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Research Article

Effect of Algae Based Diets on Growth Performance, Body Composition and Fatty Acid Profile of Indian Major Carp, Rohu (*Labeo Rohita*, Hamilton)

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Abstract: Six iso-energetic (352 kcal gross energy/100 g feed) and iso-nitrogenous (32% crude protein) experimental diets containing one of the four algae (*Anabaena cylindrica,* AN; *Nostoc salbasa,* NS; *Spirulina platensis*, SP; *Westleopsis prolifica,* WS) as the major dietary ingredient (40% of the total composition) or algal meal mixture with supplemental protein (AMM+PS) or only algal meal mixture (AMM) were tested against a control diet to find out the suitability of algal meal to develop practical diet for rohu fingerling. The experimental fish of average weight 2.0 ± 0.1 g were fed at a rate equaling 4% of body wt/day for 8 weeks at an experimental temperature of 28 ± 2°C. Significant ($p<0.05$) enhancement in growth rate was observed in the groups fed with WS (571%) followed by the groups fed with NS (517.6%) and SP (502.8%) as compared to that of the groundnut oil, rice bran and soybean meal based control diet (406.4%). Fingerlings fed with algal meal mixture (AMM) gained least (255.2%). Diet with WS performed well in terms of FCR, PER, HSI, MR and CF when AMM showed least efficiency. Fish fed with SP showed better protein utilization (ANPU%) as compared to the other dietary groups. Carcass protein was not affected by any diet. The MUFA and PUFA of liver and PUFA of muscle increased over the initial value fed with control and experimental diets. But the MUFA of muscle with these diets are less than the initial. The SFA content in all diets of both the tissues is either same or less than initial value except with AMM diet. The total n-3PUFA in liver increased with all diets over the initial value. But this increase in muscle was observed only with AN, NS, WS and AMM+PS diets. The n-6PUFA of liver and muscle remained either same or increased with all diets over initial value. This study showed that individual algal diets gave better result in growth performance and PUFA content. Among all the algal diets WS found to be the best.

Keywords: Algal diets; Growth; Fatty acid; Rohu; *Labeo rohita*

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Introduction

Fresh water and marine fish are often considered to be healthy component of human diet due to relatively high ratio of polyunsaturated to saturated fatty acids (PUFA:SFA) compared to other animal food sources (Margaret *et al*., 2014). In fact fish contains high concentration of n-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which is identified as essential for human health. Fresh water fish differ significantly from marine fish with respect to fatty acid content and requirement (Harel & Place, 2003). It has been observed that the reason for this difference is the diet (Ahlgren *et al*., 1992) as the tissue fatty acids are influenced by dietary fatty acid composition (Cengiz *et al*., 2003) at all developmental stages (Kiessling *et al*., 2001). However, fish lack the ability for *de novo* synthesis of n-3 and n-6 PUFAs, which are essential for their growth and development (Mishra, 2000). But the fish has ability to elongate and desaturate the parent fatty acids of these PUFAs. To know the origin of these PUFA in fish, the first step is to examine the pattern of fatty acids (FA) in algae (Ahlgren *et al*., 1992).

Due to stagnation in wild fisheries, aquaculture is expected to fill the gap in supplies of fish as human food (Bakhshalizadeh et al., 2011; Bakhshalizadeh et al., 2015, FAO, 2014). Among the three Indian major carps cultured, rohu (*Labeo rohita*) is the most preferred species because of its taste and market value. Locally available ingredients, predominantly of plant origin, are used to formulate fish feed which are rich in saturated, monounsaturated fatty acids (MUFA) of n-6 type (Veerina *et al*., 1999). In a comparative fatty acid profile study of wild and farmed rohu, Sharma *et al*. (2010) reported significantly higher lipid with SFA and MUFA content in cultured species but wild fish was found to have higher n-3 and n-6 PUFA, which may be due to the natural food like algae that the wild fish consumes. Few earlier studies suggest that nutrient contents of farmed fish is more uniform than wild and that fat content of farmed fish exceeds that of wild (Hossain, 2011). Growth performance of rohu fingerlings were reported to be better using value added feed containing four different algae compared to conventional control diet (Mukherjee *et al*., 2011). Teimouri *et al*. (2016) observed significant increase in n-3/n-6 ratio and muscle quality of rainbow trout (*Oncorhynchus mykiss*) by supplementation of the algae *Spirulina platensis*. Based on the nutritional value of marine algae (Brown *et al*., 1997) their use in mariculture has been emphasized (Harel and Place, 2003). However no systematic study on use of algae for freshwater fish to improve their fatty acid profile has been worked out. The present study is thus proposed to study the efficacy of algal meal based diets on the growth performances and tissue fatty acid profile of fresh water fish rohu, *Labeo rohita*.

Material and Methods

Algal meal

Six freshwater micro algae namely *Anabaena cylindrica* (AN), *Nostoc salbasa* (NS), *Spirulina platensis* (SP), *Westleopsis prolifica* (WS), *Gracillaria edulis* (GE) and *Enteromorpha intestinalis* (EI) were isolated and identified by the members of the Algal Research Laboratory, Berhampur University, Odisha. The pure strain of algae were cultured and harvested during the log phase as reported earlier (Mishra and Samantaray, 2000), washed thoroughly, centrifuged at 4000 g, hand pressed and soaked in blotting paper to remove excess water. Then the algae were kept in oven for 15 min at 60°C followed by air drying at room temperature for 45 min and ground to get the algal meal. The algal meals were kept in sealed polythene bags inside the refrigerator. The proximate composition and fatty acid profile of individual algae, used in this study for diet formulation, were reported earlier (Mishra, 2000; Mishra and Samantaray, 2000).

Experimental diets

Six experimental and one control iso-energetic (352 kcal gross energy/100 g of feed) and iso-nitrogenous (32% crude protein) diets were prepared. The control diet (Diet 1) was formulated using the locally available rice polish (crude protein 15.7%, crude fat 5%) groundnut oil cake (crude protein 44.2%, crude fat 10%) and soybean meal (crude protein 46.3%, crude fat 1.3%) **(Table 1)**. In diet 2 to 5, 40% of the ingredients of the control diet were replaced by one of the four freshwater algae, namely AN (diet 2), NS (diet 3), SP (diet 4) and WS (diet 5) that were found to be potential in protein and lipid content. Sunflower oil (Agro Tech Foods Ltd., India) and cod liver oil (Seven Seas, Ireland, UK) were used at a minimum level of 1% each of the diet 1 to 5. *Gracillaria edulis* (GE) and *Enteromorpha intestinalis* (EI) along with the above four algae were used to prepare an algal meal mixture which was used to replace the major portion of the ingredients of the control diet to make diet 6 (AMM+PS). A complete algal meal mixture was taken in diet 7(AMM) without any supplemental source of protein (PS) from rice polish or GNOC. Diet 6 and 7 were devoid of any additional oil source.

For each diet, the ingredients in required quantity were ground in an electrical grinder along with 0.05% butylated hydroxy toluene (BHT) before the dough was prepared. The pellets were prepared as described by (Samantaray and Mohanty, 1997). The pellets were kept in airtight containers under a stream of nitrogen, inside refrigerator. The diets were prepared on weekly ration basis in order to avoid nutrient loss.

Experimental animal and design

Induced bred rohu of an average weight 2.0 ± 0.1 g (mean ± SD) and length 4.1+0.6 cm were stocked randomly at a rate 14 to 16 per tank to have a total biomass of 30 g ± 2 g in each of the 14 nos 65L capacity FRP tanks under a flow through system at a flow rate adjusted to 90 ml/min to test the seven diets in duplicate. Before the beginning of the experiment the fish were acclimated to the experimental condition and diet for 2 weeks when they were fed at a rate 4% of their body weight with the control diet (32% protein and 352 kcal gross energy) of which 20% of the ingredients were replaced by algal meal mixture so that the fish get used to the experimental diet.

The experimental tanks were aerated for 8 hrs a day on regular basis throughout the experimental period of 56 days. Water temperature was maintained at 25 ± 2°C in the experimental tanks

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Table 1: Ingredients and proximate composition of the test diets, expressed as % of dry weight.

Ingredient	Dietary Code[1]						
	C	AN	NS	SP	WS	AMM+PS	AMM
Algae	-	40.0	40.0	40.0	40.0	68.0	96.0
Rice polish	25.4	-	-	27.0	-	6.0	-
GNOC	33.5	27.0	27.0	27.0	27.0	22.0	-
Soybean meal	35.1	27.0	27.0	-	27.0	-	-
Vegetable oil	1.0	1.0	1.0	1.0	1.0	-	-
Cod liver oil	1.0	1.0	1.0	1.0	1.0	-	-
α-Cellulose	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Carboxymethyl Cellulose[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin[3] and mineral mixture[4]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
			Nutrient Content				
Crude protein	32.4	32.5	33.0	32.4	32.4	32.5	32.3
Crude lipid	8.8	6.0	6.0	8.5	8.7	5.6	4.4
Gross energy (kcal/100 kg)	354	351	355	356	351	350	348
P/E ratio (mg protein/kcal energy)	91.5	92.5	92.9	91.0	92.3	92.8	92.8

[1]C-Control, AN-Anabaena, NS-Nostoc, SP-Spirulina, WS-Westleopsis, AMM+PS-Algal meal mixture+protein source, AMM-Algal meal mixture.
[2]Carboxymethyl cellulose, sodium salts (high viscosity).
[3]To supply per 100 g diet, vitamin A IP (as acetate) 10000 IU, cholecalciferol (vit. D_3) 1000 IU, menadione (vit. K) 30 mg, thiamin mononitrate IP 10.0 mg, riboflavin IP 10.0 mg, pyridoxine hydrochloride 60.0 mg, cyanocobalamin IP 30.0 mg, nicotinamine IP 200.0 mg, ascorbic acid 300 mg, tocopherol acetate 50.0 mg, biotin USP 0.50 mg, inositol 20 mg, choline chloride 400 mg, calcium pantothenate 20 mg,
[3]To supply per 100 g diet: Calcium phosphate, 258.0 mg; magnesium oxide light, 120.0 mg; dried ferrous sulphate, 64.08 mg; manganese sulphate, 4.06 mg; total phosphorous in the preparation, 50.16 mg. Trace elements: Copper sulphate, 6.78 mg; zinc sulphate, 4.40 mg; sodium molybdate, 0.50 mg; sodium borate, 1.76 mg.

using thermostatically controlled electric aquarium heater. The dissolved oxygen and pH were recorded regularly for the entire experimental period and was found to vary between 6 to 8 ppm and 7.7 to 8.1, respectively. A 12:12 hr light & dark cycle were maintained through incandescent lighting for the entire period of experiment.

The experimental fish were fed twice daily at 9:00 and 15:00 hrs, 7 days a week, throughout the experiment at a level of 4% of body weight. The uneaten feed, if any were removed from each tank separately, two hours after each feeding, oven-dried and weighted to record actual feed consumption. Fish were weighed individually at the beginning and at the end of the experiment to record the growth parameters but batch weighing was done, at fifteen days interval for feed adjustment.

In the last week of the experiment, 1% of α-cellulose was replaced by chromium oxide, which was used as an external marker in the diet to study the nutrient digestibility. The fecal matter was collected every day before first feeding, dried, and pooled for each tank. The chromium oxide in the feed and fecal matter was estimated as per the method (Furukawa and Tsukahara, 1966). At the end of the experiment 10 fish from each tank were sacrificed for subsequent carcass analysis (AOAC, 1975), liver somatic index (HSI%), muscle ratio (MR%) and fatty acid analysis (Boberg *et al*., 1985) of dorsal muscle and liver. Six representative samples of fish were sacrificed at the beginning of the experiment to record the initial carcass composition and liver and muscle fatty acid profile.

Analytical Methods

Growth analysis

The growth rates in terms of weight gain (%) daily weight gain (gm), specific growth rate (%SGR) and feed conversion ratio (FCR), protein efficacy ratio (PER), apparent net protein utilization (%ANPU) or percent protein deposited were calculated as per standard formulae (Samantaray and Mohanty, 1997).

Biometrics analysis

Morphological and anatomical measurements such as Condition Factor (CF), Muscle Ratio (MR) and Hepatosomatic Index (HSI) were calculated using the formula given by Mustafa *et al*., 1995.

Gross composition analysis

Algal protein was analysed according to the micro-burette method (Rausch, 1981; Meyer and Walther, 1988) which is recommended for algae. The algal samples were extracted with chloroform methanol mixture (2:1) containing 0.005% BHT (Boberg *et al.*, 1985). The homogenate was added with 15 ml of 0.2 m sodium dihydrogen phosphate. After thorough mixing the non-aqueous chloroform layer was pipetted off, evaporated to dryness, and weighed to determine algal total lipid. The % crude

protein and lipid of the algae were found to be 23.8 and 1.4 for *Anabaena*, 24.06 and 1.77 for *Nostoc*, 42.25 and 3.8 for *Spirulina*, 39.4 and 4.5 for *Westleopsis*, 15.31 and 2.2 for *Enteromorpha* and 20.13 and 1.1 for *Gracillaria*, respectively.

Experimental diets and fish carcasses were analysed for their proximate composition such as the moisture, crude protein, crude fat, and ash content following the method (AOAC, 1975). Moisture was determined by oven drying at 100˚C to constant weight. Crude protein was determined based on kjeldahl nitrogen (Crude protein =N × 6.25). Crude fat was determined by extraction with petroleum ether for 6 hours in soxhlet apparatus. Ash content was determined by inscernating the sample to muffle furnace for 6 hrs at 550˚C. The moisture, crude fat, and ash content of the three algae were done as per the method described above. The gross energy content of the diets was measured in Reico plain oxygen bomb calorimeter. Dietary gross energy levels were almost adjusted by subtracting the energy contribution of added cellulose (4.0 kcal/g) which was considered to be indigestible filler, so that the energy levels reflected more accurately the gross energy of the foodstuffs used. The chromium oxide in the diet was analysed spectrophotometrically and the apparent digestibility of the protein and lipid was calculated as per the method (Furukawa and Tsukahara, 1966).

Fatty acid (FA) analysis

The total lipids of the samples (algae, diets, and fish tissues) were extracted as described earlier for algae. The fatty acids in the lipid extract were transmethylated at 60˚C overnight after addition of 2 ml of 5% H_2 SO_4 in methanol (Boberg *et al.*, 1985). After addition of 1.5 ml of distilled water, the methyl esters were extracted in 3ml of petroleum ether (B.P. 40-60˚C) containing 0.05% BHT. After thorough mixing the phases were separated by centrifugation at 1500Xg for 10 minutes. The petroleum ether phase was pipetted off and the solvent was evaporated under nitrogen. The methyl esters were then redissolved in 1ml hexane to which a known amount (25 mg) of an internal standard (22.0) was added and analysed by injecting fixed quantity in a HP 5890 Gas Liquid Chromatograph equipped with 25 m megabore (530 µ) methyl silicon fused column with film thickness 2.65 mm (Hewelett-Packard-1) and an FID detector. Nitrogen was used as carrier gas at a regulated pressure of 20 ml/m. The run method was through a temperature gradient of 150-250˚C with the injector and detector temperature at 200˚C and 250˚C, respectively.

The individual FAs were identified by comparing the retention times with commercially available reference standards of FA mixture (Supelco 37, component, Supelco, USA) and individual FA standard (Sigma, USA) as described by Gopakumar and Nair, 1972 and comparing the area of peaks with the peak of internal standard (22:0) (Ahlgren *et al.*, 1992). Fatty acids whose standards were unavailable could not be identified and mentioned as others. The FA composition of experimental diets is shown in **Table 2**.

Statistical Analysis

Using analysis of variance (ANOVA) made statistical analysis of the results of the feeding trial. Duncan's Multiple Range Test (Duncan, 1955) was used to evaluate the mean difference among individual diets at 0.05 significant levels.

Regression analysis was done between growth (% weight gain) and dietary n3/n6 ratio and PUFA of liver and muscle of the fish with dietary PUFA level. All the statistical analysis was done IBM compatible PC-AT using SPSS statistical package (ver.10.0).

Results

The effects of algal meal based diets on the survival, growth performance, feed efficiency and nutrient digestibility are shown in **Table 3**. All the algae based diets, except AMM (diet 7) were consumed well by the experimental fish like that of the control diet. In comparison to the control diet (406.4%) significant increase in growth rate was observed with the WS diet (571.0%) followed by the NS diet (517.6%) and SP diet (502.8%). Fingerlings fed with AMM gained least (255.2%) among all diets. However, some improvement in the growth could be achieved by addition of supplemental protein to the algal meal mixture (AMM+PS) (320%). The rate of survival in all the treatment groups was 100% except with AMM+PS (diet 6, 80%) and AMM (diet 7, 72%). The protein and lipid digestibility was higher for fish fed with control diet which was followed by WS, NS, SP and AN diets. Lowest feed conversion ratio (FCR) and highest protein efficiency ratio (PER) and specific growth rate (SGR %) were recorded for WS group followed by NS, SP, control and AN groups. Apparent net protein utilization (ANPU %) was observed to be better with SP diet than WS, control and NS diets.

In general, the body weight and body length increased with the various algal meal diets but HSI% and MR% were not affected much by feeding algae **(Table 4)**. The CF was influenced by the algal diets. The fishes fed with WS, NS and SP diets were found to have higher CF values than control.

The carcass moisture and protein content was not found to be dependent on the diet and remained almost unchanged **(Table 5)**. The SP group has insignificantly higher muscle protein content than that of WS, NS, and AN groups. The AMM, AMM+PS and control diets could induce lowest protein deposition in the muscle. On the other hand, body lipid content did not show any distinct pattern except few marked differences in some groups. The control, AN and NS diets were able to deposit more fat in the muscle but WS could significantly decrease the muscle fat content over the initial value. The ash content had decreased from the initial value when the fingerlings were fed with all diets except with AMM.

The mean FA compositions (expressed as percentage of total FA) of liver and muscle of experimental fish along with the initial FA profiles are presented in (**Tables 6 and 7)**, respectively. As expected, dietary FA composition greatly affected the FA profile of fish liver and muscle. When the liver SFA decreased or remained same, as compared to the initial level, the MUFA and PUFA levels increased in all the dietary groups except AMM where SFA content increased significantly with lower PUFA level. Similar trend was observed in muscle SFA and PUFA level. The myristic acid (14:0) level was high in initial liver and muscle, but

Table 2: Fatty acid profile of the experimental diet, expressed as % of total fatty acid (Mean of six samples ± SE).

Fatty acid	Dietary code						
	Control	AN	NS	SP	WS	AMM ± PS	AMM
12:0	6.1 ± 0.04	-	0.04	0.11	-	-	-
14:0	10.6^{c} ± 0.1	20.4^{a} ± 0.3	17.3^{b} ± 0.1	16.0^{b} ± 0.2	16.4^{b} ± 0.2	17.2^{b} ± 0.2	20.0^{a} ± 0.2
14:1	-	8.2 ± 0.04	5.1 ± 0.02	8.1 ± 0.06	6.2 ± 0.04	12.3 ± 0.1	16.4 ± 0.1
15:0	1.8 ± 0.01	0.5	2.3 ± 0.01	0.1	-	1.5 ± 0.01	2.6 ± 0.02
15:1	3.2 ± 0.01	-	-	0.8	0.1	0.8	1.1
16:0	16.9^{c} ± 0.2	14.5^{d} ± 0.2	22.9^{a} ± 0.2	18.0^{b} ± 0.2	14.4^{d} ± 0.2	16.2^{c} ± 0.2	18.0^{b} ± 0.3
16:1	10.2^{a} ± 0.1	1.6^{c} ± 0.01	3.2^{b} ± 0.01	3.8^{b} ± 0.01	3.3^{b} ± 0.01	0.4^{d}	11.9^{a} ± 0.1
17:0	3.0 ± 0.01	-	0.9	-	-	0.1	-
17:1	-	0.8	-	0.6	-	-	0.6
18:0	18.0^{a} ± 0.4	12.9^{cd} ± 0.1	13.2^{c} ± 0.1	12.0^{d} ± 0.1	15.6^{b} ± 0.2	10.2^{e} ± 0.1	9.1^{f} ± 0.08
18:1n7	-	-	-	-	-	5.0 ± 0.03	5.0 ± 0.03
18:1n9	8.8^{d} ± 0.06	10.0^{b} ± 0.1	12.0^{a} ± 0.1	6.9^{e} ± 0.04	9.3^{c} ± 0.06	6.9^{e} ± 0.04	2.4^{f} ± 0.01
18:2n6	1.0^{d}	5.3^{b} ± 0.03	4.8^{c} ± 0.02	6.6^{a} ± 0.04	4.6^{c} ± 0.03	6.5^{a} ± 0.05	1.6^{d} ± 0.01
18:3n6	-	2.4^{b} ± 0.01	-	4.5^{a} ± 0.02	-	1.3^{c} ± 0.01	2.3^{b} ± 0.01
18:3n3	1.2^{d} ± 0.01	4.1^{b} ± 0.02	3.1^{c} ± 0.02	-	6.0^{a} ± 0.04	4.1^{b} ± 0.03	0.03^{e}
20:0	0.6	1.7 ± 0.01	1.1	1.2	0.07	2.2 ± 0.01	-
20:2n6	4.4^{b} ± 0.02	-	5.3^{a} ± 0.03	2.1^{d} ± 0.01	5.2^{a} ± 0.04	4.1^{b} ± 0.02	3.0^{c} ± 0.02
20:3n6	2.8^{c} ± 0.02	4.1^{b} ± 0.04	-	5.3^{a} ± 0.06	2.6^{c} ± 0.01	1.6^{d} ± 0.01	-
20:3n3	4.1^{b} ± 0.03	3.0^{c} ± 0.01	3.5^{c} ± 0.02	1.1^{c} ± 0.01	6.2^{a} ± 0.04	4.0^{b} ± 0.02	2.0^{d} ± 0.01
20:4n6	-	-	-	-	-	-	-
20:5n3	2.1^{b} ± 0.01	2.3^{b} ± 0.01	2.4^{b} ± 0.01	1.0^{c}	3.1^{a} ± 0.02	2.3^{b} ± 0.01	1.1^{c} ± 0.01
22:0	2.1 ± 0.01	1.0	0.6	-	-	0.1	-
22:6n3	1.1^{a} ± 0.01	1.1^{a}	1.1^{a}	0.06^{c}	0.05^{c}	1.0^{a}	0.89^{b}
24:1n9	-	-	-	5.6 ± 0.03	-	-	-
ΣFA	98.0 ± 2.1	93.9 ± 2.4	98.8 ± 2.6	93.8 ± 2.1	93.1 ± 2.3	97.8 ± 2.1	98.0 ± 2.4
Others	2.0^{b} ± 0.01	6.1^{a} ± 0.04	1.2^{c}	6.2^{a} ± 0.04	6.9^{a} ± 0.05	2.2^{b} ± 0.01	2.0^{b} ± 0.01
ΣSFA	59.1^{a} ± 1.0	51.0^{b} ± 1.1	58.3^{a} ± 1.2	47.4^{c} ± 0.9	46.5^{c} ± 0.5	47.5^{c} ± 0.5	49.7^{b} ± 0.5
ΣMUFA	22.2^{c} ± 0.3	20.6^{d} ± 0.2	20.3^{d} ± 0.2	25.8^{b} ± 0.3	18.9^{d} ± 0.2	25.4^{b} ± 0.2	37.4^{a} ± 0.3
ΣPUFA	16.7^{c} ± 0.2	22.3^{b} ± 0.2	20.2^{b} ± 0.2	20.6^{b} ± 0.2	27.7^{a} ± 0.2	24.9^{a} ± 0.2	10.9^{d} ± 0.01
Σn3	8.5^{c} ± 0.06	10.5^{b} ± 0.1	10.1^{b} ± 0.1	2.2^{e} ± 0.02	15.4^{a} ± 0.1	11.4^{b} ± 0.09	4.0^{d} ± 0.01
Σn6	8.2^{e} ± 0.06	11.8^{cd} ± 0.1	10.1^{d} ± 0.1	18.4^{a} ± 0.2	12.3^{bc} ± 0.1	13.5^{b} ± 0.1	6.9^{f} ± 0.06
Σn3/Σn6	1.0^{a}	0.9^{b}	1.0^{a}	0.1^{d}	1.2^{a}	0.8^{b}	0.6^{c}

Mean values within the same row with the same superscripts are not significantly different (P<0.05). Standard errors below 0.01 are not given.

Table 3: Growth, survivability and nutrient digestibility of rohu fingerling fed with experimental diets for 56 days. (Mean of six samples for each diet ± SE).

	Dietary Code						
Parameters	**C**	**AN**	**NS**	**SP**	**WS**	**AMM+PS**	**AMM**
Initial Weight (g)	1.88 ± 0.02	1.9 ± 0.1	2.1 ± 0.02	1.8 ± 0.02	2.0 ± 0.02	2.26 ± 0.03	1.94 ± 0.02
Final Weight (g)	9.52^{bc} ± 0.3	8.93^{c} ± 0.28	12.97^{a} ± 0.3	10.85^{b} ± 0.42	13.42^{a} ± 0.55	9.49^{bc} ± 0.3	6.89^{d} ± 0.21
Weight gain (%)	406.4^{d} ± 5.4	370^{e} ± 3.8	517.6^{b} ± 5.1	502.8^{c} ± 4.6	571^{a} ± 5.4	320^{f} ± 3.6	255.2^{g} ± 3.8
ADG (%)	13.64^{c} ± 0.6	12.55^{c} ± 0.61	19.42^{a} ± 0.65	16.16^{b} ± 0.54	20.40^{a} ± 0.75	12.91^{c} ± 0.66	8.83^{d} ± 0.3
FCR	1.45^{c} ± 0.01	1.91^{d} ± 0.03	1.3^{b} ± 0.01	1.36^{b} ± 0.01	1.12^{a} ± 0.01	2.26^{e} ± 0.01	2.97^{f} ± 0.03
SGR (%)	2.89^{b} ± 0.02	2.76^{bc} ± 0.02	3.25^{a} ± 0.04	3.15^{a} ± 0.02	3.35^{a} ± 0.01	2.56^{c} ± 0.01	2.20^{d} ± 0.02
PER	2.10^{b} ± 0.03	1.65^{c} ± 0.02	2.44^{ab} ± 0.02	2.17^{b} ± 0.02	2.66^{a} ± 0.03	1.33^{d} ± 0.01	1.04^{e} ± 0.01
ANPU (%)	29.47^{c} ± 0.9	23.33^{d} ± 0.77	24.26^{d} ± 1.0	46.39^{a} ± 1.3	35.70^{b} ± 1.06	15.48^{e} ± 0.84	13.26^{f} ± 0.3
Survival (%)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	80^{b}	72^{c}
Digestibility							
Protein	79.98^{a} ± 1.6	72.77^{ab} ± 1.4	75.49^{a} ± 1.1	75.06^{a} ± 1.63	76.23^{a} ± 1.24	70.02^{b} ± 1.1	70.56^{b} ± 1.1
Lipid	79.43^{a} ± 1.4	71.9^{b} ± 1.1	72.32^{ab} ± 1.6	72.04^{b} ± 1.44	74.49^{a} ± 1.5	70.0^{b} ± 1.1	70.41^{b} ± 1.13

Mean values within the same row with the same superscripts are not significantly different (p<0.05).

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Table 4: Effects of feeding algae on the morphological and anatomical parameters in rohu fingerling (Mean of six samples for each diet ± SE).

	C	AN	NS	SP	WS	AMM+PS	AMM
Body weight (g)	$9.52^{bc} \pm 0.3$	$8.93^{c} \pm 0.28$	$12.97^{a} \pm 0.3$	$10.85^{b} \pm 0.42$	$13.42^{a} \pm 0.55$	$9.49^{bc} \pm 0.3$	$6.89^{d} \pm 0.21$
Body length (cm)	$7.75^{b} \pm 0.13$	$7.67^{b} \pm 0.16$	$8.34^{a} \pm 0.2$	$7.91^{b} \pm 0.14$	$8.42^{a} \pm 0.11$	$7.74^{b} \pm 0.1$	$6.9^{c} \pm 0.2$
Condition factor (CF)	$2.04^{b} \pm 0.01$	$1.98^{c} \pm 0.01$	$2.23^{a} \pm 0.02$	$2.19^{a} \pm 0.02$	$2.24^{a} \pm 0.03$	$2.04^{b} \pm 0.02$	$2.02^{b} \pm 0.01$
Muscle ratio (MR %)	$36.5^{a} \pm 1.1$	$36.62^{a} \pm 1.3$	$36.75^{a} \pm 1.1$	$36.91^{a} \pm 1.28$	$37.6^{a} \pm 1.14$	$35.9^{a} \pm 1.36$	$35.15^{a} \pm 1.2$
Hepato-somatic index (HIS %)	$1.18^{a} \pm 0.02$	$1.1^{a} \pm 0.01$	$1.13^{a} \pm 0.01$	$1.16^{a} \pm 0.01$	$1.20^{a} \pm 0.01$	$1.1^{a} \pm 0.01$	$0.85^{b} \pm 0.004$

Mean values within the same row with the same superscript are not significantly different ($p<0.05$).

Table 5: Carcass composition of rohu fingerling fed with experimental diet for 56 days expressed as % dry weight. (Mean of six samples for each diet ± SE).

Parameter	Initial	Dietary Code						
		C	AN	NS	SP	WS	AMM+PS	AMM
Moisture	$75.17^{a} \pm 1.8$	$72.53^{a} \pm 1.3$	$75.26^{a} \pm 1.6$	$75.08^{a} \pm 1.4$	$75.83^{a} \pm 1.8$	$73.94^{a} \pm 1.3$	$75.16^{a} \pm 1.4$	$76.39^{a} \pm 1.6$
Crude Protein	$71.07^{a} \pm 1.1$	$72.14^{a} \pm 1.34$	$72.06^{a} \pm 1.19$	$72.15^{a} \pm 1.3$	$73.0^{a} \pm 1.43$	$72.6^{a} \pm 1.18$	$71.91^{a} \pm 1.2$	$71.7^{a} \pm 1.2$
Crude Lipid	$13.25^{c} \pm 0.4$	$15.2^{a} \pm 0.11$	$15.21^{a} \pm 0.16$	$14.9^{ab} \pm 0.3$	$14.59^{bc} \pm 0.24$	$13.76^{c} \pm 0.3$	$14.15^{bc} \pm 0.4$	$13.09^{c} \pm 0.4$
Ash	$15.68^{a} \pm 0.4$	$12.66^{c} \pm 0.25$	$12.73^{c} \pm 0.3$	$12.95^{c} \pm 0.3$	$12.41^{c} \pm 0.35$	$13.64^{bc} \pm 0.4$	$13.94^{b} \pm 0.4$	$15.21^{a} \pm 0.44$

Mean values within the same row with the same superscripts are not significantly different ($p<0.05$).

its level reduced in both the tissues due to the algal diets. In both the tissues, palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1n9) were present in predominate quantity among all the SFA and MUFA.

In general the level of n-3 FA was found to be higher than that of n-6 in both muscle and liver of all the dietary groups. The total n-3PUFA in liver increased with all diets over the initial value. But this increase in muscle was observed only with AN, NS, WS and AMM+PS diets. The n-6PUFA of liver and muscle remained either same or increased with all diets over initial value.

Further, such dietary effect could be visualized with respect to the high accumulation level of gamma-linolenic acid (18:3n6) in both liver and muscle with SP diet. The wide differences in 18:2n6 and 18:3n3 levels in the diets could reflect similar difference in liver and muscle and influenced the distribution pattern of other important long chain PUFA of those two series (20:4n6, 20:5n3, 22:6n3), particularly in liver. Approximately 2 fold increase in EPA (20:5n3) and DHA (22:6n3) in liver and muscle with WS diet and arachidonic acid (20:4n6) with SP diet in muscle emphasizes the significance of these algae as dietary ingredient for freshwater fish.

Even though no significant correlation was found between dietary PUFA levels with that of liver, a significant positive correlation was evident between the dietary PUFA and muscle PUFA content **(Table 8).**

Discussion

Most commercial fish feed ingredients are costly and their non-availability pose problem for fish farmers to take up aquaculture practices. So, a lot of research has been undertaken to find out the suitable alternative cheaper ingredients of wide availability to boost the culture. Even though algae are cheaper source of protein, lipid and other nutrients and their culture is easy, they are yet to be considered as a major food item for fish. Algae has been proved to be one of the most important food sources and feed additives in the commercial rearing of aquatic animals, especially fishes and shrimps (Khatoon *et al.*, 2010). Kumar *et al.* (2010) analyzed the effect of periphyton on the growth performance of Nile tilapia (*Oreochromis niloticus*). Algae can be used directly as live culture or as value added fish feed supplement (Mukherjee *et al.*, 2011). Based on growth performances, algae based (Spirulina and Enteromorpha) value added feeds were reported to be better than conventional control diet. Teimouri *et al.* (2016) evaluated the effect of the diets containing 0 to 100 g of *Spirulina*/kg of feed (on 25% increment level) on proximate composition, fatty acid profile and lipid peroxidation of rainbow trout and 50 g and 100 g of *Spirulina*/kg of feed reported to deposite higher percentage of PUFA in muscle. Bairagi *et al.* (2004) suggested the possible incorporation of *Leucaena* leaf meal inoculated with fish intestinal bacteria in rohu diet up to 40%. Given the relative enhancement of growth parameters and significant improvement in reproductive performance of broodstock three-spot gourami, 8.1-9.6% *S. platensis* meal instead of fish meal is recommended in the diets (Khanzadeh *et al*., 2016). The effect of *Spirulina platensis* as feed supplement for growth and alternative natural carotenoid source (Teimouri *et al*., 2016) was studied in rainbow trout. Thus in the present study four individual algae were incorporated in the diet replacing 40% of the control diet and one complete algal meal mixture were tested to find out their suitability for inclusion in the rohu diet.

Mishra and Samantaray (2004) observed maximum growth of 481.8% in rohu using 32% protein at 3 digestible energy level (12 096, 13 986 and 15 876 kJ/kg of feed). Chittem and Kunda (2013) used 20 g protein and 337 kcal/100 g of feed to study the effect of n-3 fatty acid on rohu and observed maximum weight gain of 127.4%. In the present study on rohu a diet having 352 kcal energy/100g and 32% protein was used to produce a maximum growth rate of 571%. This suggests that proper energy level in the diet supports growth. The fatty acid content of freshwater

Table 6: Fatty acid profile of rohu liver fed with algae based diets for 56 days, expressed as % of total fatty acid. (Mean of six samples for each diet ± SE).

Fatty acids	Initial	Dietary Code						
		C	AN	NS	SP	WS	AMM+PS	AMM
12:0	1.9^{a}+0.01	1.8^{a} ± 0.01	1.1^{b} ± 0.01	0.13^{d}	0.03^{e}	-	1.3^{b} ± 0.01	0.63^{c}
13:0	0.26	-	0.6	3.2 ± 0.02	2.1 ± 0.01	-	6.06 ± 0.04	3.16 ± 0.02
14:0	10.7^{a}+0.14	2.2^{d} ± 0.01	6.0^{b} ± 0.07	3.2^{c} ± 0.01	4.3^{c} ± 0.03	4.4^{c} ± 0.03	3.1^{c} ± 0.01	6.2^{b} ± 0.05
14:1	2.2+0.01	-	-	-	-	-	0.33	2.3 ± 0.01
15:0	1.2 ± 0.01	0.1	0.9	3.3 ± 0.02	0.3	0.9	3.2 ± 0.02	1.0 ± 0.01
15:1	-	-	-	-	-	-	3.1 ± 0.02	-
16:0	10.5^{e} ± 0.16	16.3^{b} ± 0.11	12.2^{d} ± 0.14	14.1^{c} ± 0.11	9.6^{ef} ± 0.1	9.1^{ef} ± 0.08	8.4^{f} ± 0.06	18.3^{a} ± 0.2
16:1	2.1^{c} ± 0.01	6.0^{a} ± 0.04	5.2^{b} ± 0.03	1.67^{d} ± 0.01	5.4^{b} ± 0.04	2.2^{c} ± 0.02	2.0^{c} ± 0.01	1.1^{d} ± 0.01
17:0	-	2.4 ± 0.01	-	0.7	-	-	-	0.8
17:1	0.9	-	-	1.2 ± 0.01	-	1.1 ± 0.01	-	-
18:0	8.3^{c} ± 0.06	2.0^{d} ± 0.01	12.8^{b} ± 0.11	16.0^{a} ± 0.15	6.1^{c} ± 0.04	6.2^{c} ± 0.06	11.95^{b} ± 0.13	13.6^{b} ± 0.1
18:1n9	4.1^{c} ± 0.01	14.0^{a} ± 0.16	4.2^{c} ± 0.05	8.2^{b} ± 0.06	4.4^{c} ± 0.03	8.5^{b} ± 0.06	9.9^{b} ± 0.1	10.9^{b} ± 0.11
18:2n6	6.4^{b} ± 0.04	4.3^{c} ± 0.02	8.2^{a} ± 0.06	4.0^{c} ± 0.03	2.8^{d} ± 0.01	6.1^{b} ± 0.04	6.0^{b} ± 0.04	3.1^{c}+ ± 0.01
18:3n6	-	0.2^{c}	1.3^{b} ± 0.01	0.2^{c}	6.6^{a} ± 0.04	0.6^{c}	0.5^{c}	0.2^{c}
18:3n3	7.4^{b} ± 0.05	6.5^{c} ± 0.06	10.8^{a} ± 0.11	6.6^{c} ± 0.04	8.0^{b} ± 0.06	4.1^{d} ± 0.02	8.1^{b} ± 0.1	7.5^{b} ± 0.08
20:0	4.4^{b} ± 0.02	3.2^{c} ± 0.01	3.2^{c} ± 0.01	1.2^{e} ± 0.01	5.2^{a} ± 0.04	5.2^{a} ± 0.04	1.3^{e} ± 0.01	2.1^{d} ± 0.01
20:2n6	2.3^{b} ± 0.01	3.9^{a} ± 0.04	2.3^{b} ± 0.01	1.6^{c} ± 0.01	1.5^{c} ± 0.01	2.3^{b} ± 0.02	3.1^{a} ± 0.02	3.6^{a} ± 0.04
20:3n6	-	1.4^{c} ± 0.03	1.4^{c} ± 0.02	2.6^{b} ± 0.03	0.3^{d}	2.4^{b} ± 0.01	3.2^{a} ± 0.03	1.5^{c} ± 0.01
20:3n3	3.3^{d} ± 0.02	9.7^{b} ± 0.08	8.1^{c} ± 0.06	9.6^{b} ± 0.1	9.7^{b} ± 0.08	8.2^{c} ± 0.06	11.6^{a} ± 0.13	9.4^{b} ± 0.11
20:4n6	9.2^{ab} ± 0.1	6.9^{c} ± 0.09	4.7^{d} ± 0.03	8.4^{b} ± 0.06	10.2^{a} ± 0.1	6.7^{c} ± 0.06	5.1^{d} ± 0.03	3.1^{e} ± 0.01
20:5n3	3.4^{d} ± 0.03	6.1^{b} ± 0.05	4.5^{c} ± 0.03	6.4^{b} ± 0.04	3.1^{d} ± 0.01	8.6^{a} ± 0.06	4.6^{c} ± 0.02	5.0^{c} ± 0.03
22:0	5.3^{ab} ± 0.04	-	4.8^{b} ± 0.03	1.05^{d}	4.6^{b} ± 0.04	6.0^{a} ± 0.04	2.6^{c} ± 0.01	2.4^{c} ± 0.01
22:6n3	2.9^{b} ± 0.03	3.0^{b} ± 0.01	2.7^{b} ± 0.01	2.7^{b} ± 0.01	1.7^{c} ± 0.01	7.3^{a} ± 0.06	2.1^{b} ± 0.01	2.1^{b} ± 0.01
24:1n9	3.1 ± 0.01	6.2 ± 0.04	4.4 ± 0.02	-	4.6 ± 0.03	8.6^{a} ± 0.06	-	0.2
ΣFA	90.0 ± 1.6	96.2 ± 2.01	99.4 ± 2.8	97.0 ± 2.4	90.6 ± 1.9	98.5 ± 2.04	97.5 ± 1.81	98.2 ± 1.9
others	10.0^{a} ± 0.08	3.8^{b} ± 0.02	0.6^{e}	3.0^{bc} ± 0.01	9.4^{a} ± 0.1	1.5^{d} ± 0.01	2.5^{c} ± 0.01	1.8^{d} ± 2.02
ΣSFA	42.5^{b} ± 0.9	28.0^{e} ± 0.31	41.6^{b} ± 0.5	42.9^{b} ± 0.44	32.2^{d} ± 0.4	31.8^{d} ± 0.29	37.9^{c} ± 0.4	48.2^{a} ± 0.44
ΣMUFA	12.4^{d} ± 0.11	26.2^{a} ± 0.3	13.8^{c} ± 0.1	11.1^{d} ± 0.1	14.4^{c} ± 0.15	20.4^{b} ± 0.18	15.3^{c} ± 10.2	14.5^{c} ± 0.13
ΣPUFA	35.1^{b} ± 0.4	42.0^{a} ± 0.4	44.0^{a} ± 0.42	43.0^{a} ± 0.4	44.0^{a} ± 0.4	46.3^{a} ± .44	44.3^{a} ± 0.5	35.5^{b} ± 0.39
Σn3	17.0^{d} ± 0.2	25.3^{b} ± 0.22	26.1^{b} ± 0.29	25.3^{b} ± 0.25	22.5^{c} ± 0.2	28.2^{a} ± 0.26	26.4^{b} ± 0.3	24.0^{b} ± 0.31
Σn6	17.9^{b} ± 0.2	16.7^{b} ± 0.19	17.9^{b} ± 0.2	16.8^{b} ± 0.14	21.4^{a} ± 0.2	18.1^{b} ± 0.16	17.9^{b} ± 0.2	11.5^{c} ± 0.11
Σn3/n6	0.9	1.5	1.4	1.5	1.05	1.6	1.5	2.1

Mean values within the same row with the same superscripts are not significantly different ($p<0.05$). Standard errors below 0.01 are not given.

algae available in this region has been worked out by Mishra and Samantaray (2000). Based on the protein, lipid and fatty acid content, the algae were selected for this study to be tested either individually or in algal meal mixture.

Our results demonstrated that all algal feeds were consumed by the test fish but the increase in body weight varied when compared with the fish fed with control diet. This may be due to the differences in feed utilization and digestion efficiency. Herbivorous fish generally have low digestion efficiency when fed natural diets as compared to carnivorous fish (Brett and Groves, 1979). Omnivorous fish rohu was found to have low ability to digest algal feed particularly when the diet was completely based on algal meal (AMM) in the present study. Obviously, prepared diets facilitated digestion. By the end of the 56 days experiment the most rapidly growing groups had obtained more than 500% increase over their initial weight at stocking. The increase in growth rate due to algal diets was observed not only to be more than the control group of the present experiment but also other experimental and control diets used earlier (Hasan *et al.*, 1991; Nandeesha *et al.*, 1994). Earlier reviews of Mustafa and Nakagawa (1995) confirmed that the supplementation of macro and micro-algae meal enhanced the growth, feed utilization, lipid metabolism and carcass quality of a variety of fishes which was supported by the work of Kumar *et al.* (2010) on tilapia using periphyton.

Rohu fingerling grew more on WS, NS and SP **(Table 3)** that have more n-3 PUFA but only algal diet (AMM) was unable to produce fish efficiently. The differences in the FCR, average daily weight gain, PER, among the groups was similar to the weight gain. Then optimum lipid level resulted in improved growth, FCR, nutrient utilization and reduced nitrogen excretion (Martins *et al.*, 2007). Mishra and Samantaray (2004) reported that the diet

Table 7: Fatty acid profile of rohu muscle fed with algae based diets for 56 days expressed as % of total fatty acid (Mean of six samples for each diet ± SE).

Fatty Acids	Initial	Dietary Code						
		C	AN	NS	SP	WS	AMM+PS	AMM
12:0	3.3 ± 0.01	-	-	1.0	0.1	0.1	-	-
13:0	-	-	-	-	-	-	-	-
14:0	14.6^{c} ± 0.15	18.7^{a} ± 0.2	6.3^{f} ± 0.1	8.4^{e} ± 0.09	8.7^{e} ± 0.09	6.4^{f} ± 0.08	16.4^{b} ± 0.2	12.2^{d} ± 0.9
14:1	17.6^{a} ± 0.21	-	10.8^{b} ± 0.13	4.2^{d} ± 0.04	2.8^{e} ± 0.9	4.0^{d} ± 0.02	6.4^{c} ± 0.04	11.7^{b} ± 0.11
15:0	0.4	4.0 ± 0.09	2.8 ± 0.01	0.01	0.4	-	-	5.6 ± 0.04
15:1	-	-	-	7.2 ± 0.06	-	1.5 ± 0.01	-	3.9 ± 0.03
16:0	19.2^{a} ± 0.22	17.0^{b} ± 0.2	15.8^{c} ± 0.2	9.4^{e} ± 0.08	10.1^{d} ± 0.1	14.4^{c} ± 0.16	20.4^{a} ± 0.24	19.2^{a} ± 0.2
16:1	6.4^{b} ± 0.05	6.3^{b} ± 0.05	3.9^{c} ± 0.04	2.1^{d} ± 0.03	9.0^{a} ± 0.08	9.1^{a} ± 0.08	4.4^{c} ± 0.03	4.4^{c} ± 0.04
17:0	-	6.5 ± 0.05	-	0.4	-	-	-	-
17:1	-	-	0.3	-	0.5	-	-	-
18:0	10.2^{c} ± 0.1	13.9^{b} ± 0.14	14.3^{ab} ± 0.55	15.8^{a} ± 0.11	10.5^{c} ± 0.09	8.0^{d} ± 0.09	5.8^{e} ± 0.03	14.5^{a} ± 0.16
18:1n9	7.8^{d} ± 0.06	5.3^{e} ± 0.04	13.7^{c} ± 0.16	16.7^{b} ± 0.2	14.1^{c} ± 0.16	18.6^{a} ± 0.21	3.2^{f} ± 0.01	1.8^{g} ± 0.01
18:2n6	1.8^{b} ± 0.01	1.7^{b} ± 0.01	2.5^{a} ± 0.01	3.1^{a} ± 0.04	1.5^{b} ± 0.03	2.4^{a} ± 0.01	2.7^{a} ± 0.01	1.9^{b} ± 0.01
18:3n6	-	0.4	0.3	-	2.0 ± 0.01	0.03	-	0.05
18:3n3	3.4^{d} ± 0.02	2.6^{e} ± 0.01	5.3^{b} ± 0.03	3.9^{d} ± 0.03	3.0^{d} ± 0.02	7.0^{a} ± 0.05	6.2^{a} ± 0.03	4.4^{c} ± 0.02
20:0	-	2.3 ± 0.01	1.6 ± 0.04	1.3 ± 0.01	4.7 ± 0.02	3.3 ± 0.01	4.6 ± 0.02	-
20:2n6	1.1^{c} ± 0.01	1.2^{c} ± 0.01	0.3^{d}	2.1^{b} ± 0.01	3.6^{a} ± 0.03	3.5^{a} ± 0.02	1.1^{c} ± 0.01	1.5^{c} ± 0.01
20:3n6	-	0.5	1.9 ± 0.01	1.2 ± 0.01	0.5	0.9	0.2	0.04
20:3n3	4.4^{c} ± 0.04	5.0^{b} ± 0.05	5.5^{b} ± 0.04	5.8^{b} ± 0.04	3.7^{d} ± 0.04	3.7^{c} ± 0.03	6.8^{a} ± 0.06	4.4^{c} ± 0.03
20:4n6	1.3^{c}	0.4^{d}	2.4^{b} ± 0.02	2.3^{b} ± 0.03	3.9^{a} ± 0.04	1.1^{c}	2.8^{b} ± 0.02	1.2^{c}
20:5n3	2.4^{c} ± 0.03	5.2a ± 0.06	2.9^{c} ± 0.03	4.2^{b} ± 0.03	2.4^{c} ± 0.01	4.61^{b} ± 0.04	2.9^{c} ± 0.03	2.9^{c} ± 0.02
22:0	-	-	2.71	2.11	3.51	2.26	-	-
22:6n3	2.1^{b} ± 0.01	2.0^{b} ± 0.01	2.5^{b} ± 0.03	2.7^{b} ± 0.02	4.4^{a} ± 0.03	4.2^{a} ± 0.03	1.05^{c}	1.44^{c}
24:1n9	-	1.04^{e} ± 0.01	2.3^{d} ± 0.02	2.1^{d} ± 0.01	4.5^{c} ± 0.06	2.8^{d} ± 0.03	10.5^{a} ± 0.13	6.3^{b} ± 0.06
ΣFA	96.0 ± 2.1	94.0 ± 2.06	98.1 ± 2.5	96.0 ± 2.1	94.0 ± 1.93	97.9 ± 3.04	95.5 ± 2.88	97.4 ± 2.18
Others	4.0^{b} ± 0.02	6.0^{a} ± 0.05	1.9^{d} ± 0.01	4.0^{b} ± 0.01	6.0^{a} ± 0.04	2.1^{cd} ± 0.01	4.5^{b} ± 0.05	2.6^{c} ± 0.02
ΣSFA	47.7^{c} ± 0.61	62.4^{a} ± 0.44	43.5^{c} ± 0.41	38.4^{d} ± 0.5	38.0^{d} ± 0.48	34.5^{d} ± 0.36	47.2^{c} ± 0.53	51.5^{b} ± 0.61
ΣMUFA	31.8^{b} ± 0.4	12.6^{d} ± 0.15	31.0^{b} ± 0.3	32.3^{b} ± 0.36	30.9^{b} ± 0.4	36.0^{a} ± 0.4	24.5^{c} ± 0.3	28.1^{c} ± 0.3
ΣPUFA	16.5^{b} ± 0.2	19.0^{b} ± 0.2	23.6^{a} ± 0.18	25.3^{a} ± 0.2	25.1^{a} ± 0.22	27.4^{a} ± 0.31	23.8^{a} ± 0.3	17.8^{b} ± 0.2
Σn3	12.3^{c} ± 0.11	14.8^{b} ± 0.3	16.2^{b} ± 0.14	16.6^{b} ± 0.2	13.5^{c} ± 0.14	19.5^{a} ± 0.42	17.0^{ab} ± 0.21	13.1^{c} ± 0.13
Σn6	4.2^{d} ± 0.04	4.2^{d} ± 0.02	7.4^{bc} ± 0.09	8.7^{b} ± 0.06	11.5^{a} ± 0.11	7.9^{bc} ± 0.19	6.8^{c} ± 0.08	4.7^{d} ± 0.05
Σn3/n6	2.9^{b}	3.5^{a}	2.2^{c}	1.9^{d}	1.2^{d}	2.5^{c}	2.5^{c}	2.8^{b}

Mean values within the same row with the same superscripts are not significantly different (P<0.05). Standard errors below 0.01 are not given.

with 8% lipid at 21^{0}C produced rohu better in terms of growth performance and n-3/n-6 ration compared to higher lipid level at that temperature. In the present study at 25 ± 2°C the lipid level was 6 to 8% in control and four individual algal meal diets. AMM diet has less lipid level which may be cause of lower growth performance in AMM group. In fact, oil extracts from algae containing long-chain polyunsaturated fatty acids are already in use as nutritional supplements in human infant formulas (Cohen *et al.*, 1995). A variety of factors are involved in determining the food value of a particular alga, including cell size, digestibility and lack of toxicity (Webb and Chu, 1983). Of particular importance are the presence of the long-chain PUFA, the content of dietary protein and carbohydrate (Whyte et al., 1990). The nutritive value of 24 tropical freshwater algae has been analysed (Ahlgren *et al.*, 1992). The percentage survival (100%) of rohu in the present study with different algal diets except AMM and AMM+PS diet justifies the level of inclusion of algae for diet development.

Feeding algae increased growth rate in rohu but the MR and HSI were not affected by any algal diet except the AMM, where HSI was decreased significantly. On the other hand, the CF was found to be influenced greatly by the algal diets such as WS, AN and SP. Pradhan and Das (2015) indicated that dietary supplementation of *Chlorella vulgaris* did not negatively affect liver function, but have growth promoting effect. Supplementation of *Spirulina* had significant positive effects on growth performance, feed utilization and body composition of catfish, *Heteropneustes fossilis* (Hossain, 2014). The effects of algal diets on the morphological and anatomical parameters of rohu, in the present study, were in contrast to that recorded for red sea bream due to algal meal additive diets (Mustafa *et al.*, 1995). Insignificant variation in carcass protein content of rohu in the present study might be due to delay in absorption.

The FA of rohu and other freshwater fishes were studied by several authors (Ackman *et al.*, 2002; Prabhakara Rao *et al.*, 2010, Jakhar *et al.*, 2012). Changes in growth performance and FA profile of rohu were reported due to the effect of different vegetable oils (Karanth *et al.*, 2009) and due to the different levels of dietary n-3 (Chittem and Kunda, 2013). In both these studies rohu FA profile is found to be affected by the diet. Samantaray and Mishra (2004) reported changes in rohu FA profile due to change in experimental temperatures and dietary lipid levels. In the present study, algal meal diets also found to affect the rohu liver and muscle FA.

The total FAs in liver and muscle of hybrid tilapia fed with commercial pellets, were reported to decrease and increase, respectively with growth, while significant proportional increase of individual saturated fatty acids and monoenes in liver was noticed up to 24th day of feeding (De Silva *et al.*, 1997). In this study, rohu have decreased and increased SFA content in liver and muscle, respectively, while fed with control diet. Furthermore, decrease in SFA level and increase in PUFA content in both the tissues was noticed due to the algal diets except AMM. Proportional decrease in individual SFA in liver was noticed with algal diets. This supports the fact that fish retains the essential PUFAs in the tissues, utilizing SFA for energy.

In the present study no correlation was noticed between the dietary n-3 and n-6 ratio with growth.The growth rate of palmitto bass (striped bass X white bass) did not increase with the increase in dietary n-3 PUFA as the final fish size at the three highest n-3 PUFA (0.87%, 1.26% and 2.27%) levels were recorded to be the same (Tuncer *et al.*, 1993). From the present study it appears that the level of n-3 PUFA available from some algal diets, particularly WS and NS, is sufficient to meet the requirement of the same in rohu. In rohu an increased level of accumulation of EPA and arachidonic acid (20:4n6) over their respective initial level in liver and muscle at 21˚C and 32˚C suggested bio-transformation of the parent FAs of these series (Samantaray and Mishra, 2004). Karanth *et al.* (2009) reported high deposition of linolenic acid (18:3n3) and EPA in rohu liver feeding with high linolenic acid diet. In the present study on rohu, the dietary 18:3n3 of control and algal diets could induce higher liver EPA level. Even though the SP diet was not found to have any 18:3n, it could induce incorporation of EPA and DHA both in liver and muscle, which may be due to elongation and desaturation of other FA of n3 series. Fairly high level of n-3 FAs found in rohu liver and muscle state the ability of the fish to elongate and desaturate linolenic acid as was observed for grass carp (Cai and Curtis, 1989). Even though no significant correlation was found between dietary PUFA levels with that of liver, a significant positive correlation was evident between the dietary PUFA and muscle PUFA content **(Table 8)**. This suggests the ability of rohu to convert dietary PUFA to muscle PUFA.

Higher n-3 content in liver and muscle of rohu in the present study in WS group followed by AN and NS group and higher n-6 content in both the tissues of SP group signifies the dietary source of those FAs in fish tissues. It is also interesting to note that the

Table 8: Relationship of liver and muscle PUFA with dietary PUFA.

A	Variable X : Dietary PUFA Variable Y : Liver PUFA Y : -21.099 (± 16.753)+0.952(± 0.379)X R^2=0.557 F=6.301 Significance F=0.053
B	Variable X : Dietary PUFA Variable Y : Muscle PUFA Y=-10.847(± 7.717)+1.369(± 0.33)X R^2=0.774 F=17.136 Significance F=0.009

EPA and DHA content in WS and arachidonic acid (20:4n6) in SP group were significantly higher in liver than other dietary groups, even though no distinct pattern could be noticed in muscle. In any case the level of these fatty acids was higher in liver than muscle. Similar higher level of n-3 FA content was reported for grass carp with aquatic plant elodea (*Elodea densa*) that have higher content of this FA ((Mustafa *et al.*, 1995). The n3/n-6 ration of the FA increased and muscle atherogenic and thrombogenic indices were significantly decreased as the dietary supplement of *Spirulina* increased (Teimouri *et al.*, 2016). In the present study the diets could induce higher n-3/n-6 ratio in liver but in muscle this ratio remained same over the initial value. Fish has the ability to modulate this ratio towards n-3 which is observed in this study. It confirms that by dietary modulations a fresh water fish can be a healthy component for human like marine (Jakhar *et al*., 2012).

Conclusion

Algae can be used directly as live culture or as value added feed supplement. Formulated algal diets were found to satisfy the nutritional requirements of rohu with high acceptability of the feed. In the present study the suitability of algal meal diets was evaluated against rice polish, GNOC and soybean based control diet for promoting growth and FA content of rohu over 56 days. The feed and protein efficiency with WS, NS, and SP three algae based diets were superior to that of the control diet. Among all the diets, the WS performed well in terms of growth, FCR, HSI, and CF when AMM showed lowest efficiency. However, fish fed with SP showed better protein utilization as compared to other dietary groups. Liver and muscle PUFAs increased in rohu by feeding algal diets. If selected algae could be used as the dietary ingredient, those will not only serve as the source of protein but also the source of EFA at the same time would reduce the cost of feed. However, the selection of algae should be based on their nutritive value.

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