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Research Article

Fatty acid profile of carps fed Chlorella oil-based diets

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Abstract

Incorporating algae oil into diets provides balanced nutrition and improves growth compared to traditional diets. Fry (2.00 ± 0.50g) and fingerling (10.0±2.0g) of rohu and grass carp were fed on diets formulated as 0.67%, 1.33% and 2% *Chlorella vulgaris (C. vulgaris)* oil for two months. In growth, a significant difference (p=0.0198) was observed in final weight (FW) in fingerlings; however, feed conversion ratio (FCR) was significant (p=0.0003) in both fingerlings and fry (<.0001) regarding species. Diets containing 1.33% algae oil showed the highest weight gain (Fingerlings=216.50g, Fry=358.06g) and the lowest FCR (Fingerlings=1.42, Fry=1.30) for both species at both stages. The increasing trend was observed among all experimental groups for moisture, protein, and fat contents, showing the highest values in the group with 1.33 % of the inclusion level of *C. vulgaris* oil in the formulated diet. When comparing species, significant differences (p<.0001) were found in moisture content among fingerlings, with grass carp fingerlings having higher moisture content than rohu fingerlings. In fry, no significant difference was present in moisture, protein, fat, and ash content. Significant interactions ($p < 0.05$) were shown in moisture and protein content in fingerlings, but no significant differences in interactions were observed in moisture, protein, fat, and ash content in fry. The fatty acid profile of grass carp and rohu fingerlings revealed significant differences (p<.0001) regarding species, except for 18:2n-6 and 20:3n-6, and the ratio of n-3/n-6. The fatty acid profile analysis of grass carp and rohu fry showed significant differences ($p < 0.05$) regarding species, except for fatty acids 18:2n-9, 20:1n-7, 20:3n-9, 20:4n-3, and 20:5n-3. In conclusion, replacing fish oil with algae oil up to 1.33% is recommended for the optimal growth of grass carp and rohu.

Keywords: C*hlorella vulgaris,* Fish oil, Fatty acid analysis, Proximate composition, Growth, Coloration

Introduction

Fish oil contains long-chain polyunsaturated fatty acids LC-PUFA), EPA (Eicosapentaenoic acid and DHA (Docosahexaenoic acid), which are essential for the growth and survival of fish (Tocher, 2010). However, the usage of alternative raw materials in feed formulation is growing due to their restricted supply and growing costs (Li et al., 2009).

A diet containing algae is species- and dose-specific (Valente et al., 2006). Research has indicated that fish species with higher levels of amylase activity, such as herbivores or omnivores (Hidalgo et al., 1999), can digest algal products more effectively (Montgomery & Gerking, 1980). Examples of these species include common carp (Diler et al., 2007), gilthead sea bream (Wassef et al., 2005), and Nile tilapia (Ergün et al., 2009). These studies also showed significant increases in growth, nutrient utilization, and body composition. Furthermore, it can yield higher oil yields than other raw materials such as sunflower, soybean, and rapeseed (Demirbas, 2008). Chisti (2007) stated that of the other microalgae species, *Chlorella* sp. produces 28–32% of the oil content, while *Spirulina* sp. and *Nitzschia* sp. generate the highest oil content (% dry weight) (Demirbas, 2008).

Microalgae are a promising feedstock choice that may help aquafeeds become less reliant on fish oil and fishmeal. Microalgae can have a significant protein content up to 70% of the dry matter and the best amino acid composition that meets fish requirements, depending on the species and growth conditions. Furthermore, some species of microalgae produce rich oil which are crucial for nutrition in fish. Additionally, they may include a variety of bioactive substances, including pigments and antioxidants (Karapanagiotidis et al., 2022).

Value-added compounds such as natural pigments, lipids, carbohydrates, and protein are present in microalgae (Spolaore et al., 2006). Microalgae's nutritional quality is determined by their protein concentration and PUFA content (Reitan et al., 1997). Methionine is sometimes deficient in plant-based components but is abundantly present in microalgae like *Chlamydomonas*, *Chlorella*, *Porphyridium,* and *Isochrysis* making them suitable to replace the fish meal in aquaculture feed (Wan et al., 2019). Omega-3 fatty acids (DHA and EPA), are abundant in some microalgae and beneficial to humans and fish (Ryckebosch et al., 2012). Microalgae species that are rich in essential fatty acids (EFA), amino acids, vitamins (particularly vitamin A, vitamin E, niacin, thiamine, and ascorbic acid), minerals, and carotenoid pigments are advantageous in a diet for marine organisms. Examples of these species are *Chlorella, Spirulina, Tetraselmis, Isochrysis, and Nannochloropsis* (Seong et al., 2021). Various research has been carried out on the use of microalgae *Chlorella* in aquaculture feed, namely for Nile tilapia (Badwy et al., 2008), African catfish (Enyidi, 2017), and Crucian carp (Shi et al., 2017).

Among the green microalgae, *Chlorella vulgaris* is a single-celled organism of the Chlorellaceae family (Richmond, 2004). Protein, fats, and carbohydrates make up 61.6, 12.5, and 13.7 percent of *Chlorella*'s biomass, respectively. A trace amount of minerals and thiamine are also present (Rodriguez-Garcia & Guil-Guerrero, 2008). However, as many microalgae species have not yet been tested on fish, more trials must be carried out for each combination of the selected species and fish. In summary, it is necessary to adjust the kind and amount of microalgae in fish feed to improve feed intake and FCR in specific fish species. The present study aimed to formulate fish feed by replacing fish oil with algae oil and to examine the effects of this replacement on the growth, proximate composition, body pigmentation, and fatty acid composition of grass carp and rohu (fry and fingerlings).

Materials and Methods

Ethical approval

The ethical approval of this research was obtained from the ethical review committee of the University of Veterinary and Animal Sciences, Lahore (Serial no. DR/166, 20-5-2024).

Experimental site

The present study was conducted at Fish Hatchery, Department of Fisheries and Aquaculture, UVAS, Ravi Campus, Pattoki. The experimental animals were *Ctenophyrangodon idella* (Grass carp) and *Labeo rohita* (rohu) at the fry (2.00 ± 0.50g) and fingerling (10.0 ± 2.0g) stages.

Extraction of oil from algae

C. vulgaris was cultured in an indoor system using BG 11 media. *C. vulgaris* was dried in an incubator for thirty minutes at 80°C and subjected to an oil extraction process (Baig et al., 2018). The oil yield (weight percent) was analyzed by following the equation (Arun et al., 2017).

$$\text{Extracted oil efficiency (wt.\%)} = \frac{\text{Mass of oil extracted (grams)}}{\text{The total mass of dried algae}} \times 100$$

Feed formulation

Four experimental diets were formulated by replacing fish oil with different levels of microalgae oil (0%, 0.67%, 1.33%, and 2%) (Tables 1 and 2). *C. vulgaris* was collected from the Ecology Laboratory Research Institute of Manawan, Punjab Fisheries Department, Pakistan. All the solid components of diets were ground for twenty minutes and algal oil was gradually added to the mixture. Water (15%) was added for the preparation of dough to form the pellets (0.5 mm) with the help of a pelletizer (KENWOOD, AT283). Pellets were dried and stored in sealed bags.

Table 1. Feed formulation for *Ctenopharyngodon idella* fry and fingerlings

	% of Dry Diet of Fingerling				**% of Dry Diet of Fry**			
Ingredients	**Control**	**0.67%**	**1.33%**	**2%**	**Control**	**0.67%**	**1.33%**	**2%**
Fishmeal	14.00	14.00	14.00	14.00	15.00	15.00	15.00	16.00
Soybean meal	12.0	13.0	15.0	14.0	15.0	16.0	18.0	19.0
Maize gluten	13.0	14.0	14.0	16.0	31.0	32.0	32.0	33.0
Wheat bran	20.0	20.0	20.0	20.0	3.0	3.0	3.0	2.0
Rice polish	15.0	14.0	12.0	11.0	15.0	13.0	11.0	10.0
Maize grains	12.0	12.0	12.0	12.0	8.0	8.0	8.0	7.0
Molasses	6.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Fish oil	2.0	1.3	0.7	0.0	2.0	1.3	0.7	0.0
Chlorella vulgaris oil	0.0	0.7	1.3	2.0	0.0	0.7	1.3	2.0
*Mineral mixture	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
**Vitamin Premix	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate Analysis								
CP%	30.07	30.24	30.12	30.10	40.14	40.22	40.11	40.32
Crude Fat	5.14	5.26	5.23	5.34	4.83	4.81	4.78	4.91
Ash	9.23	8.86	8.58	8.24	8.82	8.50	8.22	7.96
Fiber	4.45	4.84	5.32	5.64	3.07	3.48	3.95	4.28
GE, Mj/kg	19.00	19.02	18.95	18.96	20.00	19.97	19.90	20.08
CP/E	1.58	1.59	1.59	1.59	2.01	2.01	2.02	2.01

Table 2. Feed formulation for *Labeo rohita* fry and fingerlings

	% of Dry Diet of Fingerling				**% of Dry Diet of Fry**			
Ingredients	**Control**	**0.67%**	**1.33%**	**2%**	**Control**	**0.67%**	**1.33%**	**2%**
Fishmeal	15.00	15.00	15.00	15.00	16.00	16.00	16.00	16.00
Soybean meal	8.0	3.0	4.0	6.0	5.0	5.0	5.0	9.0
Maize gluten	25.0	30.0	30.0	30.0	36.0	37.0	38.5	37.0
Wheat bran	4.0	4.0	4.0	4.0	2.0	2.0	2.0	2.0
Rice polishing	29.0	30.0	30.0	28.0	28.0	28.0	26.0	23.0
Maize grains	5.0	4.0	4.0	4.0	2.0	1.0	1.5	2.0
Molasses	6.0	6.0	5.0	5.0	3.0	3.0	3.0	3.0
Fish oil	2.0	1.3	0.7	0.0	2.0	1.3	0.7	0.0
Chlorella vulgaris oil	0.0	0.7	1.3	2.0	0.0	0.7	1.3	2.0
*Mineral mixture	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
**Vitamin Premix	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate Analysis								
CP%	35.06	35.37	35.03	34.92	40.09	39.91	39.92	39.65
Crude Fat	6.56	6.93	7.17	7.14	6.51	6.72	6.71	6.55
Ash	9.54	9.18	8.87	8.59	9.04	8.81	8.45	8.18
Fiber	3.16	3.29	3.71	4.18	2.59	2.99	3.34	3.91
GE, Mj/kg	19.90	20.04	20.04	19.97	20.65	20.64	20.62	20.48
Cp/E	1.76	1.76	1.75	1.75	1.94	1.93	1.94	1.94

*Mineral mixture consists of the following:
($COCl.\ 6H_2O$ 0.0816 mg/g) ($ZnSO_4.\ 7\ H_2O$ 121.33 mg/g) (KH_2PO_4 479 mg/g) ($AlCl_3.6H_2O$ 0.25 mg/g)

($MnSO_4 \cdot 5H_2O$ 116.67 mg/g) ($CuSO_4 \cdot 5H_2O$ 210.67 mg/g) ($CaCO_3$ 316 mg/g) (KH_2PO_4 479 mg/g)
($MgSO_4 \cdot 7H_2O$ 153 mg/g) (NaCl 51 mg/g)
**Vitamin premix contains:
(Calcium pantothenate 10000 mg/g) (Vitamin B_{12} 9000 mg/g) (Vitamin C 15000 mg/g) (Vitamin D_3 3 M.I.U)
(Nicotinic acid 25000 mg/g) (Vitamin K_3 4000 mg/g) (Vitamin B_6 4000 mg/g) (Vitamin A 15 M.I.U)
(Vitamin B_2 6000 mg/g) (folic acid 750 mg/g) (Vitamin B_1 5000 mg/g) (Vitamin E 6 M.I.U)

Experimental trial

Fry and fingerlings of grass carp and rohu were purchased from the Fish Farm and Hatchery Facility of the Department of Fisheries and Aquaculture, UVAS, Ravi Campus. Fishes were bathed in $KMnO_4$ (4 ppm for 10 minutes) solution and acclimatized for 15 days. After acclimation, the number of fry was estimated by using the volumetric method and stocked randomly in the aquarium (89 x 58 x 61cm) at the rate of 100 fry per liter of water. After being fasted for two days, fish fingerlings were weighed (10.0±2.0 g) and stocked at the rate of 20 fish/aquarium in 16 experimental aquariums for both species. The diet without algae oil was fed to the fish of the control group, whereas the other 3 experimental groups were given the feed replaced with 0.67%, 1.33%, and 2% *Chlorella* oil instead of fish oil. The variables of water quality, such as temperature (24–28°C), DO (6.6–7.2), and pH (7.4–8.6) of water were regularly maintained (Tayyaba et al., 2024). A daily change of 1/3rd of the volume of water was done during the trial. The feed was given to fingerlings (3% body weight) and fry (5% body weight) three times per day up to apparent satiation six days a week. After two hours of each feeding session, unconsumed feed was siphoned off in order to assess the feed intake and FCR. The trial was carried out for 90 days, and data were analyzed through factorial ANOVA using PROC GLM in SAS software (version 9.1), considering species and treatments as main effects, and their interaction was tested, too.

Growth performance

The growth performance was calculated by using equations given below (Sughra et al., 2021).

$$\text{Net weight gain} = \text{Average final weight} - \text{Average initial weight}$$

$$\text{Percent weight gain} = (\text{Net weight gain})/(\text{Initial weight}) \times 100$$

$$\text{SGR \%} = (\text{Ln (Final wet body weight} - \text{Ln (Initial wet body weight))}/(\text{No. of days}) \times 100$$

$$\text{FCR} = (\text{Feed intake})/(\text{Net weight gain})$$

Color evaluation

Fish body color evaluation such as lightness (L*), yellowness (b*), and redness (a*) was done by using a chroma meter from two sides of the fish body i) ventral side color (the place between the 2 fins of pectoral) and ii) dorsal side color (place at the start of dorsal fin) (Gouveia et al., 2003).

Proximate composition of fish and feed

After termination of the feeding trial, fish were starved for twenty-four hours, and five from each experimental group were subjected to proximate composition along with the experimental diets according to the AOAC (2016) standard procedures.

Fatty acid composition of experimental diets and fish

Five fingerlings and 10 fry were killed ethically using MS-222 (250mg/l) and were subjected to fatty acid analysis. Samples were ground and homogenized. Lipid extraction was done by chloroform/methanol in 2:1 v/v (Folch et al., 1957) and transmethylated to obtain fatty acid methyl esters (FAMES) (Christie, 1989). Gas-liquid chromatography was used for the separation of FAMES (Izquierdo et al., 1990) and quantification was done with a flame ionization detector, and was identified by comparing with characterized standards.

Statistical analysis

Data were presented as means ± standard error of means (SEM) of three replicates. Data were analyzed through factorial ANOVA using PROC GLM in SAS software (version 9.1), considering species and treatments as main effects, and their interaction was tested, too. For the comparison of significant treatment means, Duncan's Multiple Range test was applied. Superscripts on different means within row differ significantly at $P \leq 0.05$. The following mathematical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\times\beta)_{ij} + \epsilon_{ijk}$$

Where,

Y_{ijk} = observation of dependent variable recorded on i^{th} and j^{th} treatment groups

α_i = effect of i^{th} species (i = 1, 2)

β_j = effect of j^{th} treatment (j = 1,2,3,4)

$(\alpha\times\beta)_{ij}$ = interaction effect between i^{th} and j^{th} treatments

ϵ_{ijk} = residual effect associated with i^{th} and j^{th} treatments, NID ~ 0, σ^2

Results

Growth performance

Regarding species, a significant difference was observed in FW, WG%, and FCR in fingerlings. Higher WG% and best FCR were obtained in rohu as compared to grass carp fingerlings (Table 3). In fry, significant differences were observed in AWG, WG%, SGR, and FCR. Higher WG%

was obtained in rohu as compared to grass carp fry, and a better FCR was observed in rohu than in grass carp fry.

Among treatments, in fingerlings, a significant difference was observed in FW, AWG, WG%, SGR, and FCR. Higher FW, AWG, and WG% were obtained in fish fed with 1.33% algae oil in the diet, followed by fish fed with 2%, 0.67%, and the control group. Higher SR and best FCR were noted in fish fed with 1.33% algae oil in the diet in fingerlings. Similarly, in fry significant difference was observed in FW, AWG, WG%, SGR, FCR, and SR. Higher FW, AWG, and WG% were obtained in fish fed with 1.33% algae oil in the diet followed by fish fed with 2%, 0.67%, and the control group. Higher SR and best FCR were noted in fish fed with 1.33% algae oil in the diet.

Species and treatment analysis in fingerlings showed significant differences in FCR. However, in fry significant difference was shown in FW, AWG, WG%, and FCR.

Table 3. Growth performance of Grass carp and rohu (fingerling and fry) fed with different levels of algae (Mean ± SEM)

Traits	**Species**		**Treatment**				**SEM**	**P- value**		
	Grass carp	**Rohu**	**Control**	**0.67%**	**1.33%**	**2%**		**Species**	**Treatment**	**Interaction**
Fingerling										
IW (g)	11.29^{a}	9.98^{b}	10.51	10.53	10.74	10.77	0.16	<0.0001	0.5520	0.5973
FW (g)	30.37^{a}	28.69^{b}	25.63^{c}	28.62^{b}	33.91^{a}	29.97^{b}	0.72	0.0198	<0.0001	0.1688
AWG(g)	19.13	18.71	15.12^{c}	18.09^{b}	23.17^{a}	19.30^{b}	0.67	0.4984	<0.0001	0.1093
WG (%)	169.23^{b}	187.40^{a}	144.45^{c}	171.61^{b}	216.50^{a}	180.69^{b}	6.40	0.0052	<0.0001	0.0618
SGR	1.32	1.31	1.22^{c}	1.30^{b}	1.40^{a}	1.33^{b}	0.02	0.4624	<0.0001	0.0837
FCR	1.68^{a}	1.65^{b}	1.94^{a}	1.72^{b}	1.42^{d}	1.59^{c}	0.04	0.0003	<0.0001	<0.0001
SR (%)	93.17	93.83	94.33^{a}	93.67^{a}	97.33^{a}	88.67^{b}	0.84	0.6066	0.0017	0.9359
Fry										
IW (g)	1.52^{a}	1.46^{b}	1.49^{b}	1.49^{b}	1.49^{b}	1.50^{a}	0.01	<.0001	0.0500	0.0377
FW (g)	6.10	6.12	5.59^{d}	6.19^{b}	6.82^{a}	5.85^{c}	0.10	0.6027	<.0001	0.0206
AWG(g)	4.59^{b}	4.66^{a}	4.11^{d}	4.70^{b}	5.33^{a}	4.35^{c}	0.10	0.0275	<.0001	0.0527
WG (%)	302.28^{b}	314.37^{a}	276.30^{c}	316.71^{b}	358.06^{a}	282.22^{c}	7.21	0.0140	<.0001	0.2689
SGR	0.70^{b}	0.71^{a}	0.65^{d}	0.71^{b}	0.77^{a}	0.68^{c}	0.01	0.0417	<.0001	0.0965
FCR	1.42^{b}	1.57^{a}	1.70^{a}	1.53^{b}	1.30^{d}	1.45^{c}	0.03	<.0001	<.0001	<.0001
SR (%)	94.50	93.83	94.67^{b}	94.00^{b}	97.67^{a}	90.33^{c}	0.59	0.2011	<.0001	0.8275

Coloration

Coloration of grass carp and rohu (fingerling and fry) was given in Table 4. In both fingerlings and fry, significant differences were present in L*, a*, and b* regarding species and treatment groups. Grass carp (fingerling and fry) showed higher coloration than rohu (fingerling and fry). Among treatments, the highest coloration was observed in treatment with a 2% addition of algae oil in the diet, followed by 1.33%, 0.67%, and then the control group. The analysis of species and treatment interaction showed that no significant differences were observed in both fingerling and fry. The coloration becomes more pronounced with the increase in the level of algae oil in the diet.

Table 4. Coloration of grass carp and rohu (fry and fingerling) fed with different levels of algae (Mean ± SEM)

Traits	Species		Treatment				SEM	P- value		
	Grass carp	Rohu	Control	0.67%	1.33%	2%		Species	Treatment	Interaction
Fingerling										
L*	54.10[a]	52.57[b]	45.74[d]	50.77[c]	56.99[b]	59.99[a]	1.17	0.0019	<.0001	0.8515
a*	21.49[a]	20.32[b]	12.00[d]	18.39[c]	23.34[b]	29.89[a]	1.39	0.0124	<.0001	0.3566
b*	21.57[a]	19.71[b]	13.28[d]	17.21[c]	23.58[b]	28.49[a]	1.25	0.0003	<.0001	0.3816
Fry										
L*	34.34[a]	31.72[b]	25.54[d]	29.95[c]	34.85[b]	41.79[a]	1.30	<.0001	<.0001	0.3241
a*	18.23[a]	17.07[b]	8.90[d]	15.89[c]	20.31[b]	25.50[a]	1.29	0.0133	<.0001	0.4535
b*	17.56[a]	16.15[b]	9.35[d]	13.75[c]	19.28[b]	25.03[a]	1.25	0.0034	<.0001	0.8456

Proximate composition

Proximate analysis of grass carp and rohu (fingerling and fry) was given in Table 5. Species comparison showed a significant difference in moisture in fingerlings. Grass carp fingerlings showed higher moisture content than rohu fingerlings. In fry, no significant difference was present in moisture, protein, fat, and ash content. Regarding treatment groups, a significant difference was recorded in all treatments except fat in fingerlings. Higher protein and moisture content was noted in treatment with 1.33% algae oil in the diet followed by 2%, 0.67%, and control group. Higher ash content was observed in the control group and the lowest ash content was noted in treatment with 1.33% algae oil in diet. In fry, a significant difference was observed in moisture and fat contents. The highest moisture content was recorded in treatment with 1.33% algae oil in the diet followed by 0.67%, control group, and then 2% treatment level.

Significant interactions were shown in moisture and protein content in fingerlings. However, no significant differences in interactions were observed in moisture, protein, fat, and ash content in fry.

Table 5. Proximate composition of grass carp and rohu (fry and fingerling) fed with different levels of algae (Mean ± SEM)

Traits	Species		Treatment				SEM	P- value		
	Grass carp	Rohu	Control	0.67%	1.33%	2%		Species	Treatment	Interaction
Fingerling										
Moisture	88.66[a]	86.30[b]	81.93[d]	83.62[c]	94.92[a]	89.45[b]	1.13	<.0001	<.0001	0.0038

Protein	15.94	16.19	10.69^{d}	15.53^{c}	19.77^{a}	18.29^{b}	0.77	0.5552	<.0001	0.0040
Fat	4.10	3.77	3.01d	4.16c	4.95a	3.63b	0.19	0.2289	0.0006	0.7842
Ash	4.61	4.48	4.98^{a}	4.67^{ab}	4.07^{c}	4.46^{bc}	0.09	0.3836	0.0032	0.9325
Fry										
Moisture	72.72	72.63	71.34^{b}	73.51^{a}	74.61^{a}	71.26^{b}	0.34	0.8253	<.0001	0.9999
Protein	18.12	17.64	17.28	17.90	18.35	17.99	0.20	0.2670	0.3508	0.8176
Fat	8.80	8.04	8.09^{b}	8.70^{ab}	9.45^{a}	7.45^{b}	0.25	0.0925	0.0240	0.8999
Ash	4.63	4.35	4.82	4.56	3.98	4.60	0.19	0.5228	0.5472	0.9699

Fatty acid composition of the diet

The diet without algae oil had the highest EPA (eicosapentaenoic acid) content (20:5n-3), consequently the highest levels of EPA/ARA (Arachidonic acid) and EPA/DHA (Docosahexaenoic acid), as well as the highest content of linoleic acid (LNA = 18:3n-3). The diets with algae oil had higher levels of n-3 LC-PUFA, specifically DHA (22:6n-3) and n-6 DPA (22:5n-6), as well as a higher n-3/n-6, than the diet without algae oil. The diet with 1.33% inclusion of algae oil had the highest levels of n-6 DPA and n-3 LC-PUFA (long chain polyunsaturated fatty acid). The diet containing 2% algal oil had the highest content of SFA (saturated fatty acid), especially palmitic acid (16:0), while the diet containing 0.67% algae oil had a slightly higher content of n-3/n-6 fatty acids. Thus, compared to the control diet, the inclusion of 2% or 1.33% algae oil doubled the contents of SFA and all the algae oil-containing diets increased the DHA content by almost fourfold (Table 6 and Table 7).

Table 6. Fatty acid composition of the diet of grass carp (fry and fingerlings)

	Fry				**Fingerlings**			
Fatty acids (% total FA)	**Control**	**0.67%**	**1.33%**	**2%**	**Control**	**0.67%**	**1.33%**	**2%**
14:0	0.51	1.16	1.51	1.15	0.65	1.19	1.65	1.19
14:1n-7	0.01	0.01	0.01	0.01	0.05	0.04	0.05	0.05
14:1n-5	0.05	0.01	0.01	0.01	0.09	0.04	0.02	0.04
15:0	0.09	0.53	0.13	0.19	0.13	0.67	0.17	0.25
15:1n-5	0.01	0.01	0.01	0.01	0.05	0.05	0.04	0.03
16:0ISO	0.05	0.02	0.01	0.01	0.09	0.06	0.03	0.03
16:0	10.24	27.39	14.24	31.64	13.28	29.55	17.27	34.68
16:1n-7	0.48	0.24	0.42	0.17	1.52	0.28	0.46	0.27
7716:1n-5	0.14	0.02	0.01	0.01	0.18	0.06	0.05	0.05
16:2n-4	0.15	0.02	0.01	0.01	0.19	0.05	0.04	0.04
17:0	0.07	0.02	0.01	0.01	0.08	0.05	0.02	0.05

16:3n-4	0.17	0.03	0.04	0.04	0.18	0.07	0.08	0.08
16:3n-3	0.11	0.02	0.02	0.02	0.15	0.06	0.06	0.06
16:3n-1	0.10	0.09	0.08	0.07	0.14	0.13	0.12	0.09
16:4n-3	0.06	0.01	0.01	0.03	0.09	0.05	0.04	0.07
16:4n-1	ND	0.01	0.01	0.01	ND	0.05	0.03	0.03
18:0	2.74	3.47	2.05	3.25	3.78	3.52	3.09	3.39
18:1n-9	25.04	5.28	8.88	4.41	29.08	7.33	11.93	7.45
18:1n-7	1.50	1.02	1.12	0.86	2.54	1.06	1.27	0.89
18:1n-5	0.07	0.02	0.03	0.02	0.12	0.06	0.07	0.06
18:2n-9	0.07	0.01	0.03	0.00	0.13	0.04	0.07	0.00
18:2n-6	23.93	20.75	21.50	18.82	26.98	23.79	24.65	21.97
18:2n-4	0.07	0.02	0.01	0.01	0.12	0.05	0.03	0.05
18:3n-6	0.21	0.06	0.13	0.08	0.26	0.09	0.17	0.13
18:3n-4	0.14	0.02	0.01	0.01	0.18	0.06	0.05	0.05
18:3n-3	3.20	2.00	2.61	1.78	4.24	2.04	2.76	1.84
18:3n-1	ND	ND	0.01	0.01	ND	ND	0.02	0.03
18:4n-3	0.40	0.06	0.16	0.08	0.54	0.09	0.19	0.13
18:4n-1	0.16	0.04	0.01	0.01	0.19	0.08	0.05	0.04
20:0	0.28	0.29	0.22	0.22	0.32	0.33	0.27	0.27
20:1n-9	0.31	0.07	0.10	0.06	0.45	0.09	0.14	0.09
20:1n-7	2.31	1.30	1.34	1.25	3.45	1.45	1.48	1.39
20:1n-5	0.15	0.03	0.04	0.02	0.19	0.07	0.08	0.07
20:2n-9	0.10	0.03	0.07	0.01	0.14	0.08	0.09	0.05
20:2n-6	0.65	0.07	0.15	0.06	0.6	0.09	0.19	0.09
20:3n-9	0.05	0.06	0.20	0.09	0.09	0.09	0.25	0.13
20:3n-6	0.15	0.04	0.01	0.02	0.29	0.07	0.05	0.06
20:4n-6	0.24	0.74	0.84	0.25	0.39	0.78	0.98	0.29
20:3n-3	0.28	0.18	0.19	0.08	0.32	0.23	0.23	0.12
20:4n-3	0.49	0.29	0.48	0.25	0.53	0.34	0.53	0.29
20:5n-3	1.55	0.82	1.22	0.86	2.69	0.87	1.26	0.89
22:1n-1	0.69	0.06	0.21	0.26	1.81	0.09	0.25	0.29
22:1n-9	0.40	0.23	0.28	0.02	0.54	0.27	0.33	0.06
22:4n-6	0.18	0.05	0.06	0.02	0.16	0.09	0.09	0.06
22:5n-6	0.18	2.36	6.25	4.37	0.22	3.50	9.29	6.40

22:5n-3	0.86	0.16	0.41	0.10	0.99	0.19	0.45	0.15
22:6n-3	4.37	20.61	22.48	19.24	5.52	23.65	25.53	22.29
SFA	15.20	33.19	19.35	36.66	18.25	36.23	22.39	39.70
MUFA	25.79	8.52	12.75	7.31	38.83	10.68	15.89	9.49
n-3	12.89	24.31	27.73	22.51	15.92	27.35	30.87	25.65
n-6	26.72	25.30	31.26	24.83	29.86	28.35	34.39	27.87
n-9	26.28	5.78	9.65	4.67	29.32	8.88	12.69	7.70
n-3/n-6	0.50	0.94	0.88	0.90	0.54	0.99	0.93	0.95
n-3 LC-PUFA	8.73	22.14	24.86	20.53	9.98	25.18	27.89	23.67
n-6 LC PUFA	1.62	2.35	8.39	3.70	1.66	4.49	9.54	6.85
EPA/ARA	6.2	1.0	1.1	2.0	8.6	1.2	1.5	3.5
EPA/DHA	0.3	0.1	0.1	0.1	0.7	0.1	0.1	0.1

Table 7. Fatty acid composition of diet of rohu (fry and fingerlings)

	Fry				**Fingerlings**			
Fatty acids (% total FA)	**Control**	**0.67%**	**1.33%**	**2%**	**Control**	**0.67%**	**1.33%**	**2%**
14:0	0.49	1.14	1.49	1.13	0.52	1.17	1.52	1.16
14:1n-7	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
14:1n-5	0.03	0.01	0.01	0.01	0.06	0.02	0.01	0.01
15:0	0.07	0.50	0.10	0.15	0.10	0.54	0.14	0.20
15:1n-5	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01
16:0ISO	0.03	0.01	0.01	0.01	0.06	0.03	0.01	0.01
16:0	9.23	26.35	13.20	30.53	11.25	28.40	15.25	32.65
16:1n-7	0.45	0.22	0.40	0.15	0.49	0.25	0.43	0.18
7716:1n-5	0.13	0.02	0.01	0.01	0.15	0.03	0.02	0.02
16:2n-4	0.13	0.01	0.01	0.01	0.16	0.03	0.01	0.01
17:0	0.04	0.02	0.01	0.01	0.08	0.02	0.01	0.01
16:3n-4	0.13	0.02	0.03	0.03	0.18	0.04	0.05	0.05
16:3n-3	0.8	0.02	0.02	0.02	0.12	0.03	0.03	0.03
16:3n-1	0.9	0.08	0.07	0.05	0.11	0.10	0.09	0.08
16:4n-3	0.04	0.01	0.01	0.02	0.07	0.01	0.01	0.04
16:4n-1	ND	0.01	0.01	0.01	ND	0.01	0.01	0.01
18:0	2.73	2.46	2.00	3.23	2.75	3.48	3.06	3.26
18:1n-9	24.02	4.25	7.80	4.39	26.05	6.29	9.89	5.42
18:1n-7	1.49	1.01	1.10	0.80	1.51	1.03	1.13	0.87

18:1n-5	0.05	0.01	0.02	0.02	0.08	0.03	0.04	0.03
18:2n-9	0.05	0.01	0.02	0.00	0.08	0.01	0.04	0.00
18:2n-6	22.90	19.73	20.48	17.80	24.94	21.76	22.51	19.83
18:2n-4	0.05	0.02	0.01	0.01	0.08	0.02	0.01	0.01
18:3n-6	0.19	0.05	0.10	0.06	0.22	0.07	0.14	0.09
18:3n-4	0.12	0.02	0.01	0.01	0.15	0.03	0.02	0.02
18:3n-3	2.18	1.09	2.58	1.75	3.21	2.01	2.62	1.79
18:3n-1	ND	ND	0.01	0.01	ND	ND	0.01	0.01
18:4n-3	0.39	0.04	0.12	0.05	0.41	0.07	0.17	0.09
18:4n-1	0.14	0.02	0.01	0.01	0.17	0.05	0.02	0.01
20:0	0.25	0.25	0.19	0.20	0.29	0.30	0.23	0.23
20:1n-9	0.31	0.05	0.08	0.05	0.32	0.08	0.11	0.07
20:1n-7	2.29	1.26	1.32	1.23	2.32	1.31	1.35	1.26
20:1n-5	0.13	0.02	0.02	0.02	0.16	0.04	0.05	0.03
20:2n-9	0.08	0.02	0.05	0.01	0.11	0.04	0.08	0.01
20:2n-6	0.54	0.05	0.13	0.05	0.66	0.08	0.16	0.07
20:3n-9	0.04	0.04	0.18	0.06	0.06	0.07	0.21	0.10
20:3n-6	0.13	0.03	0.01	0.02	0.16	0.05	0.02	0.03
20:4n-6	0.23	0.71	0.81	0.23	0.25	0.75	0.95	0.26
20:3n-3	0.25	0.15	0.15	0.06	0.29	0.19	0.20	0.09
20:4n-3	0.45	0.25	0.44	0.24	0.50	0.30	0.49	0.26
20:5n-3	1.53	0.80	1.20	0.84	1.56	0.83	1.23	0.87
22:1n-1	0.66	0.05	0.19	0.24	0.68	0.07	0.22	0.27
22:1n-9	0.38	0.20	0.25	0.02	0.41	0.24	0.29	0.03
22:4n-6	0.15	0.03	0.05	0.02	0.13	0.06	0.07	0.03
22:5n-6	0.15	2.33	5.21	3.35	0.19	2.37	7.26	4.38
22:5n-3	0.84	0.13	0.39	0.09	0.87	0.17	0.42	0.11
22:6n-3	3.36	19.60	21.43	17.21	4.38	21.62	23.49	20.25
SFA	14.19	31.15	18.33	35.65	16.21	34.20	20.36	37.67
MUFA	24.78	7.50	11.74	6.30	26.80	9.53	13.76	8.32
n-3	11.87	22.29	26.70	21.49	13.90	25.32	28.74	23.52
n-6	24.69	23.28	30.24	23.80	27.73	26.31	32.27	25.84
n-9	23.25	4.75	8.63	4.65	27.29	6.79	10.66	5.68
n-3/n-6	0.48	0.92	0.83	0.89	0.51	0.95	0.89	0.91
n-3 LC-PUFA	7.72	21.12	23.83	19.52	8.84	23.15	25.87	21.54
n-6 LC-PUFA	1.60	2.24	7.35	3.69	1.63	3.36	8.40	4.71
EPA/ARA	5.1	1.0	1.1	2.0	6.3	1.0	1.2	2.1
EPA/DHA	0.2	0.1	0.1	0.1	0.4	0.1	0.1	0.1

ND, not determined

Fatty acid profile of the whole body

The fatty acid analysis of grass carp and rohu fingerlings, as depicted in Table 8, revealed significant differences regarding species, except for 18:2n-6 and 20:3n-6, and the ratio of n-3/n-6. Across various treatment groups, significant differences were observed in most fatty acids, except for 18:2n-6. The analysis of species and treatments interaction showed significant differences in numerous fatty acids, including 14:1n-7, 14:1n-5, 15:00, 16:00, 16:1n-7, 18:00, 18:1n-9, 18:1n-7, 18:2n-9, 18:3n-6, 20:1n-7, 20:5n-3, 22:5n-6, 22:6n-3, n-3, n-6, n-9, and n-3/n-6.

The fatty acid analysis of grass carp and rohu fry, as presented in Table 9, showed significant differences regarding species, except for fatty acids 18:2n-9, 20:1n-7, 20:3n-9, 20:4n-3, and 20:5n-3. Among treatments, a significant difference was observed in most fatty acids, except in 18:2n-9, 20:3n-9, and 20:4n-3. The analysis of species and treatments showed significant differences in specific fatty acids, including 16:00, 16:1n-5, 17:00, 16:3n-1, 18:00, 18:1n-9, 18:2n-6, and 20:2n-6.

Table 8. Fatty acid profile of grass carp and rohu fingerlings (Mean ± SEM)

Traits	**Species**		**Treatment**				**SEM**	**P- value**		
	Grass carp	**Rohu**	**Control**	**0.67%**	**1.33%**	**2%**		**Species**	**Treatment**	**Interaction**
14:00	0.60^{a}	0.47^{b}	0.57^{a}	0.57^{a}	0.54^{b}	0.46^{c}	0.02	<.0001	<.0001	1.0000
14:1n-7	0.19^{a}	0.14^{b}	0.20^{b}	0.17^{c}	0.26^{a}	0.03^{d}	0.02	<.0001	<.0001	<.0001
14:1n-5	0.28^{a}	0.17^{b}	0.31^{a}	0.27^{b}	0.30^{a}	0.03^{c}	0.03	<.0001	<.0001	<.0001
15:00	0.38^{a}	0.30^{b}	0.46^{a}	0.38^{b}	0.34^{c}	0.18^{d}	0.02	<.0001	<.0001	<.0001
15:1n-5	0.21^{a}	0.18^{b}	0.20^{c}	0.26^{b}	0.28^{a}	0.04^{d}	0.02	<.0001	<.0001	0.4182
16:0ISO	0.18^{a}	0.16^{b}	0.20^{b}	0.24^{a}	0.20^{b}	0.04^{c}	0.02	<.0001	<.0001	0.6876
16:00	16.83^{a}	13.83^{b}	14.40^{c}	16.03^{b}	11.56^{d}	19.33^{a}	0.66	<.0001	<.0001	0.0258
16:1n-7	0.90^{a}	0.79^{b}	0.96^{a}	0.83^{c}	0.70^{d}	0.91^{b}	0.02	<.0001	<.0001	<.0001
16:1n-5	0.26^{a}	0.23^{b}	0.29^{b}	0.31^{a}	0.27^{c}	0.11^{d}	0.02	<.0001	<.0001	0.6876
16:2n-4	0.35^{a}	0.32^{b}	0.32^{c}	0.38^{a}	0.36^{b}	0.28^{d}	0.01	<.0001	<.0001	1.0000
17:00	0.24^{a}	0.21^{b}	0.29^{a}	0.25^{c}	0.27^{b}	0.09^{d}	0.02	<.0001	<.0001	1.0000
16:3n-4	0.28^{a}	0.25^{b}	0.30^{b}	0.28^{c}	0.32^{a}	0.17^{d}	0.01	<.0001	<.0001	0.7722
16:3n-3	0.23^{a}	0.19^{b}	0.22^{c}	0.26^{b}	0.32^{a}	0.06^{d}	0.02	<.0001	<.0001	1.0000
16:3n-1	0.76^{a}	0.73^{b}	0.67^{d}	0.81^{a}	0.74^{c}	0.78^{b}	0.01	<.0001	<.0001	0.4661
16:4n-3	0.40^{a}	0.38^{b}	0.49^{a}	0.36^{c}	0.41^{b}	0.31^{d}	0.01	<.0001	<.0001	0.7722
16:4n-1	0.18^{a}	0.15^{b}	0.23^{b}	0.26^{a}	0.09^{c}	0.09^{c}	0.02	<.0001	<.0001	1.0000
18:00	8.87^{a}	6.86^{b}	7.50^{c}	7.99^{b}	6.96^{d}	9.01^{a}	0.26	<.0001	<.0001	0.0198
18:1n-9	13.22^{a}	11.23^{b}	16.46^{a}	10.35^{c}	9.25^{d}	12.85^{b}	0.62	<.0001	<.0001	<.0001
18:1n-7	2.84^{a}	1.54^{b}	2.35^{a}	2.10^{c}	2.00^{d}	2.32^{b}	0.15	<.0001	<.0001	<.0001
18:1n-5	0.22^{a}	0.19^{b}	0.25^{ab}	0.26^{a}	0.24^{b}	0.07^{c}	0.02	<.0001	<.0001	1.0000
18:2n-9	1.79^{a}	1.01^{b}	2.44^{a}	0.85^{c}	0.43^{d}	1.88^{b}	0.20	<.0001	<.0001	<.0001
18:2n-6	14.69	23.24	14.91	33.20	10.88	16.89	5.29	0.4417	0.5017	0.4377
18:2n-4	0.21^{a}	0.17^{b}	0.23^{b}	0.22^{b}	0.28^{a}	0.04^{c}	0.02	<.0001	<.0001	0.4182
18:3n-6	2.20^{a}	1.67^{b}	2.76^{a}	1.31^{b}	0.94^{c}	2.76^{a}	0.19	<.0001	<.0001	<.0001
18:3n-4	0.23^{a}	0.20^{b}	0.22^{c}	0.25^{b}	0.31^{a}	0.09^{d}	0.02	<.0001	<.0001	0.7722
18:3n-3	1.02^{a}	0.99^{b}	1.23^{b}	0.68^{d}	0.78^{c}	1.34^{a}	0.06	<.0001	<.0001	0.6876

18:4n-3	0.37^{a}	0.34^{b}	0.44^{a}	0.38^{b}	0.33^{c}	0.27^{d}	0.01	<.0001	<.0001	1.0000
18:4n-1	0.19^{a}	0.16^{b}	0.19^{b}	0.24^{a}	0.24^{a}	0.05^{c}	0.02	<.0001	<.0001	0.7722
20:00	0.43^{a}	0.40^{b}	0.49^{a}	0.38^{c}	0.44^{b}	0.37^{d}	0.01	<.0001	<.0001	0.4182
20:1n-9	0.28^{a}	0.24^{b}	0.35^{a}	0.28^{b}	0.28^{b}	0.14^{c}	0.02	<.0001	<.0001	0.7722
20:1n-7	1.33^{a}	1.27^{b}	1.60^{a}	1.17^{d}	1.21^{c}	1.23^{b}	0.04	<.0001	<.0001	<.0001
20:1n-5	0.28^{a}	0.25^{b}	0.31^{a}	0.27^{b}	0.31^{a}	0.17^{c}	0.01	<.0001	<.0001	0.7722
20:2n-9	0.49^{a}	0.46^{b}	0.70^{a}	0.44^{c}	0.29^{d}	0.48^{b}	0.03	<.0001	<.0001	1.0000
20:2n-6	0.62^{a}	0.59^{b}	0.80^{a}	0.62^{b}	0.54^{c}	0.46^{d}	0.03	<.0001	<.0001	0.7722
20:3n-9	0.25^{a}	0.23^{b}	0.22^{c}	0.41^{a}	0.30^{b}	0.04^{d}	0.03	<.0001	<.0001	0.7722
20:3n-6	0.89	0.99	1.24^{a}	0.63^{c}	0.91^{b}	0.98^{b}	0.06	0.2016	0.0003	0.0826
20:4n-6	1.81^{a}	1.77^{b}	1.65^{c}	1.75^{b}	2.12^{a}	1.65^{c}	0.04	<.0001	<.0001	0.5505
20:3n-3	0.43^{a}	0.40^{b}	0.42^{c}	0.57^{a}	0.44^{b}	0.25^{d}	0.02	<.0001	<.0001	0.7722
20:4n-3	0.45^{a}	0.42^{b}	0.60^{a}	0.27^{d}	0.48^{b}	0.39^{c}	0.03	<.0001	<.0001	1.0000
20:5n-3	2.16^{a}	1.63^{b}	3.03^{a}	1.38^{c}	1.36^{d}	1.82^{b}	0.18	<.0001	<.0001	<.0001
22:1n-1	0.82^{a}	0.79^{b}	0.68^{b}	0.52^{c}	1.78^{a}	0.24^{d}	0.12	<.0001	<.0001	0.9906
22:1n-9	0.96^{a}	0.92^{b}	0.97^{b}	0.84^{c}	1.56^{a}	0.39^{d}	0.09	<.0001	<.0001	0.6792
22:4n-6	0.45^{a}	0.42^{b}	0.51^{b}	0.51^{b}	0.52^{a}	0.22^{c}	0.03	<.0001	<.0001	0.7722
22:5n-6	4.90^{a}	3.61^{b}	1.25^{d}	4.94^{b}	7.22^{a}	3.62^{c}	0.48	<.0001	<.0001	<.0001
22:5n-3	1.07^{a}	1.04^{b}	1.75^{a}	0.79^{c}	0.60^{d}	1.08^{b}	0.09	<.0001	<.0001	0.6876
22:6n-3	23.16^{a}	20.31^{b}	14.38^{d}	26.29^{b}	29.47^{a}	16.80^{c}	1.35	<.0001	<.0001	<.0001
SFA	26.25^{a}	23.97^{b}	24.26^{c}	26.18^{b}	20.65^{d}	29.36^{a}	0.70	<.0001	<.0001	<.0001
MUFA	20.54^{a}	18.50^{b}	25.24^{a}	16.78^{d}	18.19^{b}	17.88^{c}	0.73	<.0001	<.0001	0.2618
n-3	28.45^{a}	25.92^{b}	21.58^{d}	30.84^{b}	34.20^{a}	22.14^{c}	1.17	<.0001	<.0001	<.0001
n-6	24.38^{a}	22.10^{b}	22.63^{c}	21.60^{d}	22.64^{b}	26.09^{a}	0.43	<.0001	<.0001	<.0001
n-9	16.52^{a}	14.60^{b}	21.38^{a}	13.07^{c}	12.58^{d}	15.23^{b}	0.76	<.0001	<.0001	<.0001
n-3/n-6	1.08	1.20	0.68^{b}	1.44^{a}	1.57^{a}	0.88^{b}	0.09	0.1317	<.0001	0.0268
n-3 LC-PUFA	27.01^{a}	24.23^{b}	19.72^{c}	28.71^{b}	33.38^{a}	20.70^{c}	1.24	<.0001	<.0001	0.4587

n-6 LC-PUFA	8.96^{a}	6.94^{b}	5.43^{d}	8.17^{b}	11.28^{a}	6.93^{c}	0.50	<.0001	<.0001	0.7722
EPA/ARA	1.09^{a}	1.06^{b}	1.83^{a}	0.80^{c}	0.65^{d}	1.04^{b}	0.09	<.0001	<.0001	0.2526
EPA/DHA	0.14^{a}	0.12^{b}	0.24^{a}	0.07^{c}	0.06^{c}	0.15^{b}	0.02	<.0001	<.0001	0.7722

Table 9. Fatty acid profile of grass carp and rohu fry (Mean ± SEM)

Traits	**Species**		**Treatment**				**SEM**	**P-value**		
	Grasscarp	**Rohu**	**Control**	**0.67%**	**1.33%**	**2%**		**Species**	**Treatment**	**Interaction**
14:00	0.46^{a}	0.43^{b}	0.48^{a}	0.48^{a}	0.45^{b}	0.37^{c}	0.01	<.0001	<.0001	0.6876
14:1n-7	0.13^{a}	0.11^{b}	0.16^{a}	0.13^{b}	0.17^{a}	0.02^{c}	0.01	0.0021	<.0001	0.2526
14:1n-5	0.17^{a}	0.15^{b}	0.22^{a}	0.18^{b}	0.21^{a}	0.02^{c}	0.02	0.0021	<.0001	0.2526
15:00	0.29^{a}	0.26^{b}	0.37^{a}	0.29^{b}	0.30^{b}	0.14^{c}	0.02	<.0001	<.0001	0.6876
15:1n-5	0.17^{a}	0.15^{b}	0.16^{c}	0.22^{b}	0.24^{a}	0.03^{d}	0.02	0.0006	<.0001	0.7722
16:0ISO	0.15^{a}	0.13^{b}	0.17^{b}	0.21^{a}	0.16^{b}	0.03^{c}	0.01	0.0021	<.0001	0.6876
16:00	12.55^{a}	10.53^{b}	10.86^{c}	12.49^{b}	7.01^{d}	15.78^{a}	0.69	<.0001	<.0001	0.0413
16:1n-7	0.77^{a}	0.75^{b}	0.91^{a}	0.73^{c}	0.60^{d}	0.80^{b}	0.02	0.0002	<.0001	1.0000
16:1n-5	0.21^{a}	0.19^{b}	0.23^{b}	0.27^{a}	0.22^{b}	0.08^{c}	0.02	0.0002	<.0001	0.4182
16:2n-4	0.30^{a}	0.27^{b}	0.27^{c}	0.33^{a}	0.31^{c}	0.23^{d}	0.01	<.0001	<.0001	0.7722
17:00	0.19^{a}	0.17^{b}	0.24^{a}	0.20^{c}	0.22^{b}	0.05^{d}	0.02	0.0002	<.0001	0.4182
16:3n-4	0.23^{a}	0.21^{b}	0.25^{b}	0.23^{c}	0.27^{a}	0.12^{d}	0.01	<.0001	<.0001	0.7722
16:3n-3	0.17^{a}	0.16^{b}	0.17^{c}	0.20^{b}	0.26^{a}	0.03^{d}	0.02	0.0006	<.0001	0.7722
16:3n-1	0.70^{a}	0.64^{b}	0.59^{b}	0.75^{a}	0.60^{b}	0.73^{a}	0.02	0.0007	<.0001	0.0019
16:4n-3	0.34^{a}	0.32^{b}	0.43^{a}	0.29^{c}	0.35^{b}	0.25^{d}	0.01	0.0002	<.0001	1.0000
16:4n-1	0.13^{a}	0.12^{b}	0.19^{a}	0.20^{a}	0.06^{b}	0.06^{b}	0.01	0.0021	<.0001	0.6876
18:00	5.84^{a}	4.80^{b}	4.95^{c}	5.44^{b}	4.39^{d}	6.51^{a}	0.20	<.0001	<.0001	0.0001
18:1n-9	9.66^{a}	8.63^{b}	12.41^{a}	7.71^{c}	6.19^{d}	10.29^{b}	0.51	<.0001	<.0001	0.0124
18:1n-7	1.51^{a}	1.48^{b}	1.29^{d}	1.49^{b}	1.45^{c}	1.77^{a}	0.04	<.0001	<.0001	0.0677
18:1n-5	0.16^{a}	0.15^{b}	0.20^{a}	0.19^{a}	0.19^{a}	0.03^{b}	0.01	0.0021	<.0001	0.2526
18:2n-9	0.99	11.84	1.38	0.80	0.38	23.11	5.46	0.3337	0.3995	0.4183

18:2n-6	10.88^{a}	10.13^{b}	11.34^{b}	8.96^{c}	8.32^{d}	13.40^{a}	0.43	<.0001	<.0001	<.0001
18:2n-4	0.15^{a}	0.14^{b}	0.19^{b}	0.15^{c}	0.21^{a}	0.03^{d}	0.01	0.0021	<.0001	0.2526
18:3n-6	1.65^{a}	1.62^{b}	2.20^{a}	1.25^{b}	0.89^{c}	2.20^{a}	0.12	<.0001	<.0001	0.2863
18:3n-4	0.18^{a}	0.15^{b}	0.17^{c}	0.21^{b}	0.24^{a}	0.04^{d}	0.02	<.0001	<.0001	0.1084
18:3n-3	0.95^{a}	0.93^{b}	1.18^{b}	0.62^{d}	0.71^{c}	1.25^{a}	0.06	<.0001	<.0001	0.7722
18:4n-3	0.31^{a}	0.29^{b}	0.39^{a}	0.30^{b}	0.28^{c}	0.22^{d}	0.01	<.0001	<.0001	0.7722
18:4n-1	0.14^{a}	0.13^{b}	0.11^{b}	0.19^{a}	0.20^{a}	0.03^{c}	0.01	0.0075	<.0001	0.2863
20:00	0.37^{a}	0.35^{b}	0.41^{a}	0.32^{c}	0.39^{b}	0.32^{c}	0.01	<.0001	<.0001	0.7722
20:1n-9	0.22^{a}	0.20^{b}	0.31^{a}	0.23^{b}	0.22^{b}	0.09^{c}	0.02	0.0002	<.0001	0.4182
20:1n-7	1.27	1.26	1.53^{a}	1.14^{c}	1.18^{b}	1.21^{b}	0.03	0.1501	<.0001	0.8850
20:1n-5	0.22^{a}	0.20^{b}	0.25^{a}	0.22^{b}	0.25^{a}	0.13^{c}	0.01	0.0006	<.0001	0.7722
20:2n-9	0.42^{a}	0.39^{b}	0.61^{a}	0.37^{c}	0.23^{d}	0.41^{b}	0.03	<.0001	<.0001	0.2526
20:2n-6	0.55^{a}	0.53^{b}	0.71^{a}	0.56^{b}	0.48^{c}	0.40^{d}	0.02	<.0001	<.0001	0.0568
20:3n-9	2.43	0.18	0.15	0.35	4.70	0.02	1.12	0.3281	0.4033	0.4175
20:3n-6	0.96^{a}	0.94^{b}	1.19^{a}	0.81^{d}	0.85^{c}	0.95^{b}	0.03	<.0001	<.0001	0.7722
20:4n-6	1.76^{a}	1.74^{b}	1.60^{d}	1.70^{b}	2.09^{a}	1.62^{c}	0.04	0.0002	<.0001	0.4182
20:3n-3	0.38^{a}	0.36^{b}	0.36^{c}	0.51^{a}	0.39^{b}	0.21^{d}	0.02	0.0006	<.0001	0.7722
20:4n-3	0.39	2.74	0.54	0.22	0.41	5.09	1.19	0.3394	0.4323	0.4205
20:5n-3	1.61	1.61	2.01^{a}	1.34^{c}	1.32^{d}	1.76^{b}	0.06	1.0000	<.0001	1.0000
22:1n-1	0.75^{a}	0.73^{b}	0.61^{b}	0.45^{c}	1.71^{a}	0.19^{d}	0.12	<.0001	<.0001	0.6876
22:1n-9	0.89^{a}	0.86^{b}	0.91^{b}	0.78^{c}	1.50^{a}	0.32^{d}	0.09	<.0001	<.0001	0.4182
22:4n-6	0.38^{a}	0.35^{b}	0.44^{a}	0.44^{a}	0.44^{a}	0.14^{b}	0.03	<.0001	<.0001	0.0965
22:5n-6	3.07^{a}	2.29^{b}	1.19^{d}	2.88^{b}	4.65^{a}	2.01^{c}	0.28	<.0001	<.0001	<.0001
22:5n-3	1.02^{a}	0.99^{b}	1.70^{a}	0.72^{c}	0.56^{d}	1.04^{b}	0.09	<.0001	<.0001	0.7722
22:6n-3	18.36^{a}	16.58^{b}	10.82^{d}	21.25^{b}	24.42^{a}	13.39^{c}	1.18	<.0001	<.0001	<.0001
SFA	21.44^{a}	19.68^{b}	20.71^{b}	20.16^{c}	16.59^{d}	24.80^{a}	0.64	<.0001	<.0001	<.0001
MUFA	15.72^{a}	14.71^{b}	20.70^{a}	11.23^{d}	14.63^{b}	14.32^{c}	0.72	<.0001	<.0001	0.7722
n-3	23.64^{a}	22.13^{b}	18.54^{c}	24.80^{b}	30.15^{a}	18.05^{d}	1.05	<.0001	<.0001	<.0001
n-6	20.32^{a}	18.81^{b}	18.58^{d}	19.06^{c}	19.61^{b}	21.03^{a}	0.25	<.0001	<.0001	<.0001

n-9	12.80[a]	11.78[b]	19.06[a]	9.53[c]	8.95[d]	11.65[b]	0.85	<.0001	<.0001	0.1546
n-3/n-6	1.17[a]	1.15[b]	0.89[c]	1.39[b]	1.51[a]	0.83[d]	0.06	0.0002	<.0001	1.0000
n-3 LC-PUFA	21.94[a]	20.42[b]	15.14[d]	24.62[b]	28.32[a]	16.64[c]	1.15	<.0001	<.0001	<.0001
n-6 LC-PUFA	5.88[a]	4.86[b]	2.88[d]	5.61[b]	8.70[a]	4.31[c]	0.46	<.0001	<.0001	0.0615
EPA/ARA	1.03[a]	1.01[b]	1.75[a]	0.72[c]	0.59[d]	1.01[b]	0.09	0.0002	<.0001	0.0266
EPA/DHA	0.10[a]	0.09[b]	0.19[a]	0.04[c]	0.04[c]	0.12[b]	0.01	0.0081	<.0001	0.4832

Discussion

The Atlantic salmon (*Salmo salar* L.) were given a diet containing 5% *Schizochytrium* sp. oil acquired 31% more weight than those fed a diet without it (Sharawy et al., 2020a). Research was performed to check the effect of replacing FO with algae oil (AO) (*Schizochytrium* sp.) in farmed Atlantic salmon on growth. Four diets were formulated, three of which contained algal prime and one as the control. For every treatment, the weight of the fish increased by about eight times. For FCR with an algal diet, a significant change (p = 0.021) was observed (Zatti et al., 2023). FO was substituted with SO and PO in seven different diets of Asian seabass at percentages of 0%, 25%, 37.5%, 50%, 62.5%, 75%, and 100%, respectively. Compared to other feeding groups, the fish fed a diet that substituted 37.5% of the fish oil had better weight gain, FCR, and SGR (Rahman et al., 2022). Lee et al. (2022) reported that the reason for the decrease in fish growth beyond the ideal level of replacement of fish oil might be due to rigid cell wall of microalgae. In the present research, the growth performance of grass carp and rohu (fingerling and fry) was given in Table 3. Regarding species, a significant difference was recorded in FW, WG%, and FCR in fingerlings. Higher WG% and best FCR were obtained in rohu as compared to grass carp fingerlings. In fry signific ant differences were observed in AWG, WG%, SGR, and FCR. Higher WG% was obtained in rohu as compared to grass carp fry and a better FCR was observed in rohu than grass carp fry. Among treatments, in fingerlings, a significant difference was observed in FW, AWG, WG%, SGR, and FCR. Higher FW, AWG, and WG%, were obtained in fish fed with 1.33% algae oil in diet followed by fish fed with 2%, 0.67%, and control group. Higher SR and best FCR were noted in fish fed with 1.33% algae oil in the diet in fingerlings. Similarly, in fry significant differences was recorded in FW, AWG, WG%, SGR, FCR, and SR. Higher FW, AWG, and WG%, were obtained in fish fed with 1.33% algae oil in their diet followed by fish fed with 2%, 0.67%, and control group. Higher SR and best FCR were noted in fish fed with 1.33% algae oil in their diet. The result of the analysis of species and treatment interactions in fingerling showed significant differences in FCR. However, in fry significant difference was shown in FW, AWG, WG%, and FCR.

It is known that microalgal biomasses can change the color of fish. The most widely used microalgae for color enhancement is *Haematococcus pluvialis* because of its high astaxanthin content (Chen et al., 2017). Güroy et al. (2019) reported that the addition of microalgae in diet of fish improves coloration of the fillet to a control diet. The inclusion of *C. vulgaris* in fish feed increased the market value as it contains carotenes and xanthophylls (Gille et al., 2018). Fish-fed diets high in *C. vulgaris* show a general tendency of improvement in color parameters

(L and b) (Gouveia & Rema, 2005). The study was conducted to determine how various feeding carotenoid sources such as astaxanthin (20, 40, and 60 $mgkg^{-1}$), *Dunaliella salina* extract (DSE) (200, 400, and 600 $mgkg^{-1}$), crayfish meal (*Cherax quadricarinatus*) (10, 20 and 30 gkg^{-1}), and *squilla* sp meal (10, 20 and 30 gkg^{-1}) would effect on pigmentation of *Oreochromis* spp. In comparison to the control, reddish values (4.22) were improved by 30g of crayfish meal. When compared to DSE at 200 mg, astaxanthin showed greater chroma values and yellowness at 20 mg. In conclusion, the four sources of carotenoids significantly improved the commercial value of this cultivated species, with a focus on *Dunaliella salina*, and had a substantial impact on pigmentation (Arous et al., 2014). In the present study, the coloration of grass carp and rohu (fingerling and fry) was given in Table 4. In both fingerlings and fry, significant differences were present in L*, a*, and b* regarding species and treatment groups. Grass carp (fingerling and fry) showed higher coloration than rohu (fingerling and fry). Among treatments, the highest coloration was observed in treatment with a 2% addition of algae oil in the diet followed by 1.33%, 0.67%, and then the control group. The analysis of species and treatment interaction showed no significant differences in both fingerling and fry. The coloration becomes more pronounced with the increase in the level of algae oil in the diet.

Research was carried out to determine the fat content, by completely substituting terrestrial alternative oils for fish oil (FO). Three diets were made by substituting linseed oil, soybean oil, and lard (designed as FO, LO, SO, and lard, respectively) and the control diet contains 6% FO. In comparison to the fish-fed diet containing FO, fish-fed LO and SO diets showed significantly greater levels of whole-body lipids (Sankian et al., 2019). FO was replaced with SO and PO in seven different diets of Asian seabass at percentages of 0%, 25%, 37.5%, 50%, 62.5%, 75%, and 100%, respectively. The final fish carcass's moisture, ash content, and crude protein were not significantly effected by replacement of fish oil replacement using a combination of SO and PO. The higher lipid content was noticed with a 100% FO replacement diet as compared to other diets (Rahman et al., 2022). The purpose of the study was to determine how various feeding levels of carotenoid source such as astaxanthin (20, 40, and 60 $mgkg^{-1}$), *Dunaliella salina* extract (DSE) (200, 400, and 600 $mgkg^{-1}$), crayfish meal (*Cherax quadricarinatus*) (10, 20 and 30 gkg^{-1}), and *squilla* sp meal (10, 20 and 30 gkg^{-1}) would effect on crude protein of *Oreochromis* spp. Among all the treatments, the 20g crayfish exhibited the highest levels of crude protein (Arous et al., 2014). Juvenile black seabream, *Acanthopagrus schlegeli*, was used in a feeding experiment to assess the effects of replacing SO for FO in the diet on fish liver. Fish were given four different diets in triplicate, with soybean oil replacing fish oil in the amounts of 0%, 60%, 80%, and 100%. The addition of soybean oil increased the liver's crude lipid content; the amount of fish oil replacement had no significant effect on liver proximate composition (Peng et al., 2008). Mukherjee et al. (2011) reported that increase in protein content in fish from experimental groups results from more protein in algal-based experimental diets. In the current study, the proximate analysis of grass carp and rohu (fingerling and fry) was given in Table 5. Species comparison showed a significant difference in moisture in fingerlings. Grass carp fingerlings showed higher moisture content than rohu fingerlings. In fry, no significant difference was present in moisture, protein, fat, and ash content. Regarding treatment groups, a significant difference was noted in all treatments except for fat content in fingerlings. Higher protein and moisture values were noted in treatment with 1.33% algae oil in the diet followed by 2%, 0.67%, and control group. Higher ash content was noted in the control group and the lowest ash content was observed in treatment with 1.33% algae oil in diet. In fry, a significant difference was recorded in moisture and fat contents. The highest moisture value was shown in treatment with 1.33% algae oil in the diet followed by 0.67% in the control group and then 2% treatment level. Significant interactions were shown in moisture and protein content in fingerlings. However, no significant differences in interactions were noted in moisture, protein, fat, and ash content in fry.

The addition of dietary algae increased the quantity of LC-PUFA in tissues of fish. As C. vulgaris also contains LC-PUFA, it also improves the fatty acid profile of fish (Norambuena, 2015). To check the effects of replacement of fish oil in the diet with a combination of *Schizochytrium* sp. and *Microchloropsis* sp. and replacing fishmeal in the diet with *C. vulgaris* on the fatty acid profile of *Sparus aurata's* muscle, Karapanagiotidis et al. (2022) experimented. They found that replacing fish oil with a mixture of *Schizochytrium* sp. and *Microchloropsis* sp. enhanced the n-6 PUFA, especially 20:4n-6 and 22:5n-6 in the muscle. An experiment was performed to check the results of full replacement of FO with AO (*Schizochytrium* sp.) in farmed Atlantic salmon on fatty acid profile. Four diets were formulated, three of which contained algal prime and one as the control. The majority of fatty acid analyses conducted on the entire body revealed significant differences with algal diets (Zatti et al., 2023). FO was replaced with SO and PO in seven different diets of Asian seabass at percentages of 0%, 25%, 37.5%, 50%, 62.5%, 75%, and 100%, respectively. Fish fed a diet that had 37.5% FO replacement showed improved levels of arachidonic acid (20:4n-6), EPA (20:5n-3), DHA (22:6n-3), and the n-3/n-6 ratio (Rahman et al., 2022). The purpose of the study was to evaluate poppy seed oil's potential application as the primary lipid source in typical carp diets. The common carp were given five different experimental diets. The diets included varying amounts of blended fish oil and poppy seed oil as oil sources. The findings showed that poppy seed oil increased the amount of linoleic acid (18: 2 ω−6) in the diet (Kesbiç et al., 2023). The diet without algae oil had the highest EPA content (20:5n-3), consequently the highest levels of EPA/ARA and EPA/DHA, as well as the highest content of LNA (18:3n-3). The diets with algae oil had higher levels of n-3 LC-PUFA, specifically DHA (22:6n-3) and n-6 DPA (22:5n-6), as well as a higher n-3/n-6, than the diet without algae oil. The diet with 1.33% inclusion of algae oil had the highest levels of n-6 DPA and n-3 LC-PUFA. The diet containing 2% algal oil had the highest content of SFA, especially palmitic acid (16:0), while the diet containing 0.67% algal oil had a slightly higher content of n-3/n-6 fatty acids. Thus, in comparison to the control group, the inclusion of 2% or 1.33% algae oil doubled the contents of SFA, and all the algae oil-containing diets increased the DHA content by fourfold (Table 6 and Table 7).

Due to the high DHA and EPA content of the microalgae, diets containing *Schizochytrium* sp. have been shown to boost the efficiency of EPA+DHA retention. The three diets were as follows: diet 1 had higher levels of n-3 LC-PUFA from marine sources (10% EPA+DHA, FO), diet 2 had higher levels of n-3 LC-PUFA supplemented with *Schizochytrium* sp. (AO) (5% FO+5% AO), and the control diet was similar to commercial feed (7.5% EPA+DHA, FO). Overall improvements in body weight were observed across all diet groups, suggesting that the inclusion of microalgae had no detrimental effects. The salmon-fed enriched microalgae diet resulted in higher levels of oleic acid, linoleic acid, and DHA in the fillets' composition. The salmon fillets from the diets with higher n-3 LC PUFA levels had the highest levels of EPA (Gard, 2023). Juvenile black seabream, *Acanthopagrus schlegeli*, was used in a feeding trial to assess the effects of replacing soybean oil with fish oil in the diet on fatty acids. Fish were given four diets in triplicate, with soybean oil replacing fish oil in the amounts of 0%, 60%, 80%, and 100%. Fish-fed soybean oil diets showed large increases in linoleic and linolenic acid; nevertheless, the addition of soybean oil to the diet resulted in significant reductions in DHA, EPA, and the ratio n−3/n−6 (Peng et al., 2008). In the current research, the fatty acid profile analysis of grass carp and rohu fingerlings, as depicted in Table 8, revealed significant differences regarding species, except for 18:2n-6 and 20:3n-6, and the ratio of n-3/n-6. Across various treatment groups, significant differences were observed in most fatty acids, except for 18:2n-6. The analysis of interaction of species and treatments showed significant differences in numerous fatty acids, including 14:1n-7, 14:1n-5, 15:00, 16:00, 16:1n-7, 18:00, 18:1n-9, 18:1n-7, 18:2n-9, 18:3n-6, 20:1n-7, 20:5n-3, 22:5n-6, 22:6n-3, n-3, n-6, n-9, and n-3/n-6. The fatty acid profile analysis of grass carp and rohu fry, as presented in Table 9, showed significant differences regarding species, except for fatty acids 18:2n-9, 20:1n-7, 20:3n-9, 20:4n-3, and

20:5n-3. Among treatments, a significant difference was observed in most fatty acids, except in 18:2n-9, 20:3n-9, and 20:4n-3. The interaction analysis between species and treatment showed significant differences in specific fatty acids, including 16:00, 16:1n-5, 17:00, 16:3n-1, 18:00, 18:1n-9, 18:2n-6, and 20:2n-6.

Conclusion

The current study aimed to formulate fish feed by replacing animal source oil with plant-based oil, as well as their impacts on grass carp and rohu growth, body pigmentation, survival rate, and immunology. The replacement of fish oil with algae oil improved WG and reduced FCR. It also enhances immunity and flesh quality in fish, making it an alternative source of fish oil. The performance of grass carp and rohu (fry and fingerlings) can be enhanced by replacing fish oil with algae oil up to 1.33-2% inclusion level. Including 1.33% *C. vulgaris* in the diet yields the highest weight gain, protein and fat content; however, 2% inclusion enhances coloration. Microalgae oil can reduce reliance on fish oil in aquafeeds and could have sparked significant research and commercial interest. Therefore, 1.33-2% of *C. vulgaris* oil in the diet is recommended at an industrial scale to improve the growth of fish.

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