae inclusion levels (beyond 3%) in fish diets (T_3) have been reported due to presence of anti-nutritional factors (protease inhibitors and anti-vitamins) which might interfered with the digestibility and bioavailability of nutrients (Moutinho *et al.* 2018). Vizcaíno *et al.* (2019) reported the significantly lower (P < 0.05) protease activity in Senegalese sole (*Solea senegalensis*) when fed with diet containing 5% algae. The improvement of digestive enzyme activities in this study up to inclusion level of 3% *Spirulina* meal and subsequent decline provided a relative reasonable explanation to the better growth performance of the fishes at 3% incorporation.

There were significantly (P<0.05) higher level with respect to apparent digestibility coefficient (ADC) in T_2 group, while no significant differences in dry matter among all treatment groups (Table 6). The low ash and crude fiber

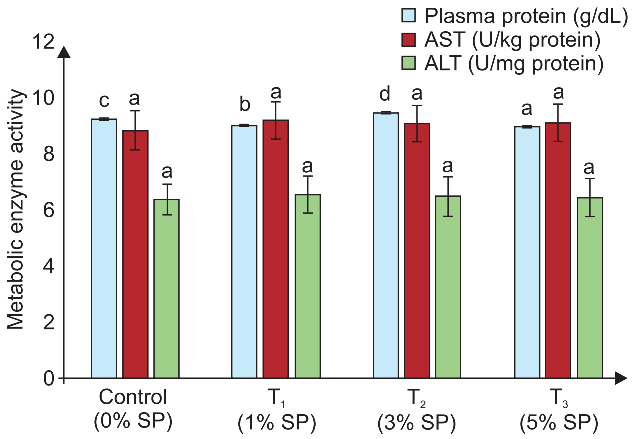

Fig. 1. Metabolic enzyme activity of GIFT tilapia fed with experimental diets for eight weeks. The bar with different superscript letters within the same treatment indicating the significant differences (P<0.05), (*n*=30) (SP, *Spirulina* meal).

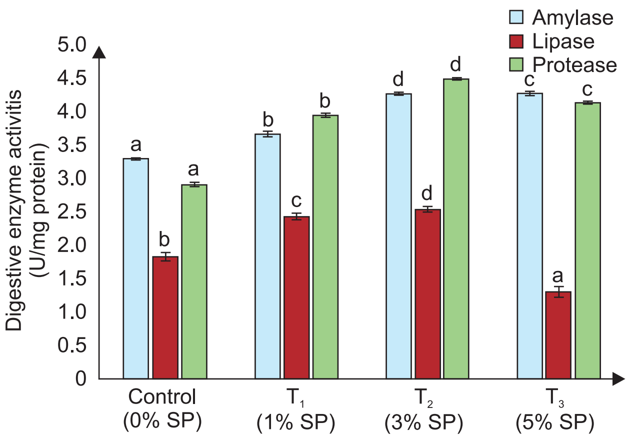

Fig. 2. Digestive enzyme activities of GIFT tilapia fed with experimental diets for eight weeks. The Bar with different superscript letters within the same treatment indicating the significant differences (P<0.05), (*n*=30) (SP, *Spirulina* meal).

contents (Table 3), high nutrients absorption that influence higher digestive enzyme activities, intestinal villi length and muscular thickness in fish were related to high nutrients digestibility. Therefore, the high apparent digestibility values registered in this study confirm GIFT Tilapia's ability to digest nutrients in algae. The results were in agreement with those reported in Nile tilapia, *O. niloticus* (Ngugi *et al.* 2017) when fed with diets containing amaranth leaf protein concentrates and herring meal respectively.

The histological changes in fish can be influenced by feed ingredients composition, diseases and poor handling management. In the present study, the results in histological changes of liver and intestine in fish are in harmony with that of Bin-Dohaish *et al.* (2018) who reported that, there were no histological changes. In addition, the intestinal villi length and muscular layer thickness of fish in T_2 were significantly higher compared to other treatments and the control group (Fig. 3). The results may be attributed by the algae composition with consequent potential for proliferation of villi structures (Mohan *et al.* 2006). Therefore, the improvement of the villi structure enhanced the nutrients absorption and overall fish performance. The present finding was in corroborated with results reported by Khalila *et al.* (2018) in Nile Tilapia, *O. niloticus* when fed soybean meal supplemented with *Spirulina*.

Based on the results, it may be concluded that the supplementation of *S. plantesis* meal in fish diet at 3% improved growth performance and survival, fish body proximate composition, digestive enzyme activities, nutrients digestibility, intestinal villi structure and lowed level of low metabolic enzyme activities resulting into improved health status of GIFT Tilapia. Therefore, the use of *Spirulina platensis* as a feed ingredient in GIFT Tilapia culture can be widely adopted by fish farmers.

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Table 6. Fecal matter proximate composition and apparent digestibility of fish fed with experimental diets for eight weeks

Parameter	Experimental diet			
	Control (0% SP)	T_1 (1% SP)	T_2 (3% SP)	T_3 (5% SP)
Fecal matter proximate composition (g/kg)				
Dry matter	924.53	920.79	928.01	927.17
Crude protein	133.17	141.80	126.42	121.30
Ether extract	21.17	21.92	19.01	13.67
Crude fiber	190.15	173.0	198.07	197.37
Gross Energy (KJ/kg)	13.22	13.03	13.43	13.31
Apparent nutrients digestibility (%)				
Dry matter	98.03±0.08[a]	98.13±0.07[a]	98.08±0.01[a]	98.07±0.03[a]
Crude protein	89.19±0.04[a]	89.35±0.01[b]	89.63±0.003[c]	89.27±0.03[a]
Ether extract	88.64±0.06[a]	88.83±0.09[a]	89.31±0.04[b]	88.75±0.03[a]
Crude fiber	88.27±0.67[a]	91.88±0.15[b]	94.68±0.14[c]	89.73±0.13[a]
Gross Energy	88.48±0.06[a]	88.88±0.01[b]	89.27±0.01[c]	88.50±0.03[a]

Values are means±standard error (*n*=30). Means with different superscripts letters within each row are statistically significant (P<0.05). (SP, *Spirulina*).

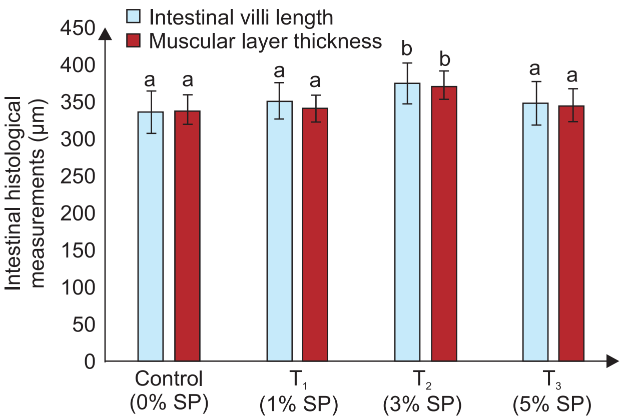

Fig. 3. Intestinal histological changes on GIFT tilapia fed with experimental diets for eight weeks. The bar with different superscript letters within the same treatment indicating the significant differences (P<0.05), (*n*=30) (SP, *Spirulina* meal).

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