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Composite algal supplementation in nutrition of *Oreochromis mossambicus*

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Abstract

Composite algal mix has been used as feed supplement in nutrition of *Oreochromis mossambicus* and its effect on growth performance, feed efficiency, nutrient utilisation and body composition was investigated in a 12 week feeding trial. Three isonitrogenous diets containing 0 % (conventional feed, CF), 35% (value added feed, VAF) and 100% (algal feed, AF) algal supplementation were used in combination with other conventional fish feed ingredients. The algal genera used were *Phormidium valderianum*, *Spirulina subsalsa*, *Navicula minima*, *Chlorococcum infusionum* and *Rhizoclonium riparium* in a ratio of 35:35:12:12:6 for experimental feed formulation. Fish fed with VAF showed an increased growth performance ($P < 0.05$), feed efficiency and nutrient utilisation compared to 0 % and 100% supplemented diets. Carcass lipid levels decreased at 35% supplementation level. The results suggested that, 35 % supplementation of conventional feed with composite algal mix can be used in mossambique tilapia diet.

Introduction

Fish has long been used as the cheapest source of protein for human nutrition worldwide – still with a gap in production and supply (Tidwell and Allan, 2001). A major determinant of successful growth and intensification of aquaculture production depends on aqua feed. Feed costs make up about 60% of an intensive aquaculture regimen (Güroy *et al*. 2007). Traditionally, animal protein sources, particularly fishmeal are used as the major ingredients of aqua feeds (Glencross *et al.* 2007). Fishmeal is one of the most expensive ingredients in formulated fish feeds in one hand and its regular use reduces the diversity of marine fauna on the other.

Therefore, to reduce the cost of aqua feeds, non conventional sources of proteins are in high demand. Plant feed stuffs are considered as economically viable and environment friendly feed ingredients and can be considered as essential biomass for future aquaculture feed formulation (Tacon *et al*. 2006).

Algae have emerged as an alternative protein source since long back and their use is quite popular in modern aquaculture industry .Potential algal genera contain about 30-70% protein, 10-20% lipid, 5-15% carbohydrate and high amount of carotenoids with antioxidant property (Becker, 2004). Most commonly used algal genera include microalgae like, *Chlorella*, *Nannochloropsis*, *Pavlova*, *Tetraselmis*, *Thalassiosira*, *Isochrysis*, *Dunaliella*, *Haematococcus*, *Scenedesmus*, etc. (Brown *et al*. 1997; Yamaguchi, 1997; Borowitzka, 1998; Apt and Behrens 1999; Muller-Feuga, 2000; Soletto *et al*. 2005). But most of the reports are available on use of single algal genus as fish feed ingredients ,therefore availability of algal biomass may be the major constrain in using them (Appler and Jauncey, 1983; Appler, 1985; Güroy *et al*. 2007; Azaza *et al*. 2008).Moreover, the production cost including the harvesting of the unicellular micro-algae are quite expensive making them almost unaffordable in developing country like India and others. Therefore, exploration of local algae for aquaculture usage is much more relevant for useful cheap production of aquaculture feed.

In India, most of the fish farmers use the mixture of rice bran and mustard oil cake for tilapia farming together with natural fauna of the pond. In the present study to formulate cheap and better feed, 35% supplementation with algal biomass was tested for value addition to the conventional feed using Tilapia (*Oreochromis mossambicus*) as model fish. Five algal taxa with high growth rate and relatively cheap production cost were chosen as feed ingredients. The new composite mixture was formulated after several trials. A total (100%) replacement of conventional feed by composite algal biomass was also used to understand the efficacy of algal meal as fish feed. Three marine genera viz. *Phormidium valderianum, Spirulina subsalsa* and *Navicula minima* and two fresh water genera *Chlorococcum infusionum and Rhizoclonium riparium* were taken as experimental materials.

Materials and methods

A mixture of two cyanobacterial genera viz. *Phormidium valderianum* Gomon, 1892, *Spirulina subsalsa* Ørsted ex Gomont 1892 and three eukaryotic algal genera viz. *Navicula minima* Grunow in Van Heurck, 1880 (Bacillariophyceae), *Chlorococcum infusionum* Schrank 1811 (Chlorophyceae) and *Rhizoclonium riparium* (Roth) Kützing ex Harvey 1849 (Chlorophyceae) were used as the experimental feed supplements. *Oreochromis mossambicus* Peters 1852 was used as model fish.

Collection and culturing of algae

The experimental algal genera were collected from eastern parts of India, isolated following standard protocols and micro algal cultures were raised for biomass utilization. Unialgal batch cultures of Cyanobacteria were grown using ASN III (Ott, 1965), for *Navicula* ASN III media was supplemented with SiO_4 and for *Cholorococcum* BBM (Bold, 1942) were used. *Rhizoclonium* was collected from Kakdweep, West Bengal and used in feed ingredients as natural resource.

Experimental fish, rearing condition and feeding regime

Juvenile *Oreochromis mossambicus* of same age group (1.7-1.8 g ± 0.54) were purchased from a government fish breeding farm (Kolkata, India). They were acclimatized for 2 weeks in the laboratory condition and were fed with commercial diet for proper rearing. The experimental trial was conducted in large rectangular flow through fibre reinforced plastic (FRP) tanks (0.6 x 0.5 x 0.38 m^3) containing 80 L water with a flow rate of about 1L min^{-1}. All the fishes were starved for 24h prior to the onset of the feeding trial. A 12- weeks feeding trial was carried out in triplicate groups of 20 fishes each (1.8-1.9 g). Three experimental sets were prepared for each experimental diet. Set 1 was fed with conventional feed (CF) and considered as control feed; set 2 was fed with algal feed (AF) with 100% supplementation and set 3 was fed with value added feed (VAF) with 35% algal supplementation. All groups of fish were fed their respective diets to apparent satiation twice a day at 0900h and 1700 h. The uneaten food was collected after 1h of feeding and dry matter content was determined (AOAC, 1995) for feed consumption calculation. The fishes were weighed every fortnight after a 24-h starvation period to determine the daily ration. Water quality was monitored throughout the feeding experiments with the water temperature, dissolved oxygen and pH being maintained at 26±1°C, 6.8±1.1 mg L^{-1} and 7.1±0.4 respectively.

Experimental diets

The pre-weighed dry algae were mixed in a proportion of *Phormidium*: *Spirulina*: *Navicula*: *Chlorococcum*: *Rhizoclonium* :: 35:35:12:12:6 to prepare the composite algal mix using a food mixer. The algae free conventional feed (CF) and 2 experimental diets viz. value added feed (VAF) containing 35% replacement of CF with composite algal mix and algal feed (AF) i.e. 100% replacement of CF were prepared using a feed pelletiser machine. All the feeds were formulated to meet the 40% protein requirement of juvenile tilapia (Fitzsimmons, 1997). The formulation and chemical composition of feeds are shown in table 1.

Analytical procedures

Analysis of dry matter (by oven drying at 105 °C for 24 h), crude protein (Kjeldahl apparatus, nitrogen X 6.25), crude lipid (extraction with petroleum ether by Soxhlet apparatus), crude fibre (by an automatic analyzer, Fibertec, Tecator, Sweden) and ash (incineration in a muffle furnace at 600° C for 4 h) were performed for both feed and carcass (AOAC, 1995). Water quality parameters were measured following APHA (1998).

Evaluation of growth performance

Growth performance and feed utilization of experimental fish were evaluated by standard methods (Siddhuraju and Becker, 2003) in terms of final fish weight (g), weight gain (WG, %), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR) and protein efficiency ratio (PER). Fishes were randomly selected from each experimental tank and decapitated for such analysis after 84 days of experimentation. The formulae used are as follows:

1. WG = [(Final body weight (g) - Initial body weight (g))/ Initial body weight (g)] x 100

2. SGR= [(ln Final body weight (g) - ln Initial body weight (g) / Number of days] x 100

3. FCR = Dry feed fed (g) / Live body weight gain (g)

4. PER = Body weight gain (g) / Crude protein fed (g) x 100

Statistical Analysis

All growth data were subjected to one-way analysis of variance (ANOVA). The significance of difference between means was determined by Duncan’s multiple range test ($P < 0.05$) using SPSS for Windows (Version 10.0) (Duncan, 1955).

Results

The nutritive values of the formulated feeds are shown in table 1. A 35% replacement of CF with algal mix (VAF) showed maximum crude lipid (10.32%), gross energy (24.29 kJ g^{-1}), calcium (7.08%) and phosphorus

(1.46%). AF contained higher amount of carotenoid (0.48%), manganese (1.79 mg k^{-1}) and zinc (6.39 mg k^{-1}) whereas CF was enriched with carbohydrate (12.35%) and iron (24.03 mg k^{-1}). Diet palatability was assessed by direct observation of fish behaviour and feeding responses. All the 3 diets were well accepted by the fishes. On an average, the VAF was well accepted (0.43 ± 0.14 g/ fish /day) ,followed by AF (0.42 ± 0.17 g/ fish /day) and CF (0.39 ± 0.11 g/ fish /day).

Table 1: Percentage and proximate composition of experimental diets (dry weight basis)*

	Feed Type		
Ingredients (%)	Conventional Feed (CF)	Algal Feed (AF)	Value Added Feed (VAF)
Algae mix	0	100	35
Rice bran	63	0	50
Mustard oil cake	37	0	15
Nutrient contents (DM %)			
Crude Protein (%)	40	40	40
Crude Lipid (%)	6.8	6.17	10.32
Carbohydrate (%)	12.35	8.25	10.48
Carotenoid (%)	0.001	0.48	0.36
Gross Energy (kJ g^{-1})	20.23	19.83	24.29
Copper (mg k^{-1})	0.38	0.6	0.56
Manganese (mg k^{-1})	1.31	1.79	1.67
Iron (mg k^{-1})	24.03	6.39	10.68
Zinc (mg k^{-1})	0.9	6.52	3.1
Calcium (%)	4.52	3.2	7.08
Phosphorus (%)	0.7	0.9	1.46

***nutrient values are mean of triplicate determination**

Survival percentage, growth performances and feed utilization of tilapia fingerlings of control and experimental sets are given in table 2. Survival rates were significantly higher in VAF fed fishes (99.7%) followed by AF (98.8 %) and CF (96.5 %). No external clinical symptoms were recorded in the experimental fishes during the study. Weight gain (from 261.11% to 303.27%), SGR (0.649 to 0.706), FCR (from 1.85 to 1.59) and PER (1.19 to 1.67) of fish fed with VAF were significantly (P < 0.05) higher than that of others indicating the best results among the 3 diets used.

Table 2: Growth performance and nutrient utilisation of *O.mossambicus* fed with experimental diets *

Parameters	Conventional Feed (CF)	Algal Feed (AF)	Value Added Feed (VAF)
Initial Weight (g)	1.85	1.85	1.85
Final Weight (g)	6.5^{a}	7.11^{a}	7.96^{b}
Weight gain Percentage (%)	261.11^{a}	261.6^{a}	303.27^{b}
SGR (% day^{-1})	0.649^{a}	0.664^{a}	0.706^{b}
FCR	1.85^{a}	1.78^{a}	1.59^{a}
PER	1.19^{a}	1.32^{a}	1.67^{b}
Survival (%)	96.5^{a}	98.8^{b}	99.7^{c}

***values are mean of triplicate determination. Values on the same row with different superscripts are significantly different (*P* < 0.05).), SGR (specific growth rate), FCR (feed conversion ratio), PER (protein efficiency ratio)**

The carcass proximate composition as affected by the test diets are summarized in table 3. All the diets resulted in improvement of carcass protein showing significant (11.26%) changes in VAF fed fishes than the other two diets. VAF also showed significant increase in moisture level but with a significant decrease in lipid content (3.1 %) .Glycogen content was significantly higher in CF fed fishes (21.37%), whereas, carotenoid was more in fishes fed with VAF (0.91 %), which was statistically significant also. A rise in carcass ash content was observed in VAF fed fishes (P < 0.05). Variations between carcass protein, lipid and glycogen content due to three dietary treatments can be well understood from figure 1 also.

Table 3: Carcass proximate composition of *O.mossambicus* (% wet weight basis) during the experimental trial *

		Feed Type		
Parameters (%)	Initial value	Conventional Feed (CF)	Algal Feed (AF)	Value Added Feed (VAF)
Moisture	77.29	74.14[a]	75.94[a]	78.27[b]
Crude protein	6.48	8.51[a]	10.54[b]	11.26[b]
Crude Lipid	1.35	3.89[a]	3.4[b]	3.1[b]
Crude Glycogen	8.1	21.37[a]	10.3[b]	15.49[c]
Carotenoid	0.18	0.24[a]	0.66[b]	0.91[c]
Ash	3.47	5.72[a]	3.68[b]	6.93[c]

*** values are mean of triplicate determination. Values on the same row with different superscripts are significantly different (*P* < 0.05)**

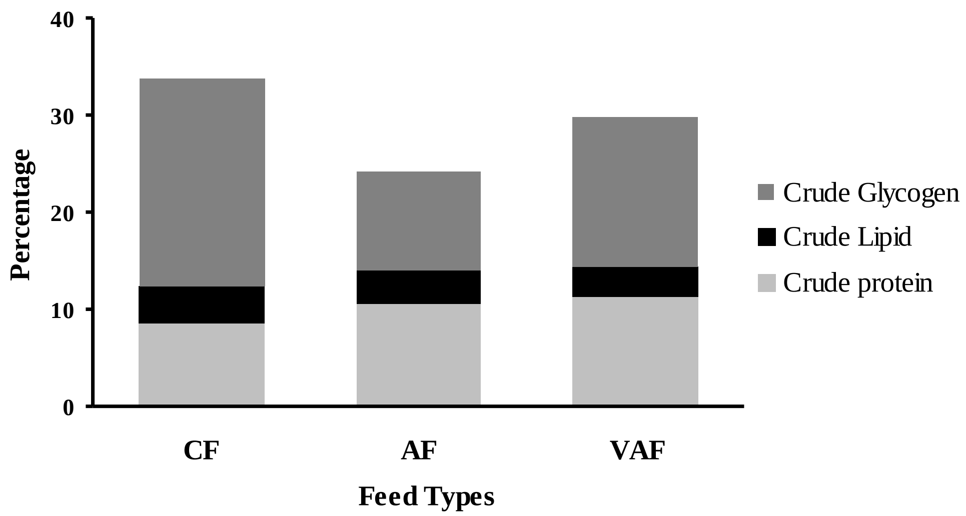

Fig 1: Crude glycogen, lipid and protein content of fish fed with different experimental diets.

Discussion

The advantages of partial inclusion of algal supplement can be attributed to the balance of dietary fibers, lipid, carbohydrates, minerals and carotenoid together with basic nutritional requirements in fish diet in comparison to commercial diet (Güroy *et al*. 2007; Azaza *et al*. 2008; Ergün *et al*. 2008; Tartiel *et al*. 2008). Growth performance of fish fed with VAF significantly surpassed the growth performances of fish subjected to CF and AF indicating the importance of composite algal mix supplementation (35 %). It has been reported earlier that intake of feeds containing different protein sources and their dietary level affect the energy content differently (Morales *et al*. 1994). In the present study differences in feed intake was insignificant ($P > 0.05$) in spite of the differences in gross energy content of commercial and experimental feed due to similar dietary protein levels.

In case of growth performances and feed utilization the SGR, FCR and PER value of VAF was better than the other two diets, although there was no statistically significant differences between CF and AF . This improved SGR and PER in VAF fed fishes may be due to high dietary lipid content in algae based formulated VAF , having same protein level and low level of carbohydrate to that of conventional feed. The protein sparing effect of dietary lipid and carbohydrate in fish has already been widely demonstrated (Cho and Kaushik, 1990; Sanz *et al*. 1993; Vergara *et al*. 1996). Fitzsimmons *et al*. (1997) reported carbohydrate as alternate source of energy in mossambique tilapia using upto 8% natural lipid as fish ingredients. Higher dietary carbohydrate content of conventional feed resulted in increased level of glycogen of controlled fish, whereas, higher lipid and carbohydrate level of formulated feed increased the protein level of fish carcass. Substantial amount of vitamin C obtained from algal biomass helped in lipid metabolism of fish tissue. Vitamin C is known to promote the lipid metabolism therefore ,reducing the carcass lipid level together with increase in protein levels (Miyasaki *et al*. 1995; Ji *et al*. 2003). Similar results have been observed in VAF fed fishes where protein levels have increased and lipid levels have decreased.

Therefore, the mixed algal biomass used in the present experiment as fish feed ingredient promoted the body weight, SGR, FCR, PER and nutritious value of fish tissue with higher protein level.The cost of production of the formulated feed was also less than the market available algae based feed as the filamentous algal genus

(*Rhizoclonium*) was collected from natural resources and the other two genera (*Phormidium*, *Spirulina*) were produced in open tank culture using low cost method. Moreover, the use of natural filamentous algal biomass can also be possible to apply as formulated feed only as in natural pond they clog the gills of the fishes, hence, always being avoided by the fish fauna.

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